DEPARTMENT OF MICROBIOLOGY
KARPAGAM ACADEMY OF HIGHER EDUCATION
(Established Under Section 3 of UGC Act, 1956)
Eachanari Post, Coimbatore, Tamil Nadu, India – 641 021

I M.Sc MICROBIOLOGY - 2017 – 2019 Batch

FUNDAMENTALS OF MICROBIOLOGY AND CLASSIFICATION (17MBP101)

SEMESTER – I **4H – 4C**

SYLLABUS

UNIT – I

History and scope of Microbiology. Microbial evolution and Diversity – Taxonomic ranks - Classification system – Phenetic and Phylogenetic Haeckel's three–kingdom concept, Whittaker's Five-kingdom concept, Three-domain concept of Carl Woese.

UNIT – II

Microscopy –Simple, Compound, Dark-field, Phase contrast, Fluorescent and Electron microscopes. (SEM and TEM), Confocal microscopy – Principles and their applications. Stains and Staining techniques: Simple and Differential staining methods.

UNIT – III

Classification of bacteria - Bergey's manual and its importance. Classification of algae Clamydomonas, volvox, diatoms, red and brown algae. Classification of virus – DNA, RNA viruses. Classification and taxonomy of fungi – Alexopolous. Economical importance of Fungi. Classification of protozoa – *Entamoeba histolytica, Giardia, Trichomonas, Plasmodium.*

UNIT – IV

Sterilization and disinfection, culture methods: Auxenic and synchronous, aerobic and anaerobic, culture media and nutritional types, growth curve, generation time and growth kinetics. Factors influencing microbial growth. Preservation methods and quality control.

UNIT – V

Modern Microbiology: Molecular taxonomy, 16S/18S rRNAs and its importance in identification of microorganisms. Phylogenetic tree, Molecular tools in assessing microbial diversity, probiotics and their applications, microbial fuel cells.

SUGGESTED READINGS

TEXT BOOKS

1. Dubey, R.C., and Maheswari, D.K., (2010). *A Text book of Microbiology*. (3rd Ed), S. Chand and Company, New Delhi.
2. Modi, H. A. (1996). *Elementary Microbiology*. Vol.2, AKTA Prakashan Nadiad, Gujarat
3. Powar, C.B., and Daginawala, H.F., (2008). *General Microbiology*. Vol: 2. Himalaya Publishing House.
4. Singh, R.P. (2007). *General Microbiology*. Kalyani Publishers, New Delhi.
5. Frobisher, H., Hinsdil, R.D., Crabtree, K.T., and Goodhert, D.R., (2005). *Fundamentals of Microbiology*, Saunder and Company, London.

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1. Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T., and Williams, S.T., (2000). *Bergey's Manual of Determinative Bacteriology*. (9th ed.). Lippincott Williams and Wilkins Publishers. Baltimore.
2. Pelczar Jr. M.J., Chan, E.C.S., and Kreig, N.R., (2004). *Microbiology*. (5th ed.). Tata McGraw-Hill Publishing Company, New Delhi.
3. Prescott, L.M., Harley, J.P., and Klein, C.A., (2003). *Microbiology*, (5th ed.). McGraw Hill Publishing Company Limited, New York.
4. Salle, A.J. (2007). *Fundamental Principles of Bacteriology*. (7th ed.)., Envins Press, New York.
5. Tortora, G.J., Funke, B.R., and Case, C.L., (2010). *Microbiology: An Introduction*. (10th ed.). Pearson Education, Singapore.
6. Alcomo, I.E., (2006). *Fundamentals of Microbiology*. (8th ed.). Jones and Bartlett Publishers, Sudbury, Massachusetts.
7. Stanier, R.Y., Ingraham, J.L., Wheelis, M.L., and Painter, P.R., (2008). *General Microbiology*.
   (5th ed.). Macmillan Press Ltd, London.
8. Talaro, K.P., and Talaro, A., (2006). *Foundations in Microbiology*. (6th ed.). McGraw-Hill College, Dimensi.

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LECTURE PLAN

Unit I

S.No	Duration	Topic	Reference
1	2	History of Microbiology	R1: pgs ;1-2, W1, R2:pgs 3-17:
2	2	Microbial evolution and Diversity	R1:pgs; 422 -425, W2
3	2	Taxonomic ranks ,Classification system	R1:pgs; 425 -426, W2
4	2	Phenetic and Phylogenetic	R1:pgs; 426 -428, W3
5	2	Haeckel's three–kingdom concept,	R1:pgs: 435 - 439
6	1	Whittaker's Five-kingdom concept,	R1:pgs: 435 - 439
7	1	Three-domain concept of Carl Woese.	R1:pgs: 435 - 439
8	1	Recapitulation and discussion of question	
	Total Hrs: 13		

R1: Prescott, L.M., J.P. Harley and C.A. Klein, 2003. Microbiology, 5th Edition McGraw Hill Publishing Company Limited. New York.

R2: Pelczar Jr. M.J., E.C.S. Chan and N.R. Kreig, 2004. Microbiology. 5th Edition. Tata McGraw-Hill Publishing Company. New Delhi.

W1: quizlet.com/1691173/brief-history-of-microbiology

W2: mcdb.colorado.edu

W3: www.blackwellpublishing.com

UNIT - I

History and scope of Microbiology

Introduction

Microbiology, the study of microorganisms derives its name from three greek words - mikros (small), bios (life) and logos (study). This means that microbiology deals with the study of microorganisms. What are microorganisms? Organisms which are so tiny and invisible to the naked eye constitute micro organisms (microbes). If any object is smaller than 0.lmm, the human eye can not perceive it and even at a size of 1.0mm very little details of an object can be seen with the naked eye. Hence we can say that study of organisms with a size of 1mm or less comes under the preview of microbiology. Microorganisms can be looked into and studies only with the help of microscope. Scientists opine that microbes originated on our planet about three or four billion years ago from complex organic materials present in ocean waters or possibly in vast cloud banks. As the first forms of life they are regarded as ancestral to all forms of life on earth.

Microbiology is an exceptionally broad discipline encompassing specialties as diverse as biochemistry, cell biology, genetics, taxonomy, pathogenic bacteriology, food and industrial microbiology, and ecology. A microbiologist must be acquainted with many biological disciplines and with all major groups of microorganisms: viruses, bacteria, fungi, algae, and protozoa.

Microbiology often has been defined as the study of organisms and agents too small to be seen clearly by the unaided eye that is, the study of microorganisms. Because objects less than about one millimeter in diameter cannot be seen clearly and must be examined with a microscope, microbiology is concerned primarily with organisms and agents this small and smaller. Its subjects are viruses, bacteria, many algae and fungi, and protozoa. Yet other members of these groups, particularly some of the algae and fungi, are larger and quite visible. For example, bread molds and filamentous algae are studied by microbiologists, yet are visible to the naked eye. The difficulty in setting the boundaries of microbiology led Roger Stanier to suggest that the field be defined not only in terms of the size of its subjects but also in terms of

its techniques. A microbiologist usually first isolates a specific microorganism from a population and then cultures it. Thus microbiology employs techniques such as sterilization and the use of culture media that are necessary for successful isolation and growth of microorganisms.

Beginning of Microbiology

Although microbes are most ancient and they had the planet all for themselves initially, even after the advent of man they have been influencing his life both for good and bad since time immemorial. In a lighter vein one can say that ever since the first toast was proposed and the first loaf of bread was baked man has known the influence of microbes.

In spite of the above statement it seems incredible that the scientific study of microbes-microbiology is an infant science. In fact but for one single instrument viz., the microscope, the entire microbial world would have been unknown to us. Microbes were observed for the first time by Leeuwenhoek a little more than 300 years ago and even then their role in human life was never contemplated. They were just thought to be cute tiny animalcules and their study was a mere curiosity. There was indeed a lapse of more than 200 years since their discovery before they were studied seriously as notorious pathogens. Conclusive refutation of the theory of spontaneous generation by Louis Pasteur, and the germ theory of disease by Robert Koch may be mentioned as the two most significant discoveries that made many scientists truly open their eyes towards microorganisms and their role in human welfare. The discovery of Louis Pasteur that microbes are not the result but cause of fermentation opened up entirely new vistas-microbes as agents of chemical change.

Historical Events of Microbiology

- **Van Leeuwenhoek (1673):** Developed the simple microscopes. First to observe microbes (animalcules). Father of Microbiology
  - **Spontaneous generation:** Living organisms can arise from nonliving matter Ex: Moist soil toads, snakes, mice
  - **Biogenesis:** Living organisms can arise only from preexisting living organisms
- **Francesco Redi:** Set up an experiment to disprove spontaneous generation of maggots. Filled 2 jars with decaying meat sealed one and left the other open (only the open jar

developed maggots). Another experiment was set up in which a jar was covered with a fine mesh instead of being sealed so that fresh air could enter the jar (again, only the open jar developed maggots). Proved that the flies had to come in contact with the meat and lay their eggs on it, maggots did not arise spontaneously.

- **John Needham:** Seemed to help prove the case for spontaneous generation by heating nutrient broth and pouring it into covered flasks (developed microbial growth) he claimed that the "vital force" had been destroyed by the heating.
- **Lazzaro Spallanzani:** He showed that broth that was heated after being put into a sealed flask did not develop microbial growth.
- **Louis Pasteur**
  1) Microorganisms can be present in nonliving matter (even in air).
  2) Spontaneous generation disproved.
  3) Heat can destroy microbes
  - **Aseptic Techniques:** Pasteur used to prevent the contamination by unwanted microorganisms.
  - **Water purification, sewage collection and treatment:** Romans developed sophisticated drinking water and sewer systems (even indoor plumbing), but these ideas were lost during the Dark Ages. Most villages, towns, and cities had problems with certain diseases due to fecal contamination of their drinking water because they lacked sewer systems. Development of good public sanitation facilities during the 18th and 19th centuries in what are now "developed" countries, drastically decreased their incidence of epidemics of cholera, typhoid and dysentery.
- **Bassi (1830s-40s):** Perhaps the first to demonstrate a microbial cause of disease. Showed that a microscopic fungus causes a disease in silkworms. Suggested that human diseases could be caused by microbes also
- **Semmelweiss (1840s)**
  1. Suspected doctors/midwives were transmitting childbed fever to women during childbirth.
  2. Required hand washing in a chloride of lime solution. Incidence of childbed fever dropped from up to 50% to 1-3% viciously criticized Died in exile and shame.

- **Joseph Lister (1860s)**
  1. Used a disinfectant (phenol) to clean wounds, surgical dressings and instruments.
  2. Large decrease in wound infections.
  3. Concept of Asepsis (lack of germs) finally accepted.
- **Koch (1876)**
  1. Developed pure culture techniques for growing bacteria in the lab.
  2. Proved Germ Theory of Disease by showing that a specific type of bacteria causes anthrax.
  3. Koch's Postulates: Steps for determining the cause of any infectious disease.
     - **Smallpox inoculation by Chinese physicians:** Used intranasal inoculation of individuals with dried small pox pustules from patients with mild cases Records seem to indicate the practice started as early as the 6th century in China Became widely used in China in the 16th and 17th centuries. In the 17th century, Western civilizations began to take notice
- **Jenner (1796):** Milkmaids who had had cowpox seemed to be protected from smallpox Showed that inoculation with material from cowpox lesions leads to immunity from smallpox (1st vaccine) 3. Steady decline in smallpox cases until eradicated in 1977.
- **Louis Pasteur:** Found that certain microbes that lost their ability to cause disease due to repeated culturing in the lab could be used to generate immunity Anthrax, cholera, rabies vaccines.
- **Fleming (1928):** Discovered fungi produced penicillin which killed bacteria.
- **Chain and Florey (1941):** Showed penicillin was effective against bacterial infections in patients 1 Commercial production of penicillin - Just in time for World War II.
- **1970s-1990s:** Most drug companies stop or greatly reduce antibiotic research.
- **Today - Major problem with antibiotic resistant bacteria, few new antibiotics**
- **Genetics and Molecular Biology (Avery, Macleod, and McCarty):** Showed that DNA was the molecule encoding heredity.
- **Watson and Crick:** Discovered the structure of DNA.
- **Mullis:** Developed the polymerase chain reaction (PCR).

Scope of Microbiology

Since the microbes are living, it follows that microbiology deals with a group of particular life forms and it comes under the broad domain of biology which includes the study of all aspects of living beings including man.

Where can we fit in microbes in the hierarchy of living beings? Traditionally living beings are divided into plants and animals. But members of microbes can be accommodated in both plants (fungi) and animals (protozoa) and some cannot be accommodated in either plants or animals as they share the characters of both. (For instance Euglena was a disputed property till recently between botanists and zoologists.)

In one of the earlier attempts to resolve this problem, Haeckel (1866) a German Zoologist suggested that there should be a third kingdom besides Plantae (plants) and Animalia (animals) to include all the microorganisms. He gave the name Protista to this kingdom to include all unicellular microorganisms that are neither plants nor animals.

Haeckel's classification raised some questions like how to distinguish a fungus from a bacterium or from an alga. The discovery in late 1940s of the prokaryotic and eukaryotic nature of the cells rendered the three kingdom classification unsatisfactory.

A recent and comprehensive classification proposed by R.H. Whittaker (1969) has five kingdoms of living beings

Kingdom Monera

Kingdom Protista

Kingdom Fungi

Kingdom Animalia

Kingdom Plantae

Microorganisms include three of the (Monera, Protista and Fungi) five kingdoms mentioned above. At present it is agreed that within the preview of microbiology, five major groups of microorganisms-viruses, bacteria, fungi, algae and protozoa are dealt with.

As it will be evident from the above discussion, the scope of microbiology extends to both eukaryotic as well as prokaryotic microbes. While discussing the scope of microbiology it should be evident to us that it does not deal with merely the enumeration of structural diversity or classification but extends to all aspects of microbial life.

Microbiology is concerned with their form, structure, reproduction, physiology, metabolism classification and most important their economic importance. In other words, what the microbes can do and should not be allowed to do (sometimes) as for as human beings are concerned is one of the vital aspects of microbiology on which rests human destiny.

Branches of Microbiology

It has been mentioned earlier that microbiology is not a mere study of the structural diversity and classification of microbes but encompasses the whole gamut of microbial life. The knowledge of the various aspects of microbes has been accumulating since last century and has become so vast that no microbiologist can claim familiarity with all aspects of the subject. The various aspects of microbiological study can be divided basically into the following branches.

The five Kingdom classifications of living beings

1. **Phycology**: Deals with the study of autotrophic eukaryotic organisms. I Members are generally called algae. Algae include both microscopic as well as macroscopic members. Only the microscopic algae are studied as a part of microbiology.
2. **Mycology**: The study of eukaryotic, achlorophyllous organisms generally referred to as Fungi is included in this branch. Some of the common fungi are yeasts, moulds, mushrooms, puff balls etc. Fungi are not only harmful but beneficial also.
3. **Virology**: Viruses are neither eukaryotic not prokaryotic. In fact they are on the border line between living and non living. Viruses cause disease to plants and animals including human beings. The dreaded AIDS is also caused by a virus.

4. **Protozoology**: Study of Protozoans in all their aspects comes under the perview of protozoology. Protozoans are known to cause many diseases like malaria, amoebic dysentery, sleeping sickness etc.
5. **Bacteriology**: This is the largest group among microbes not only in number but also in importance. Bacteria of both kinds Eubacteria and cyanobacteria (also known as blue green algae) are studied here. Bacteria have a profound influence on various human endeavours including health, industry, agriculture etc.
6. **Medical microbiology**: This branch deals with the pathogenic microbes, their life cycle, physiology, genetics, reproduction etc., many of the microbes also provide remedies for microbial diseases. All these aspects are studied in this branch. Some of the diseases like tuberculosis, leprosy, typhoid etc are caused by microbes and cure for them is provided by other microbes in the form of antibiotics.
7. **Agricultural microbiology**: In this branch the role of microbes in agriculture is studied from the point of view of both harm and usefulness. Many microbes - fungi, bacteria and viruses cause a number of plant diseases. From the point of view of benefit - N2 fixing activity, use of microbes as biofertilizers and several other aspects are studied.
8. **Industrial microbiology**: The role of microbes in Industrial Production is studied. Many microbes produce industrial alcohols, and acids as a part of their metabolism. The study of fermentation by microbes has contributed a great lot to alcohol manufacturing. Breweries have greatly benefited by understanding the role of specific microbes in fermentation.
9. **Food and Dairy microbiology**: Various aspects such as food processing, food preservation, canning, Pasteurization of milk, study of food borne microbial diseases and their control is studied.
10. **Aquatic microbiology**: Microbiological examination of water, water purification, and biological degradation of waste are studied in this branch.
11. **Aero microbiology**: Dispersal of disease causing microbes through air microbial population in air, their quality and quantity in air comes under the preview of this branch.
12. **Environmental microbiology**: This is one of the most important branches of micro biology. The role of microbes in maintaining the quality of the environment is studied here. Microbial influence in degradation and decay of natural waste, their role in biogeochemical cycles are

all studied. Some of the recent researches have shown that certain bacteria can help in cleaning the oil spill and this gives added significance to the study of environmental microbiology.

13. **Geochemical microbiology**: Role of microbes in coal, gas and mineral formation, prospecting for coal, oil and gas and recovery of minerals from low grade ores using microbes is included here.
14. **Biotechnology**: This is the most significant branch which may even change the course of life as we know today. Microbes are used as gene carriers to deliver specific genes to function in a different environment. New, genetically engineered microbes can produce drugs (human insulin) or in agriculture N2 fixing ability may be transferred to all the plants. The Potentialities of biotechnology are immense.
15. **Immunology**: Studied in this branch are the immune responses in organisms. How toxins are produced? How the antigens influence the formation of antibodies? How protective vaccination helps in combating the diseases? How immune system collapses (as in AIDS) are some of the questions for which immunology as a branch of microbiology is trying to find out answers.
16. **Exo microbiology**: This is a branch still in its infancy. Study of life's in outer space.

Discovery of Microorganisms

Even before microorganisms were seen, some investigators suspected their existence and responsibility for disease. Among others, The Roman philosopher Lucretius (about 98–55 B.C.) and the physician Girolamo Fracastoro (1478–1553) suggested that disease was caused by invisible living creatures. The earliest microscopic observations appear to have been made between 1625 and 1630 on bees and weevils by the Italian Francesco Stelluti, using a microscope probably supplied by Galileo. However, the first person to observe and describe microorganisms accurately was the amateur microscopist Antony van Leeuwenhoek (1632–1723) of Delft, Holland.

Leeuwenhoek earned his living as a draper and haberdasher (a dealer in men's clothing and accessories), but spent much of his spare time constructing simple microscopes composed of double convex glass lenses held between two silver plates. His microscopes could magnify

around 50 to 300 times, and he may have illuminated his liquid specimens by placing them between two pieces of glass and shining light on them at a 45° angle to the specimen plane. This would have provided a form of dark-field illumination (see chapter 2) and made bacteria clearly visible. Beginning in 1673 Leeuwenhoek sent detailed letters describing his discoveries to the Royal Society of London. It is clear from his descriptions that he saw both bacteria and protozoa.

Theory of Spontaneous generation

From earliest times, people had believed in spontaneous generation that living organisms could develop from nonliving matter. Even the great Aristotle (384–322 B.C.) thought some of the simpler invertebrates could arise by spontaneous generation. This view finally was challenged by the Italian physician Francesco Redi (1626–1697), who carried out a series of experiments on decaying meat and its ability to produce maggots spontaneously. Redi placed meat in three containers. One was uncovered, a second was covered with paper, and the third was covered with fine gauze that would exclude flies. Flies laid their eggs on the uncovered meat and maggots developed. The other two pieces of meat did not produce maggots spontaneously. However, flies were attracted to the gauze-covered container and laid their eggs on the gauze; these eggs produced maggots.

Thus the generation of maggots by decaying meat resulted from the presence of fly eggs, and meat did not spontaneously generate maggots as previously believed. Similar experiments by others helped discredit the theory for larger organisms. Leeuwenhoek's discovery of microorganisms renewed the controversy.

Some proposed that microorganisms arose by spontaneous generation even though larger organisms did not. They pointed out that boiled extracts of hay or meat would give rise to microorganisms after sitting for a while. In 1748 the English priest John Needham (1713–1781) reported the results of his experiments on spontaneous generation. Needham boiled mutton broth and then tightly stoppered the flasks. Eventually many of the flasks became cloudy and contained microorganisms. He thought organic matter contained a vital force that could confer the properties of life on nonliving matter

A few years later the Italian priest and naturalist Lazzaro Spallanzani (1729–1799) improved on Needham's experimental design by first sealing glass flasks that contained water and seeds. If the sealed flasks were placed in boiling water for 3/4 of an hour, no growth took place as long as the flasks remained sealed. He proposed that air carried germs to the culture medium, but also commented that the external air might be required for growth of animals already in the medium. The supporters of spontaneous generation maintained that heating the air in sealed flasks destroyed its ability to support life.

Several investigators attempted to counter such arguments. Theodore Schwann (1810–1882) allowed air to enter a flask containing a sterile nutrient solution after the air had passed through a red-hot tube. The flask remained sterile. Subsequently Georg Friedrich Schroder and Theodor von Dusch allowed air to enter a flask of heat-sterilized medium after it had passed through sterile cotton wool. No growth occurred in the medium even though the air had not been heated. Despite these experiments the French naturalist Felix Pouchet claimed in 1859 to have carried out experiments conclusively proving that microbial growth could occur without air contamination.

This claim provoked Louis Pasteur (1822–1895) to settle the matter once and for all. Pasteur first filtered air through cotton and found that objects resembling plant spores had been trapped. If a piece of the cotton was placed in sterile medium after air had been filtered through it, microbial growth appeared. Next he placed nutrient solutions in flasks, heated their necks in a flame, and drew them out into a variety of curves, while keeping the ends of the necks open to the atmosphere Pasteur then boiled the solutions for a few minutes and allowed them to cool. No growth took place even though the contents of the flasks were exposed to the air. Pasteur pointed out that no growth occurred because dust and germs had been trapped on the walls of the curved necks. If the necks were broken, growth commenced immediately. Pasteur had not only resolved the controversy by 1861 but also had shown how to keep solutions sterile. The English physicist John Tyndall (1820–1893) dealt a final blow to spontaneous generation in 1877 by demonstrating that dust did indeed carry germs and that if dust was absent, broth remained sterile even if directly exposed to air. During the course of his studies, Tyndall provided evidence for the existence of exceptionally heat-resistant forms of bacteria. Working independently, the

German botanist Ferdinand Cohn (1828–1898) discovered the existence of heat-resistant bacterial endospores.

The Role of Microorganisms in Disease

The importance of microorganisms in disease was not immediately obvious to people, and it took many years for scientists to establish the connection between microorganisms and illness. Recognition of the role of microorganisms depended greatly upon the development of new techniques for their study. Once it became clear that disease could be caused by microbial infections, microbiologists began to examine the way in which hosts defended themselves against microorganisms and to ask how disease might be prevented. The field of immunology was born. Recognition of the Relationship between Microorganisms and Disease

Although Fracastoro and a few others had suggested that invisible organisms produced disease, most believed that disease was due to causes such as supernatural forces, poisonous vapors called miasmas, and imbalances between the four humors thought to be present in the body.

The idea that an imbalance between the four humors (blood, phlegm, yellow bile [choler], and black bile [melancholy]) led to disease had been widely accepted since the time of the Greek physician Galen (129–199). Support for the germ theory of disease began to accumulate in the early nineteenth century.

Agostino Bassi (1773–1856) first showed a microorganism could cause disease when he demonstrated in 1835 that a silkworm disease was due to a fungal infection. He also suggested that many diseases were due to microbial infections.

In 1845 M. J. Berkeley proved that the great Potato Blight of Ireland was caused by a fungus. Following his successes with the study of fermentation, Pasteur was asked by the French government to investigate the pébrine disease of silkworms that was disrupting the silk industry. After several years of work, he showed that the disease was due to a protozoan parasite. The disease was controlled by raising caterpillars from eggs produced by healthy moths.

Indirect evidence that microorganisms were agents of human disease came from the work of the English surgeon Joseph Lister (1827–1912) on the prevention of wound infections. Lister

impressed with Pasteur's studies on the involvement of microorganisms in fermentation and putrefaction, developed a system of antiseptic surgery designed to prevent microorganisms from entering wounds. Instruments were heat sterilized, and phenol was used on surgical dressings and at times sprayed over the surgical area.

The approach was remarkably successful and transformed surgery after Lister published his findings in 1867. It also provided strong indirect evidence for the role of microorganisms in disease because phenol, which killed bacteria, also prevented wound infections.

The first direct demonstration of the role of bacteria in causing disease came from the study of anthrax (see chapter 39) by the German physician Robert Koch (1843–1910). Koch (figure 1.4) used the criteria proposed by his former teacher, Jacob Henle (1809–1885), to establish the relationship between Bacillus anthracis and anthrax, and published his findings in 1876. Koch injected healthy mice with material from diseased animals, and the mice became ill. After transferring anthrax by inoculation through a series of 20 mice, he incubated a piece of spleen containing the anthrax bacillus in beef serum.

The bacilli grew, reproduced, and produced spores. When the isolated bacilli or spores were injected into mice, anthrax developed. His criteria for proving the causal relationship between a microorganism and a specific disease are known as Koch's postulates and can be summarized as follows:

1. The microorganism must be present in every case of the disease but absent from healthy organisms.
2. The suspected microorganism must be isolated and grown in a pure culture.
3. The same disease must result when the isolated microorganism is inoculated into a healthy host.
4. The same microorganism must be isolated again from the diseased host.

Although Koch used the general approach described in the postulates during his anthrax studies, he did not outline them fully until his 1884 publication on the cause of tuberculosis.

Koch's proof that Bacillus anthracis caused anthrax was independently confirmed by Pasteur and his coworkers. They discovered that after burial of dead animals, anthrax spores survived and were brought to the surface by earthworms. Healthy animals then ingested the spores and became ill.

The Development of Techniques for Studying Microbial Pathogens

During Koch's studies on bacterial diseases, it became necessary to isolate suspected bacterial pathogens. At first he cultured bacteria on the sterile surfaces of cut, boiled potatoes. This was unsatisfactory because bacteria would not always grow well on potatoes. He then tried to solidify regular liquid media by adding gelatin. Separate bacterial colonies developed after the surface had been streaked with a bacterial sample. The sample could also be mixed with liquefied gelatin medium. When the gelatin medium hardened, individual bacteria produced separate colonies. Despite its advantages gelatin was not an ideal solidifying agent because it was digested by many bacteria and melted when the temperature rose above 28°C. A better alternative was provided by Fannie Eilshemius Hesse, the wife of Walther Hesse, one of Koch's assistants. She suggested the use of agar as a solidifying agent she had been using it successfully to make jellies for some time. Agar was not attacked by most bacteria and did not melt until reaching a temperature of 100°C. One of Koch's assistants, Richard Petri, developed the petri dish (plate), a container for solid culture media.

These developments made possible the isolation of pure cultures that contained only one type of bacterium, and directly stimulated progress in all areas of bacteriology. Isolation of

bacteria and pure culture techniques.

Koch also developed media suitable for growing bacteria isolated from the body. Because of their similarity to body fluids, meat extracts and protein digests were used as nutrient sources.

The result was the development of nutrient broth and nutrient agar, media that are still in wide use today. By 1882 Koch had used these techniques to isolate the bacillus that caused tuberculosis. There followed a golden age of about 30 to 40 years in which most of the major bacterial pathogens were isolated.

The discovery of viruses and their role in disease was made possible when Charles Chamberland (1851–1908), one of Pasteur's associates, constructed a porcelain bacterial filter in 1884. The first viral pathogen to be studied was the tobacco mosaic disease virus.

Immunological Studies

In this period progress also was made in determining how animals resisted disease and in developing techniques for protecting humans and livestock against pathogens. During studies on chicken cholera, Pasteur and Roux discovered that incubating their cultures for long intervals between transfers would attenuate the bacteria, which meant they had lost their ability to cause the disease. If the chickens were injected with these attenuated cultures, they remained healthy but developed the ability to resist the disease. He called the attenuated culture a vaccine [Latin vacca, cow] in honor of Edward Jenner because, many years earlier, Jenner had used vaccination with material from cowpox lesions to protect people against smallpox.

Shortly after this, Pasteur and Chamberland developed an attenuated anthrax vaccine in two ways: by treating cultures with potassium bichromate and by incubating the bacteria at 42 to 43°C. Pasteur next prepared rabies vaccine by a different approach. The pathogen was attenuated by growing it in an abnormal host, the rabbit. After infected rabbits had died, their brains and spinal cords were removed and dried.

During the course of these studies, Joseph Meister, a nine-year-old boy who had been bitten by a rabid dog, was brought to Pasteur. Since the boy's death was certain in the absence of treatment, Pasteur agreed to try vaccination. Joseph was injected 13 times over the next 10 days with increasingly virulent preparations of the attenuated virus. He survived. In gratitude for Pasteur's development of vaccines, people from around the world contributed to the construction of the Pasteur Institute in Paris, France. One of the initial tasks of the Institute was vaccine production.

After the discovery that the diphtheria bacillus produced a toxin, Emil von Behring (1854–1917) and Shibasaburo Kitasato (1852–1931) injected inactivated toxin into rabbits, inducing them to produce an antitoxin, a substance in the blood that would inactivate the toxin and protect against the disease. A tetanus antitoxin was then prepared and both antitoxins were used in the treatment of people.

The antitoxin work provided evidence that immunity could result from soluble substances in the blood, now known to be antibodies (humoral immunity). It became clear that blood cells were also important in immunity (cellular immunity) when Elie Metchnikoff (1845–1916) discovered that some blood leukocytes could engulf disease-causing bacteria. He called these cells phagocytes and the process phagocytosis.

The development of Microbiology.

In the late 1800s and for the first decade of the 1900s, scientists seized the opportunity to further develop the germ theory of disease as enunciated by Pasteur and proved by Koch. There emerged a **Golden Age of Microbiology** during which many agents of different infectious diseases were identified. Many of the etiologic agents of microbial disease were discovered during that period, leading to the ability to halt epidemics by interrupting the spread of microorganisms.

Despite the advances in microbiology, it was rarely possible to render life-saving therapy to an infected patient. Then, after World War II, the **antibiotics** were introduced to medicine. The incidence of pneumonia, tuberculosis, meningitis, syphilis, and many other diseases declined with the use of antibiotics.

Work with viruses could not be effectively performed until instruments were developed to help scientists see these disease agents. In the 1940s, the **electron microscope** was developed and perfected. In that decade, cultivation methods for viruses were also introduced, and the knowledge of viruses developed rapidly. With the development of vaccines in the 1950s and 1960s, such viral diseases as polio, measles, mumps, and rubella came under control.

Modern microbiology.

Modern microbiology reaches into many fields of human endeavor, including the development of pharmaceutical products, the use of quality-control methods in food and dairy product production, the control of disease-causing microorganisms in consumable waters, and the industrial applications of microorganisms. Microorganisms are used to produce vitamins, amino acids, enzymes, and growth supplements. They manufacture many foods, including fermented

dairy products (sour cream, yogurt, and buttermilk), as well as other fermented foods such as pickles, sauerkraut, breads, and alcoholic beverages.

One of the major areas of applied microbiology is **biotechnology.** In this discipline, microorganisms are used as living factories to produce pharmaceuticals that otherwise could not be manufactured. These substances include the human hormone insulin, the antiviral substance interferon, numerous blood-clotting factors and clot dissolving enzymes, and a number of vaccines. Bacteria can be reengineered to increase plant resistance to insects and frost, and biotechnology will represent a major application of microorganisms in the next century.

The steps of Koch's postulates used to relate a specific microorganism to a specific disease. (a) Microorganisms are observed in a sick animal and (b) cultivated in the lab. (c) The organisms are injected into a healthy animal, and (d) the animal develops the disease. (e) The organisms are observed in the sick animal and (f) reisolated in the lab.

As the scientist-writer Steven Jay Gould emphasized, we live in the Age of Bacteria. They were the first living organisms on our planet, live virtually everywhere life is possible, are more numerous than any other kind of organism, and probably constitute the largest component of the earth’s biomass.

The whole ecosystem depends on their activities, and they influence human society in countless ways. Thus modern microbiology is a large discipline with many different specialties; it has a great impact on fields such as medicine, agricultural and food sciences, ecology, genetics, biochemistry, and molecular biology.

For example, microbiology has been a major contributor to the rise of molecular biology, the branch of biology dealing with the physical and chemical aspects of living matter and its function. Microbiologists have been deeply involved in studies on the genetic code and the mechanisms of DNA, RNA, and protein synthesis. Microorganisms were used in many of the early studies on the regulation of gene expression and the control of enzyme activity. In the 1970s new discoveries in microbiology led to the development of recombinant DNA technology and genetic engineering. The mechanisms of DNA, RNA, and protein synthesis; Recombinant DNA and genetic engineering.

One indication of the importance of microbiology in the twentieth century is the Nobel Prize given for work in physiology or medicine. About 1/3 of these have been awarded to scientists working on microbiological problems Microbiology has both basic and applied aspects. Many microbiologists are interested primarily in the biology of the microorganisms themselves.

They may focus on a specific group of microorganisms and be called virologists (viruses), bacteriologists (bacteria), phycologists or algologists (algae), mycologists (fungi), or protozoologists (protozoa). Others are interested in microbial morphology or particular functional processes and work in fields such as microbial cytology, microbial physiology, microbial ecology, microbial genetics and molecular biology, and microbial taxonomy.

A person can be thought of in both ways (e.g., as a bacteriologist who works on taxonomic problems). Many microbiologists have a more applied orientation and work on practical problems in fields such as medical microbiology, food and dairy microbiology, and public health microbiology (basic research is also conducted in these fields). Because the various fields of microbiology are interrelated, an applied microbiologist must be familiar with basic microbiology. For example, a medical microbiologist must have a good understanding of microbial taxonomy, genetics, immunology, and physiology to identify and properly respond to the pathogen of concern.

What are some of the current occupations of professional microbiologists? One of the most active and important is medical microbiology, which deals with the diseases of humans and animals. Medical microbiologists identify the agent causing an infectious disease and plan measures to eliminate it. Frequently they are involved in tracking down new, unidentified pathogens such as the agent that causes variant Creutzfeldt-Jacob disease, the Hantavirus, and the virus responsible for AIDS. These microbiologists also study the ways in which microorganisms cause disease. Legionnaires disease; Hantavirus pulmonary syndrome; AIDS.

Public health microbiology is closely related to medical microbiology. Public health microbiologists try to control the spread of communicable diseases. They often monitor community food establishments and water supplies in an attempt to keep them safe and free from infectious disease agents.

Immunology is concerned with how the immune system protects the body from pathogens and the response of infectious agents. It is one of the fastest growing areas in science; for example, techniques for the production and use of monoclonal antibodies have developed extremely rapidly. Immunology also deals with practical health problems such as the nature and treatment of allergies and autoimmune diseases like rheumatoid arthritis.

Monoclonal antibodies and their uses, Many important areas of microbiology do not deal directly with human health and disease but certainly contribute to human welfare. Agricultural microbiology is concerned with the impact of microorganisms on agriculture.

Agricultural microbiologists try to combat plant diseases that attack important food crops, work on methods to increase soil fertility and crop yields, and study the role of microorganisms living in the digestive tracts of ruminants such as cattle. Currently there is great interest in using bacterial and viral insect pathogens as substitutes for chemical pesticides.

The field of microbial ecology is concerned with the relationships between microorganisms and their living and nonliving habitats. Microbial ecologists study the contributions of micro organisms to the carbon, nitrogen, and sulfur cycles in soil and in freshwater. The study of pollution effects on microorganisms also is important because of the impact these organisms have on the environment. Microbial ecologists are employing microorganisms in bioremediation to reduce pollution effects.

Scientists working in food and dairy microbiology try to prevent microbial spoilage of food and the transmission of food borne diseases such as botulism and salmonellosis. They also use microorganisms to make foods such as cheeses, yogurts, pickles, and beer. In the future microorganisms themselves may become a more important nutrient source for livestock and humans.

In industrial microbiology microorganisms are used to make products such as antibiotics, vaccines, steroids, alcohols and other solvents, vitamins, amino acids, and enzymes. Microorganisms can even leach valuable minerals from low-grade ores. Research on the biology of microorganisms occupies the time of many microbiologists and also has practical applications.

Those working in microbial physiology and biochemistry study the synthesis of antibiotics and toxins, microbial energy production, the ways in which microorganisms survive harsh environmental conditions, microbial nitrogen fixation, the effects of chemical and physical agents on microbial growth and survival, and many other topics.

Microbial genetics and molecular biology focus on the nature of genetic information and how it regulates the development and function of cells and organisms. The use of microorganisms has been very helpful in understanding gene function. Microbial geneticists play an important role in applied microbiology by producing new microbial strains that are more efficient in synthesizing useful products.

Genetic techniques are used to test substances for their ability to cause cancer. More recently the field of genetic engineering has arisen from work in microbial genetics and molecular biology and will contribute substantially to microbiology, biology as a whole, and medicine. Engineered microorganisms are used to make hormones, antibiotics, vaccines, and other products.

New genes can be inserted into plants and animals; for example, it may be possible to give corn and wheat nitrogen-fixation genes so they will not require nitrogen fertilizers.

Microbiology Future - A Great Career Awaits for Microbiologists

Microbiology has tremendous scope and a very bright future. Students who are pursuing their career as a Microbiologist, golden opportunities await for you. Some of the best scientific research jobs are in available in the field of Microbiology.Einstein rightly said "Imagination is powerful than knowledge". Today the innovation in science is spreading its arms like the way universe is spreading since big bang. Few 100 years ago man imagined flying in sky and today it is happening. Today he can fly in sky for days, months and years with aero plane, rockets, space labs etc. There are thousands of such examples were science has made miracles and made imagination a reality. Today you imagine something and will see that tomorrow or soon innovation in science will make it reality. What is required in today's scientific world is the vision, the imagination to innovate rest the technology and knowledge will take care.

One such branch of science is microbiology which has made many imaginations a reality. The innovations in this field has given the ability to human being to see tiny invisible organisms of unbelievable size less than 0.2 micron or even less and to study every detail of it. The scope of microbiology is immense due to its ability to control all critical points of many fields like Medical, Diary, Pharmaceutical, Industrial, Clinical, research, water industry, agriculture, nanotechnology, chemical etc.

It is true that career in microbiology is great due to its vast scope but at the same time, this is not sufficient and what is further required to have great career in microbiology is a new dimension to the thinking, new dimension to the education system, and new dimension to the way the knowledge of microbiology is applied.

Today education is considered as a way of earning money in life through jobs. From our school days it is being taught and our mind set is being made to have a good job after education. Our first aim after education is getting a white collar job through campus interview or any other mean. Is it really making sense to just get a good job with education, is it only meaning of today's education? The answer is of course not. There is a need to change the mind sets and to change the education system such that it should teach us to innovate, should teach us to imagine and to establish with our own knowledge and education beyond a mean to earn just for livelihood.

With new dimension of thinking , today microbiologist can easily innovate new diagnostic kits (e.g. Pathogen detecting, antigen detecting, receptor detecting etc), can discover new drugs with antibiotic sensitivity tests, zone of inhibitions etc., Can isolate unique species from mountains , strange areas, extreme conditions, who know you may find antibiotic properties in many of them. Hundreds of such enzyme properties, antibiotic properties within microorganisms are being detected daily and are applied in various medical, fermentation industries and in developing new products for well being of human life.

Microbiologist can apply for patents for their small-big innovations and can even sell them for million dollars, can develop their own small or big clinical laboratory, Can develop their own dairy, pharmaceutical, medical, agricultural institutes and industries simply with knowledge of applied microbiology. Who knows your imagination of isolating organisms from extreme

condition can give new drugs for today's burning issues and diseases and can save thousands of patients life's all over the world.

Today microbiologists are required in top organizations like NASA for identification of any life form for their various missions like the recent Mars curiosity mission and many more. The scope is immense; just what is needed is right application of knowledge. With such a scope in microbiology what today's students, professionals need is just a change of their mindsets , a change in their imagination , a thinking beyond circle, rest as mentioned earlier, knowledge and technology will take care to make them successful. Job is a way to apply knowledge but innovation and imagination is a way to destination and Einstein rightly said that "imagination is powerful than knowledge".

MICROBIAL EVOLUTION AND DIVERSITY

General Introduction and Overview

The bewildering diversity of living organisms, it is desirable to classify or arrange them into groups based on their mutual similarities. Taxonomy [Greek taxis, arrangement or order, and nomos, law, or nemein, to distribute or govern] is defined as the science of biological classification. In a broader sense it consists of three separate but interrelated parts: classification, nomenclature, and identification.

1. **Classification** is the arrangement of organisms into groups or taxa (s., taxon) based on mutual similarity or evolutionary relatedness.
2. **Nomenclature** is the branch of taxonomy concerned with the assignment of names to taxonomic groups in agreement with published rules.
3. **Identification** is the practical side of taxonomy, the process of determining that a particular isolate belongs to a recognized taxon.

People often think of taxonomy as trivial and boring, simply a matter of splitting hairs over names of organisms. Actually, taxonomy is important for several reasons.

- First, it allows us to organize huge amounts of knowledge about organisms because all members of a particular group share many characteristics. In a sense it is something like a

giant filing system or library catalogue that provides easy access to information. The more accurate the classification, the more information-rich and useful it is.

- Second, taxonomy allows us to make predictions and frame hypotheses for further research based on knowledge of similar organisms. If a relative has some property, the microorganism in question also may have the same characteristic.
- Third, taxonomy places microorganisms in meaningful, useful groups with precise names so that microbiologists can work with them and communicate efficiently. Just as effective written communication is not possible without adequate vocabulary; correct spelling, and good grammar, microbiology is not possible without taxonomy.
- Fourth, taxonomy is essential for accurate identification of microorganisms. Its practical importance in this respect can hardly be overemphasized. For example, it is essential to clinical microbiology ; treatment often is exceptionally difficult when the pathogen is unknown.

The term systematics often is used for taxonomy. However, many taxonomists define it in more general terms as "the scientific study of organisms with the ultimate object of characterizing and arranging them in an orderly manner." Any study of the nature of organisms, when the knowledge gained is used in taxonomy, is a part of systematics. Thus it encompasses disciplines such as morphology, ecology, epidemiology, biochemistry, molecular biology, and physiology. Microbial taxonomy is too broad a subject for adequate coverage in a single chapter. Therefore this chapter emphasizes general principles and uses examples primarily from prokaryotic taxonomy. The taxonomy of each major eucaryotic microbial group is reviewed where the group is introduced in subsequent chapters. Currently microbial taxonomy is in ferment because of the use of new molecular techniques in classifying microorganisms. Even though these new advances have generated much excitement and are drastically changing microbial taxonomy, more traditional approaches still have value and also will be outlined.

Microbial Evolution and Diversity

It has been estimated that our planet is about 4.6 billion years old. Fossilized remains of procaryotic cells around 3.5 to 3.8 billion years old have been discovered in stromatolites and sedimentary rocks. Stromatolites are layered or stratified rocks, often domed, that are formed by

incorporation of mineral sediments into microbial mats . Modern stromatolites are formed by cyanobacteria; presumably at least some fossilized stromatolites were formed in the same way. Thus procaryotic life arose very shortly after the earth cooled. Very likely the earliest procaryotes were anaerobic. Cyanobacteria and oxygen-producing photosynthesis probably developed 2.5 to 3.0 billion or more years ago. Microbial diversity increased greatly as oxygen became more plentiful. The studies of Carl Woese and his collaborators on rRNA sequences in procaryotic cells suggest that procaryotes divided into two distinct groups very early on. Figure 1.1 depicts a universal phylogenetic tree that reflects these views. The tree is divided into three major branches representing the three primary groups: Bacteria, Archaea, and Eucarya. The archaea and bacteria first diverged, then the eucaryotes developed. These three primary groups are called domains and placed above the phylum and kingdom levels (the traditional kingdoms are distributed among these three domains). The domains differ markedly from one another. Eucaryotic organisms with primarily glycerol fatty acyl diester membrane lipids and eucaryotic rRNA belong to the Eucarya. The domain Bacteria contains procaryotic cells with bacterial rRNA and membrane lipids that are primarily diacyl glycerol diesters. Procaryotes having isoprenoid glycerol diether or diglycerol tetraether lipids in their membranesand archaeal rRNA compose the third domain, Archaea.

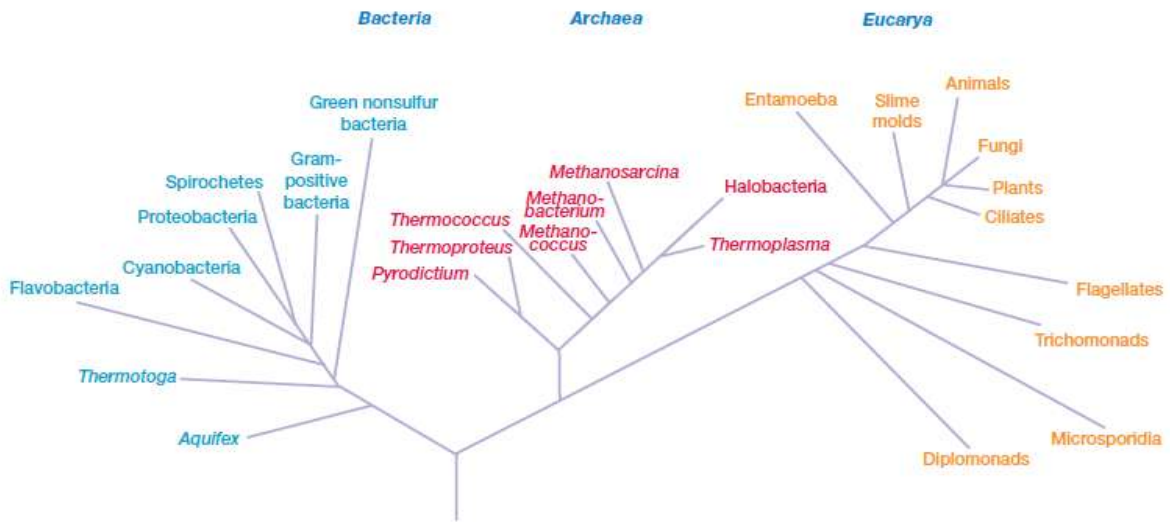


Figure 1.1: Universal Phylogenetic Tree.

It appears likely that modern eucaryotic cells arose from prokaryotes about 1.4 billion years ago. There has been considerable speculation about how eucaryotes might have developed from procaryotic ancestors. It is not certain how this process occurred, and two hypotheses have been proposed. According to the first, nuclei, mitochondria, and chloroplasts arose by invagination of the plasma membrane to form double-membrane structures containing genetic material and capable of further development and specialization. The similarities between chloroplasts, mitochondria, and modern bacteria are due to conservation of primitive procaryotic features by the slowly changing organelles. According to the more popular endosymbiotic hypothesis, the first event was nucleus formation in the pro-eucaryotic cell. The ancestral eucaryotic cell may have developed from a fusion of ancient bacteria and archaea. Possibly a gram-negative bacterial host cell that had lost its cell wall engulfed an archaeon to form an endosymbiotic association. The archaeon subsequently lost its wall and plasma membrane, while the host bacterium developed membrane infolds. Eventually the host genome was transferred to the original archaeon, and a nucleus and the endoplasmic reticulum was formed. Both bacterial and archaeal genes could be lost during formation of the eucaryotic genome. It should be noted that many believe that the Archaea and Eucarya are more closely related than this hypothetical scenario implies. They propose that the eucaryotic line diverged from the Archaea and then the nucleus formed, possibly from the Golgi apparatus. Mitochondria and chloroplasts appear to have developed later. The free-living, fermenting ancestral eucaryote with its nucleus established a permanent symbiotic relationship with photosynthetic bacteria, which then evolved into chloroplasts. Cyanobacteria have been considered the most likely ancestors of chloroplasts. More recently Prochloron has become the favorite candidate. Prochloron lives within marine invertebrates and resembles chloroplasts in containing both chlorophyll a and b, but not phycobilins. The existence of this bacterium suggests that chloroplasts arose from a common ancestor of prochlorophytes and cyanobacteria. Mitochondria arose from an endosymbiotic relationship between the free-living primitive eucaryote and bacteria with aerobic respiration (possibly an ancestor of three modern groups: Agrobacterium, Rhizobium, and Rickettsia). Some have proposed that aerobic respiration actually arose before oxygenic (oxygen-producing)photosynthesis and made use of small amounts of oxygen availableat this early stage of planetary development. The exact sequence of development is still unclear. The

endosymbiotic hypothesis has received support from the discovery of an endosymbiotic cyanobacterium that inhabits the biflagellate protist Cyanophora paradoxa and acts as its chloroplast. This endosymbiont, called a cyanelle, resembles the cyanobacteria in its photosynthetic pigment system and fine structure and it is surrounded by a peptidoglycan layer. It differsfrom cyanobacteria in lacking the lipopolysaccharide outer membrane characteristic of gram-negative bacteria. The cyanelle may be a recently established endosymbiont that is evolving into a chloroplast. Further support is provided by rRNA trees, which locate chloroplast RNA within the cyanobacteria. At present both hypotheses have supporters. It is possible that new data may help resolve the issue to everyone's satisfaction. However, these hypotheses concern processes that occurred in the distant past and cannot be directly observed. Thus a complete consensus on the matter may never be reached.

Taxonomic Ranks

In preparing a classification scheme, one places the microorganism within a small, homogeneous group that is itself a member of larger groups in a nonoverlapping hierarchical arrangement. A category in any rank unites groups in the level below it based on shared properties (figure 1.2). In procaryotic taxonomy the most commonly used levels or ranks (in ascending order) are species, genera, families, orders, classes, and phyla. Microbial groups at each level or rank have names with endings or suffixes characteristic of that level (table 1.1). Microbiologists often use informal names in place of formal hierarchical ones. Typical examples of such names are purple bacteria, spirochetes, methane-oxidizing bacteria, sulfate-reducing bacteria, and lactic acid bacteria. The basic taxonomic group in microbial taxonomy is the species. Taxonomists working with higher organisms define the term species differently than do microbiologists. Species of higher organisms are groups of interbreeding or potentially interbreeding natural populations that are reproductively isolated from other groups. This is a satisfactory definition for organisms capable of sexual reproduction but fails with many microorganisms because they do not reproduce sexually. Procaryotic species are characterized by phenotypic and genotypic differences. A prokaryotic species is a collection of strains that share many stable properties and differ significantly from other groups of strains. This definition is very subjective and can be interpreted in many ways. The following more precise definition has been proposed by some

bacterial taxonomists. A species (genomospecies) is a collection of strains that have a similar G +C composition and 70% or greater similarity as judged by DNA hybridization experiments. Ideally a species also should be phenotypically distinguishable from other similar species. A strain is a population of organisms that is distinguishable from at least some other populations within a particular taxonomic category. It is considered to have descended from a single organism or pure culture isolate. Strains within a species may differ slightly from one another in many ways. Biovars are variant procaryotic strains characterized by biochemical or physiological differences, morphovars differ morphologically, and serovars have distinctive antigenic properties. One strain of a species is designated as the type strain. It is usually one of the first strains studied and often is more fully characterized than other strains; however, it does not have to be the most representative member. The type train for the species is called the type species and is the nomenclatural type or the holder of the species name. A nomenclatural type is a device to ensure fixity of names when taxonomic rearrangements take place. For example, the type species must remain within the genus of which it is the nomenclatural type. Only those strains very similar to the type strain or type species are included in a species. Each species is assigned to a genus, the next rank in the taxonomic hierarchy. A genus is a well-defined group of one or more species that is clearly separate from other genera. In practice there is considerable subjectivity in assigning species to a genus, and taxonomists may disagree about the composition of genera.

Table 1.1: An Example of Taxonomic Ranks and Names

Rank	Example
Domain	*Bacteria*
Phylum	*Proteobacteria*
Class	γ-Proteobacteria
Order	*Enterobacteriales*
Family	*Enterobacteriaceae*
Genus	*Shigella*
Species	*S. dysenteriae*

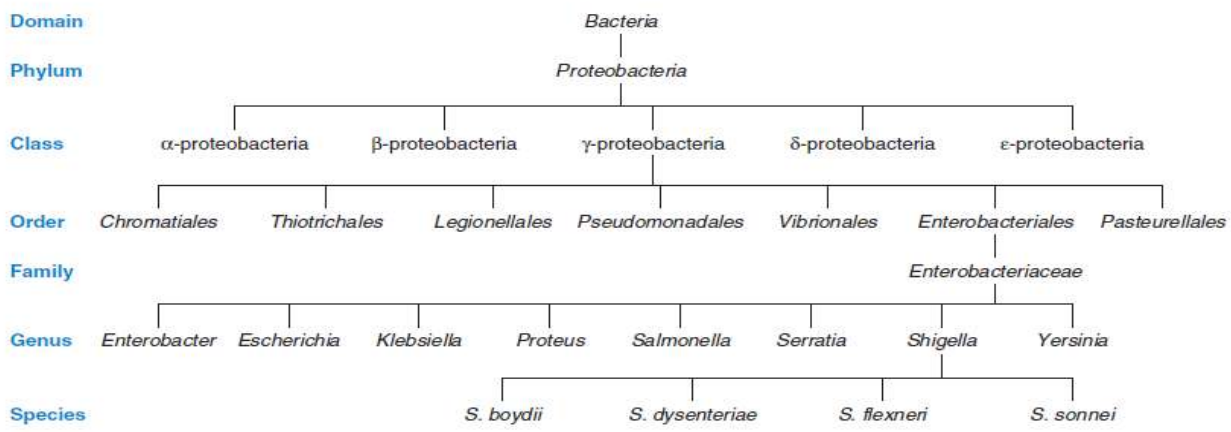


Figure 1.2: Hierarchical Arrangement in Taxonomy. In this example, members of the genus Shigella are placedwithin higher taxonomic ranks. Not all classification possibilies are given for each rank to simplify the diagram.

Classification Systems

Once taxonomically relevant characteristics of microorganisms have been collected, they may be used to construct a classification system. The most desirable classification system, called a natural classification, arranges organisms into groups whose members share many characteristics and reflects as much as possible the biological nature of organisms. Linnaeus developed the first natural classification, based largely on anatomical characteristics, in the middle of the eighteenth century. It was a great improvement over previously employed artificial systems because knowledge of an organism's position in the scheme provided information about many of its properties. For example, classification of humans as mammals denotes that they have hair, self-regulating body temperature, and milk-producing mammary glands in the female. There are two general ways in which classification systems can be constructed. Organisms can be grouped together based on overall similarity to form a phenetic system or they can be grouped based on probable evolutionary relationships to produce a phylogenetic system. Computers may be used to analyze data for the production of phenetic classifications. The process is called numerical taxonomy. This section briefly discusses phenetic and phylogenetic classifications, and describes numerical taxonomy.

Phenetic Classification

Many taxonomists maintain that the most natural classification is the one with the greatest information content or predictive value. A good classification should bring order to biological diversity and may even clarify the function of a morphological structure. For example, if motility and flagella are always associated in particular microorganisms, it is reasonable to suppose that flagella are involved in at least some types of motility. When viewed in this way, the best natural classification system may be a phenetic system, one that groups organisms together based on the mutual similarity of their phenotypic characteristics. Although phenetic studies can reveal possible evolutionary relationships, they are not dependent on phylogenetic analysis. They compare many traits without assuming that any features are more phylogenetically important than others—that is, unweighted traits are employed in estimating general similarity. Obviously the best phenetic classification is one constructed by comparing as many attributes as possible. Organisms sharing many characteristics make up a single group or taxon.

Numerical Taxonomy

The development of computers has made possible the quantitative approach known as numerical taxonomy. Peter H. A. Sneath and Robert Sokal have defined numerical taxonomy as "the grouping by numerical methods of taxonomic units into taxa on the basis of their character states." Information about the properties of organisms is converted into a form suitable for numerical analysis and then compared by means of a computer. The resulting classification is based on general similarity as judged by comparison of many characteristics, each given equal weight. This approach was not feasible before the advent of computers because of the large number of calculations involved. The process begins with a determination of the presence or absence of selected characters in the group of organisms under study. A character usually is defined as an attribute about which a single statement can be made. Many characters, at least 50 and preferably several hundred, should be compared for an accurate and reliable classification. It is best to include many different kinds of data: morphological, biochemical, and physiological. After character analysis, an association coefficient, a function that measures the agreement between characters possessed by two organisms, is calculated for each pair of organisms in the group.

The simple matching coefficient (SSM), the most commonly used coefficient in bacteriology, is the proportion of characters that match regardless of whether the attribute is present or absent (table 1.2). Sometimes the Jaccard coefficient (SJ) is calculated by ignoring any characters that both organisms lack (table 1.2). Both coefficients increase linearly in value from 0.0 (no matches) to 1.0 (100% matches). The simple matching coefficients, or other association coefficients, are then arranged to form a similarity matrix. This is a matrix in which the rows and columns represent organisms, and each value is an association coefficient measuring the similarity of two different organisms so that each organism is compared to every other one in the table (figure 1.3a). Organisms with great similarity are grouped together and separated from dissimilar organisms (figure 1.3b); such groups of organisms are called phenons (sometimes called phenoms).

The results of numerical taxonomic analysis are often summarized with a treelike diagram called a dendrogram (figure1.3c). The diagram usually is placed on its side with the X-axis or abscissa graduated in units of similarity. Each branch point is at the similarity value relating the two branches. The organisms in the two branches share so many characteristics that the two groups are seen to be separate only after examination of association coefficients greater than the magnitude of the branch point value. Below the branch point value, the two groups appear to be one. The ordinate in such a dendrogram has no special significance, and the clusters may be arranged in any convenient order.

Table 1.2: The Calculation of Association Coefficients for Two Organisms

In this example, organisms A and B are compared in terms of the characters they do and do not share. The terms in the association coefficient equations are defined as follows:

		Organism B: 1	Organism B: 0
Organism A	1	a	b
	0	c	d

a = number of characters coded as present (1) for both organisms
b and c = numbers of characters differing (1,0 or 0,1) between the two organisms
d = number of characters absent (0) in both organisms
Total number of characters compared = $a + b + c + d$

The simple matching coefficient $(S_{SM}) = \frac{a + d}{a + b + c + d}$

The Jaccard coefficient $(S_J) = \frac{a}{a + b + c}$

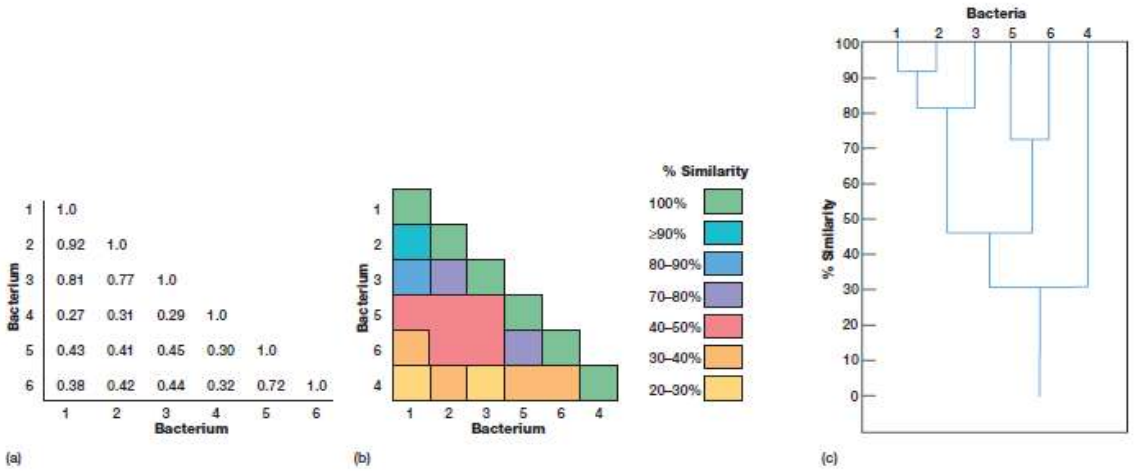


Figure 1.3: Clustering and Dendrograms in Numerical Taxonomy. (**a**) A small similarity matrix that compares six strains of bacteria. The degree of similarity ranges from none (0.0) to complete similarity (1.0). (**b**) The bacteria have been rearranged and joined to form clusters of similar strains. For example, strains 1 and 2 are the most similar. The cluster of 1 plus 2 is fairly similar to strain 3, but not at all to strain 4. (**c**) A dendrogram showing the results of the analysis in part b. Strains 1 and 2 are members of a 90-phenon, and strains 1–3 form an 80-phenon. While strains 1–3 may be members of a single species, it is quite unlikely that strains 4–6 belong to the same species as 1–3.

Major Characteristics Used in Taxonomy

Many characteristics are used in classifying and identifying microorganisms. This section briefly reviews some of the most taxonomically important properties. For sake of clarity, characteristics have been divided into two groups: classical and molecular.

Classical Characteristics

Classical approaches to taxonomy make use of morphological, physiological, biochemical, ecological, and genetic characteristics. These characteristics have been employed in microbial taxonomy for many years. They are quite useful in routine identification and may provide phylogenetic information as well.

Morphological Characteristics

Morphological features are important in microbial taxonomy for many reasons. Morphology is easy to study and analyze, particularly in eucaryotic microorganisms and the more complex procaryotes. In addition, morphological comparisons are valuable because structural features depend on the expression of many genes, are usually genetically stable, and normally (at least in eucaryotes) these do not vary greatly with environmental changes. Thus morphological similarity often is a good indication of phylogenetic relatedness. Many different morphological features are employed in the classification and identification of microorganisms. Although the light microscope has always been a very important tool, its resolution limit of about 0.2 _m (see chapter 2) reduces its usefulness in viewing smaller microorganisms and structures. The transmission and scanning electron microscopes, with their greater resolution, have immensely aided the study of all microbial groups.

Physiological and Metabolic characteristics

Physiological and metabolic characteristics are very useful because they are directly related to the nature and activity of microbial enzymes and transport proteins. Since proteins are gene products, analysis of these characteristics provides an indirect comparison of microbial genomes.

Ecological Characteristics

Many properties are ecological in nature since they affect the relation of microorganisms to their environment. Often these are taxonomically valuable because even very closely related microorganisms can differ considerably with respect to ecological characteristics. Microorganisms living in various parts of the human body markedlydiffer from one another and from those growing in freshwater, terrestrial, and marine environments. Some examples of taxonomically important ecological properties are life cycle patterns; the nature of symbiotic relationships; the ability to cause disease in a particular host; and habitat preferences such as requirements for temperature, pH, oxygen, and osmotic concentration. Many growth requirements are also considered physiological characteristics (table 19.4).

Genetic Analysis

Because most eucaryotes are able to reproduce sexually, genetic analysis has been of considerable usefulness in the classification of these organisms. As mentioned earlier, the species is defined in terms of sexual reproduction where possible. Although prokaryotes do not reproduce sexually, the study of chromosomal gene exchange through transformation and conjugation is sometimes useful in their classification.

Transformation can occur between different prokaryotic species but only rarely between genera. The demonstration of transformation between two strains provides evidence of a close relationship since transformation cannot occur unless the genomes are fairly similar. Transformation studies have been carried out with several genera: Bacillus, Micrococcus, Haemophilus, Rhizobium, and others. Despite transformation's usefulness, its results are sometimes hard to interpret because an absence of transformation may result from factors other than major differences in DNA sequence.

Conjugation studies also yield taxonomically useful data, particularly with the enteric bacteria. For example, Escherichia can undergo conjugation with the genera Salmonella and Shigella but not with Proteus and Enterobacter. These observations fit with other data showing that the first three of these genera are more closely related to one another than to Proteus and Enterobacter. Plasmids are undoubtedly important in taxonomy because they are present in most bacterial genera, and many carry genes coding for phenotypic traits. Because plasmids could have a significant effect on classification if they carried the gene for a trait of major importance in the classification scheme, it is best to base a classification on many characters. When the identification of a group is based on a few characteristics and some of these are coded for by plasmid genes, errors may result. For example, hydrogen sulfide production and lactose fermentation are very important in the taxonomy of the enteric bacteria, yet genes for both traits can be borne on plasmids as well as bacterial chromosomes. One must take care to avoid errors as a result of plasmid-borne traits.

Molecular Characteristics

Some of the most powerful approaches to taxonomy are through the study of proteins and nucleic acids. Because these are either direct gene products or the genes themselves, comparisons of proteins and nucleic acids yield considerable information about true relatedness. These more recent molecular approaches have become increasingly important in procaryotic taxonomy.

Comparison of Proteins

The amino acid sequences of proteins are direct reflections of mRNA sequences and therefore closely related to the structures of the genes coding for their synthesis. For this reason, comparisons of proteins from different microorganisms are very useful taxonomically. There are several ways to compare proteins. The most direct approach is to determine the amino acid sequence of proteins with the same function. The sequences of proteins with dissimilar functions often change at different rates; some sequences change quite rapidly, whereas others are very stable. Nevertheless, if the sequences of proteins with the same function are similar, the organisms possessing them are probably closely related. The sequences of cytochromes and other electron transport proteins, histones, heat-shock proteins, transcription and translation proteins, and a variety of metabolic enzymes have been used in taxonomic studies. Because protein sequencing is slow and expensive, more indirect methods of comparing proteins frequently have been employed. The electrophoretic mobility of proteins (see pp. 327–28) is useful in studying relationships at the species and subspecies levels. Antibodies can discriminate between very similar proteins, and immunologic techniques are used to compare proteins from different microorganisms.

The physical, kinetic, and regulatory properties of enzymes have been employed in taxonomic studies. Because enzyme behavior reflects amino acid sequence, this approach is useful in studying some microbial groups, and group-specific patterns of regulation have been found.

Nucleic Acid Base Composition

Microbial genomes can be directly compared, and taxonomic similarity can be estimated in many ways. The first, and possibly the simplest, technique to be employed is the determination of DNA base composition. DNA contains four purine and pyrimidine bases: adenine (A), guanine (G), cytosine (C), and thymine (T). In double-stranded DNA, A pairs with T, and G pairs with C. Thus the (G + C)/(A_T) ratio or G + C content, the percent of G + C in DNA, reflects the base sequence and varies with sequence changes as follows:

$$\text{Mol\% G + C} = \frac{\text{G+C}}{\text{G + C + A + T}} * 100$$

The base composition of DNA can be determined in several ways. Although the G + C content can be ascertained after hydrolysis of DNA and analysis of its bases with high-performance liquid chromatography (HPLC), physical methods are easier and more often used. The G +C content often is determined from the melting temperature (Tm) of DNA. In double-stranded DNA three hydrogen bonds join GC base pairs, and two bonds connect AT base pairs. As a result DNA with a greater G + C content will have more hydrogen bonds, and its strands will separate only at higher temperatures—that is, it will have a higher melting point. DNA melting can be easily followed spectrophotometrically because the absorbance of 260 nm UV light by DNA increases during strand separation. When a DNA sample is slowly heated, the absorbance increases as hydrogen bonds are broken and reaches a plateau when all the DNA has become single stranded.

The midpoint of the rising curve gives the melting temperature, a direct measure of the G + C content. Since the density of DNA also increases linearly with G +C content, the percent G + C can be obtained by centrifuging DNA in a CsCl density gradient. The G +C content of DNA from animals and higher plants averages around 40% and ranges between 30 and 50%. In contrast, the DNA of both eucaryotic and procaryotic microorganisms varies greatly in G +C content; procaryotic G+C content is the most variable, ranging from around 25 to almost 80%. Despite such a wide range of variation, the G + C content of strains within a particular species is constant.

If two organisms differ in their G + C content by more than about 10%, their genomes have quite different base sequences. On the other hand, it is not safe to assume that organisms with very similar G + C contents also have similar DNA base sequences because two very different base sequences can be constructed from the same proportions of AT and GC base pairs. Only if two microorganisms also are alike phenotypically does their similar G + C content suggest close relatedness. G+C content data are taxonomically valuable in at least two ways.

First, they can confirm a taxonomic scheme developed using other data. If organisms in the same taxon are too dissimilar in G + C content, the taxon probably should be divided.

Second, G+C content appears to be useful in characterizing prokaryotic genera since the variation within a genus is usually less than 10% even though the content may vary greatly between genera. For example, Staphylococcus has a G+C content of 30 to 38%, whereas Micrococcus DNA has 64 to 75% G + C; yet these two genera of gram-positive cocci have many other features in common.

Nucleic Acid Hybridization

The similarity between genomes can be compared more directly by use of **nucleic acid hybridization** studies. If a mixture of single stranded DNA formed by heating dsDNA is cooled and held at a temperature about 25°C below the Tm, strands with complementary base sequences will reassociate to form stable dsDNA, whereas non complementary strands will remain single. Because strands with similar, but not identical, sequences associate to form less temperature stable dsDNA hybrids, incubation of the mixture at 30 to 50°C below the Tm will allow hybrids of more diverse ssDNAs to form. Incubation at 10 to 15°C below the Tm permits hybrid formation only with almost identical strands. In one of the more widely used hybridization techniques, nitrocellulose filters with bound nonradioactive DNA strands are incubated at the appropriate temperature with single-stranded DNA fragments made radioactive with 32P, 3H, or 14C. After radioactive fragments are allowed to hybridize with the membrane-bound ss-DNA, the membrane is washed to remove any nonhybridized ssDNA and its radioactivity is measured.

The quantity of radioactivity bound to the filter reflects the amount of hybridization and thus the similarity of the DNA sequences. The degree of similarity or homology is expressed as the

percent of experimental DNA radioactivity retained on the filter compared with the percent of homologous DNA radioactivity bound under the same conditions. Two strains whose DNAs show at least 70% relatedness under optimal hybridization conditions and less than a 5% difference in Tm often are considered members of the same species. If DNA molecules are very different in sequence, they will not form a stable, detectable hybrid. Therefore DNA-DNA hybridization is used to study only closely related microorganisms.

More distantly related organisms are compared by carrying out DNARNA hybridization experiments using radioactive ribosomal or transfer RNA. Distant relationships can be detected because rRNA and tRNA genes represent only a small portion of the total DNA genome and have not evolved as rapidly as most other microbial genes. The technique is similar to that employed for DNA-DNA hybridization: membrane-bound DNA is incubated with radioactive rRNA, washed, and counted. An even more accurate measurement of homology is obtained by finding the temperature required to dissociate and remove half the radioactive rRNA from the membrane; the higher this temperature, the stronger the rRNA-DNA complex and the more similar the sequences.

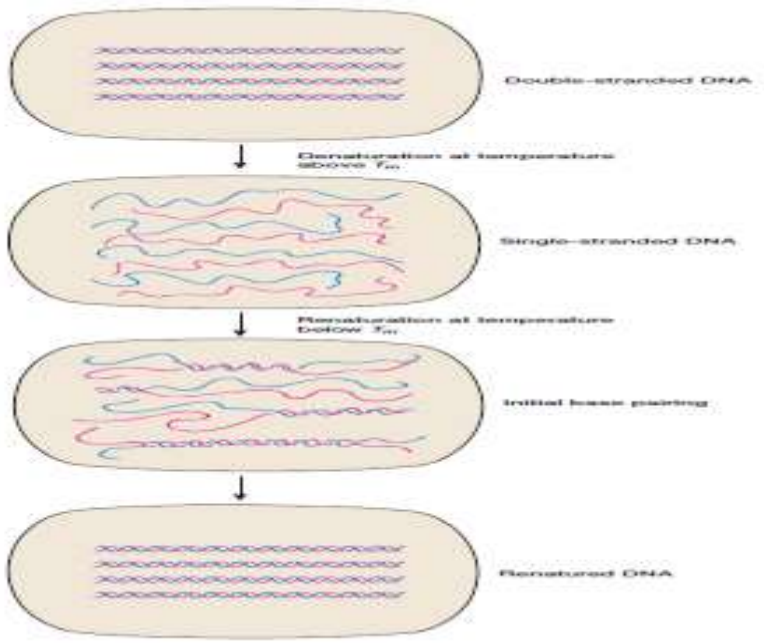


Figure 1.4: Nucleic Acid Melting and Hybridization. Complementary strands are shown in different colors.

Nucleic Acid Sequencing

Despite the usefulness of G + C content determination and nucleic acid hybridization studies, genome structures can be directly compared only by sequencing DNA and RNA. Techniques for rapidly sequencing both DNA and RNA are now available; thus far RNA sequencing has been used more extensively in microbial taxonomy. Most attention has been given to sequences of the 5S and 16S rRNAs isolated from the 50S and 30S subunits, respectively, of procaryotic ribosomes. The rRNAs are almost ideal for studies of microbial evolution and relatedness since they are essential to a critical organelle found in all microorganisms. Their functional role is the same in all ribosomes. Furthermore, their structure changes very slowly with time, presumably because of their constant and critical role. Because rRNA contains variable and stable sequences, both closely related and very distantly related microorganisms can be compared. This is an important advantage as distantly related organisms can be studied only using sequences that change little with time. There are several ways to sequence rRNA. Ribosomal RNAs can be characterized in terms of partial sequences by the oligonucleotide cataloging method as follows. Purified, radioactive 16S rRNA is treated with the enzyme T1 ribonuclease, which cleaves it into fragments. The fragments are separated, and all fragments composed of at least six nucleotides are sequenced. The sequences of corresponding 16S rRNA fragments from different procaryotes are then aligned and compared using a computer, and association coefficients (Sab values) are calculated. Complete rRNAs now are sequenced using procedures like the following. First, RNA is isolated and purified. Then, reverse transcriptase is used to make complementary DNA (cDNA) using primers that are complementary to conserved rRNA sequences. Next, the polymerase chain reaction amplifies the cDNA. Finally, the cDNA is sequenced and the rRNA sequence deduced from the results.

Assessing Microbial Phylogeny

Procaryotic taxonomy is changing rapidly. This is caused by ever increasing knowledge of the biology of procaryotes and remarkable advances in computers and the use of molecular characteristics to determine phylogenetic relationships between prokaryotic groups. This section briefly describes some of the ways in which phylogenetic relationships are determined.

Molecular Chronometers

The sequences of nucleic acids and proteins change with time and are considered to be **molecular chronometers.** This concept, first suggested by Zuckerkandl and Pauling (1965), is important in the use of molecular sequences in determining phylogenetic relationships and is based on the assumption that there is an evolutionary clock. It is thought that the sequences of many rRNAs and proteins gradually change over time without destroying or severely altering their functions. One assumes that such changes are selectively neutral, occur fairly randomly, and increase linearly with time. When the sequences of similar molecules are quite different in two groups of organisms, the groups diverged from one another a long time ago. Phylogenetic analysis using molecular chronometers is somewhat complex because the rate of sequence change can vary; some periods are characterized by especially rapid change. Furthermore, different molecules and various parts of the same molecule can change at different rates. Highly conserved molecules such as rRNAs are used to follow large-scale evolutionary changes, whereas rapidly changing molecules are employed in following speciation. Not everyone believes that molecular chronometers, and particularly protein clocks, are very accurate. Further studies will be required to establish their accuracy and usefulness.

Phylogenetic Trees

Phylogenetic relationships are illustrated in the form of branched diagrams or trees. A **phylogenetic tree** is a graph made of branches that connect nodes (**figure 1.5**). The nodes represent taxonomic units such as species or genes; the external nodes, those at the end of the branches, represent living organisms. The tree may have a time scale, or the length of the branches may represent the number of molecular changes that have taken place between the two nodes. Finally, a tree may be nrooted or rooted. An unrooted tree (figure 1.5a) simply represents phylogenetic relationships but does not provide an evolutionary path. Figure 1.5a shows that A is more closely related to C than it is to either B or D, but does not specify the common ancestor for the four species or the direction of change. In contrast, the rooted tree (figure 1.5b) does give a node that serves as the common ancestor and shows the development of the four species from this root. It is much more difficult to develop a rooted tree. For example, there are 15 possible rooted trees that connect four species, but only three possible unrooted trees. Phylogenetic trees

are developed by comparing molecular sequences. To compare two molecules their sequences must first be aligned so that similar parts match up. The object is to align and compare homologous sequences, ones that are similar because they had a common origin in the past. This is not an easy task, and computers plus fairly complex mathematics must be employed to minimize the number of gaps and mismatches in the sequences being compared.

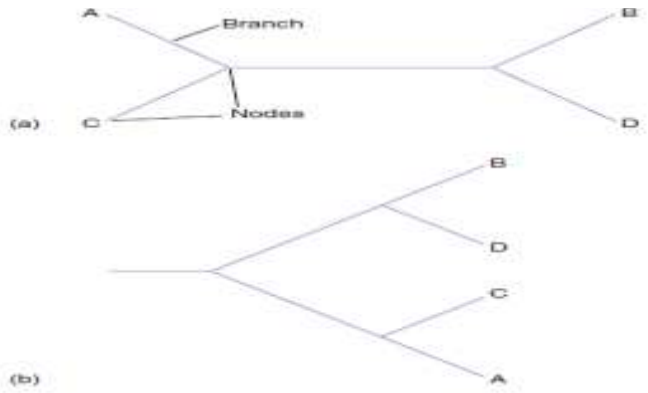


Figure 1.5 Examples of Phylogenetic Trees. (**a**) Unrooted tree joining four taxonomic units. (**b**) Rooted tree.

Once the molecules have been aligned, the number of positions that vary in the sequences can be determined. These data are used to calculate a measure of the difference between the sequences. Often the difference is expressed as the **evolutionary distance.** This is simply a quantitative indication of the number of positions that differ between two aligned macromolecules. Statistical adjustments can be made for back mutations and multiple substitutions that may have occurred. Organisms are then clustered together based on similarity in the sequences. The most similar organisms are clustered together, then compared with the remaining organisms to form a larger cluster associated together at a lower level of similarity or evolutionary distance. The process continues until all organisms are included in the tree. Phylogenetic relationships also can be estimated by techniques such as parsimony analysis. In this approach, relationships are determined by estimating the minimum number of sequence

changes required to give the final sequences being compared. It is presumed that evolutionary change occurs along the shortest pathway with the fewest changes or steps from an ancestor to

the organism in question. The tree or pattern of relationships is favored that is simplest and requires the fewest assumptions.

rRNA, DNA, and Proteins as Indicators of Phylogeny

Although a variety of molecular techniques are used in estimating the phylogenetic relatedness of procaryotes, the comparison of 16S rRNAs isolated from thousands of strains is of particular importance. Recall that either complete rRNA or rRNA fragments can be sequenced and compared (p. 432). The association coefficients or Sab values from rRNA studies are assumed to be a true measure of relatedness; the higher the Sab values obtained from comparing two organisms, the more closely the organisms are related to each other. If the sequences of the 16S rRNAs of two organisms are identical, the Sab value is 1.0. Sab values also are a measure of evolutionary time. A group of procaryotes that branched off from other prokaryotes long ago will exhibit a large range of Sab values because it has had more time to diversify than a group that developed more recently. That is, the narrower the range of Sab values in a group of procaryotes, the more modern it is. After Sab values have been determined, a computer calculates the relatedness of the organisms and summarizes their relationships in a tree or dendrogram. Ribosomal RNA sequence studies have uncovered a feature of great practical importance. The 16S rRNA of most major phylogenetic groups has one or more characteristic nucleotide sequences called oligonucleotide signatures. **Oligonucleotide signature sequences** are specific oligonucleotide sequences that occur in most or all members of a particular phylogenetic group. They are rarely or never present in other groups, even closely related ones. Thus signature sequences can be used to place microorganisms in the proper group. Signature sequences have been identified for bacteria, archaea, eucaryotes, and many major prokaryotic groups. Although rRNA comparisons are useful above the species level, DNA similarity studies sometimes are more effective in categorizing individual species and genera. These comparisons can be carried out using G+C content or hybridization studies, as already discussed. Techniques such as direct sequence analysis and analysis of DNA restriction fragment patterns also can be used. There are advantages to DNA comparisons. As with rRNA, the DNA composition of a cell does not change with growth conditions. DNA comparisons also are based on the complete genomes rather than a fraction, and make it easier to precisely define a species based on the 70%

relatedness criterion. Full sequences of genomes now are being published and will make it easier to study the impact on phylogenetic schemes of such processes as lateral gene transfer as will be discussed later. Many protein sequences are currently used to develop phylogenetic trees. This approach does have some advantages over rRNA comparisons. A sequence of 20 amino acids has more information per site than a sequence of four nucleotides. Protein sequences are less affected by organism-specific differences in G+ C content than are DNA and RNA sequences. Finally, protein sequence alignment is easier because it is not dependent on secondary structure as is an rRNA sequence. Proteins evolve at different rates, as might be expected. Indispensable proteins with constant functions do not change as rapidly (e.g., histones and heat-shock proteins), whereas proteins such as immunoglobulins evolve quite rapidly. Thus not all proteins are suitable for studying large-scale changes that occur over long periods. As mentioned earlier, there is a question about the adequacy of protein based clocks. It is clear that sequences of all three macromolecules can provide valuable phylogenetic information. However, different sequences sometimes produce different trees, and it may be difficult to decide which result is most accurate. Presumably more molecular data plus further study of phenotypic properties will help resolve uncertainties.

Polyphasic Taxonomy

Because phylogenetic results vary with the data used in analysis, many taxonomists believe that all possible valid data should be employed in determining phylogeny. In the approach called **polyphasic taxonomy,** taxonomic schemes are developed using a wide range of phenotypic and genotypic information ranging from molecular properties to ecological characteristics. The techniques that are appropriate for grouping organisms depend on the level of taxonomic resolution needed. For example, serological techniques can be used to identify strains, but not genera or species. Protein electrophoretic patterns are useful in determining species, but not genera or families. DNA hybridization and the analysis of % G+C can be used in studying species and genera. Characteristics such as chemical composition, DNA probe results, rRNA sequences, and DNA sequences can be used to define species, genera, and families. Where possible, as many properties as possible are used to get more stable and reliable results. Successful polyphasic approaches often will help one select techniques for rapid identification of

the microorganism. Because rRNA sequences have been used so extensively, we will focus mainly on the phylogenetic trees derived from rRNA studies.

The Major Divisions of Life

Since the beginning of biology, organisms have been classified as either plants or animals. However, discoveries in microbiology over the past century have shown that the two-kingdom system is oversimplified. Although not all biologists would agree, most microbiologists now believe that living forms can be divided into three distinctly different groups. We will first review this system in more detail, then turn to alternate views.

Domains

As mentioned earlier and illustrated in figure 1.1, Carl Woese and his collaborators have used rRNA studies to group all living organisms into three domains: Archaea, Bacteria, and Eucarya. Thus there are two quite different groups of procaryotes, the bacteria and the archaea. The bacteria comprise the vast majority of procaryotes. Among other properties, bacteria either have cell wall peptidoglycan containing muramic acid or are related to bacteria with such cell walls, and have membrane lipids with ester-linked, straight-chained fatty acids that resemble eukaryotic membrane lipids. The second group, the archaea differ from bacteria in many respects and resemble eucaryotes in some ways (table 19.8). Although the Archaea are described in more detail at a later point, it should be noted that they differ from bacteria in lacking muramic acid in their cell walls and in possessing:

(1) membrane lipids with ether-linked branched aliphatic chains

(2) transfer RNAs without thymidine in the T or T+C arm

(3) distinctive RNA polymerase enzymes

(4) ribosomes of different composition and shape.

Thus although archaea resemble bacteria in their prokaryotic cell structure, they vary considerably on the molecular level. Both groups differ from eucaryotes in their cell ultrastructure and many other properties. However, inspection of table 19.8 shows that both

bacteria and archaea do share some biochemical properties with eucaryotic cells. For example, bacteria and eucaryotes have ester-linked membrane lipids; archaea and eucaryotes are similar with respect to some components of the RNA and protein synthetic systems. Although the preceding view is the most widely accepted, other phylogenetic trees have been proposed. Six or more different trees relating the major domains have been proposed. **Figure 1.6** provides a simplified view of some of these. The first(figure 1.6a) indicates that the three groups are about equidistant from one another and fits with the early rRNA data. Figure 1.6b represents the currently most popular tree in which archaea and eucaryotes have a common ancestor; organisms like the bacteria may have existed before the other domains. The third tree, called the eocyte tree (figure 19.10c), is based on the proposal that sulfur-dependent, extremely thermophilic procaryotes called eocytes (dawn _ cell) are a separate group and more closely related to eucaryotes than are the archaea. Finally, some have proposed that eucaryotic cells are chimeric and arose from the fusion of a bacterium and archaeon (possibly a bacterium lacking a cell wall engulfed an eocytelike archaeon) (figure 1.6d). Clearly the situation is confused and more than one model has been proposed, though most microbiologists favor the three domain tree in figure 19.10b. When some protein sequences are

used to construct phylogenetic trees, one does not even get a three domain pattern. Many factors may account for these problems. There could be unrecognized gene duplications that occurred before the domains formed, leading to confusing patterns. Unequal rates of evolution could distort the trees. Phylogenetically important information may have been lost in some molecular sequences. There may be significant sequence variation between the same molecules from different strains of the same species. Unless several strains are analyzed, false conclusions may be drawn. Thus inaccurate universal trees may result when only the sequences from a few molecules are employed (as is usually the case). One of the most important difficulties in constructing a satisfactory tree is widespread, frequent horizontal or lateral gene transfer. Recent genome sequence studies have shown that there is extensive horizontal gene transfer within and between domains. Eucaryotes possess genes from both bacteria and archaea, and there has been frequent gene swapping between the two procaryotic domains. It appears that at least some bacteria even have acquired eucaryotic genes. Thus the pattern of microbial evolution is not as linear and treelike as previously thought. **Figure 1.7** depicts a morerealistic reticulated tree in

which horizontal gene transfer playsa major role. This tree resembles a web or network with many lateral branches linking various trunks, each branch representing the transfer of one or a few genes. Instead of having a single main trunk or common ancestor at its base, this tree has several trunks or groups of primitive cells that contribute to the original gene pool. Although there is extensive gene transfer between the two procaryotic domains throughout their development, the eucaryotic domain seldom participates in horizontal gene transfer after the formation of fungi, plants, and animals. It is possible that eucaryotic cells originated in a complex process involving many gene transfers from both bacteria and archaea. This hypothesis still allows for the formation of mitochondria and chloroplasts by endosymbiosis with _-proteobacteria and cyanobacteria, respectively. Presumably the three domains remain separate because there are many more gene transfers within each than between domains. This brief discussion of the problems in developing a true universal phylogenetic tree is intended to show the difficulty in determining phylogenetic relationships. The best results will be obtained when all possible data, both molecular and phenotypic, are used in the analysis (for example, in polyphasic taxonomy). We will usually employ trees derived from 16S rRNA sequences because these data are most extensive and are used by most microbiologists. Keep in mind that such trees may well change as further data are collected and analyzed.

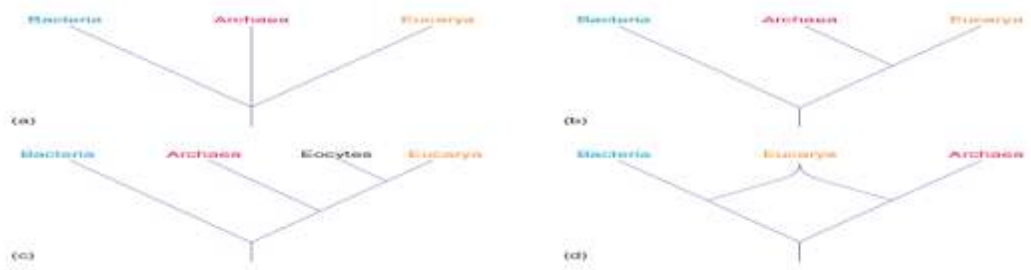


Figure 1.6: Variations in the Design of the "Tree of Life." These four alternative phylogenetic trees are discussed in the text.

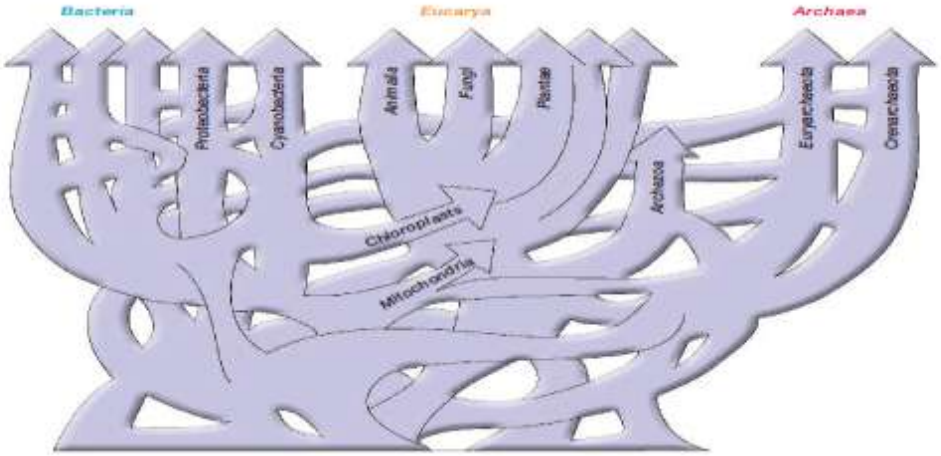


Figure 1.7: Universal Phylogenetic Tree with Frequent Horizontal or Lateral Gene Transfers.

Kingdoms

While most bacteriologists favor the three-domain system, many protozoologists, botanists, and zoologists still think in terms of five or more kingdoms. This section briefly summarizes the nature of some of these classification systems. The first classification system to have gained popularity in the last few decades is the five-kingdom system first suggested by Robert H. Whittaker in the 1960s. An overview of Whittaker's five-kingdom system is presented in **figure 1.8a.** Organisms are placed into five kingdoms based on at least three major criteria:

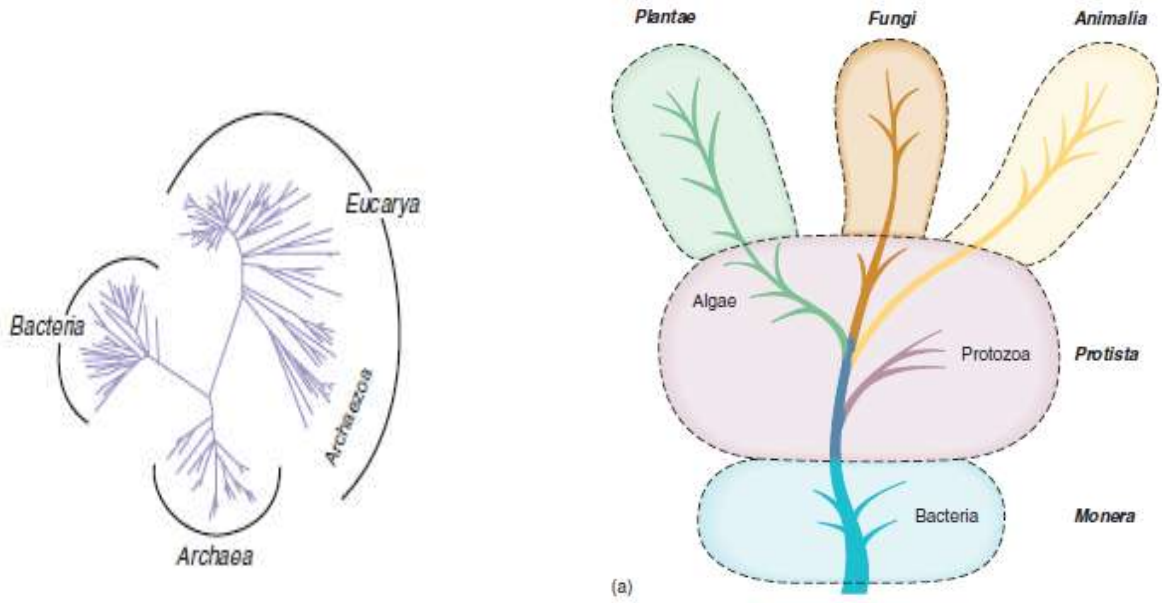
(1) cell type—procaryotic or eucaryotic,

(2) level of organization—solitary and colonial unicellular organization ormulticellular, and

(3) nutritional type.

In this system the kingdom Animalia contains multicellular animals with wall-less eukaryotic cells and primarily ingestive nutrition, whereas the kingdom Plantae is composed of multicellular plants with walled eucaryotic cells and primarily photoautotrophic nutrition. Microbiologists study members of the other three kingdoms. The kingdom Monera or Procaryotae contains all procaryotic organisms. The kingdom Protista is the least homogeneous and hardest to define. **Protists** are eucaryotes with unicellular organization, either in the form of solitary cells or

colonies of cells lacking true tissues. They may have ingestive, absorptive, or photoautotrophic nutrition, and they include most of the microorganisms known as algae, protozoa, and many of the simpler fungi. The kingdom Fungi contains eucaryotic and predominately multinucleate organisms, with nuclei dispersed in a walled and often septate mycelium; their nutrition is absorptive. The five-kingdom system is not accepted by many biologists. A major problem is its lack of distinction between archaea and bacteria. The kingdom Protista also may be too diverse to be taxonomically useful. In addition, the boundaries between the kingdoms Protista, Plantae, and Fungi are ill-defined. For example, the brown algae are probably not closely related to the plants even though the five-kingdom system places them in the Plantae. Because of such problems with the five-kingdom system, various alternatives have been suggested. The six-kingdom system is the simplest option; it divides the kingdom Monera or Procaryotae into two kingdoms, the Eubacteria and Archaeobacteria (figure1.8b). Many attempts have been made to divide the protists into several better-defined kingdoms. The eight-kingdom system of Cavalier-Smith is a good example (figure 1.8c). Cavalier-Smith believes that differences in cellular structure and genetic organization are exceptionally important in determining phylogeny; thus he has used ultrastructural characteristics as well as rRNA sequences and other molecular data in developing his classification. He divides all organisms into two empires and eight kingdoms.



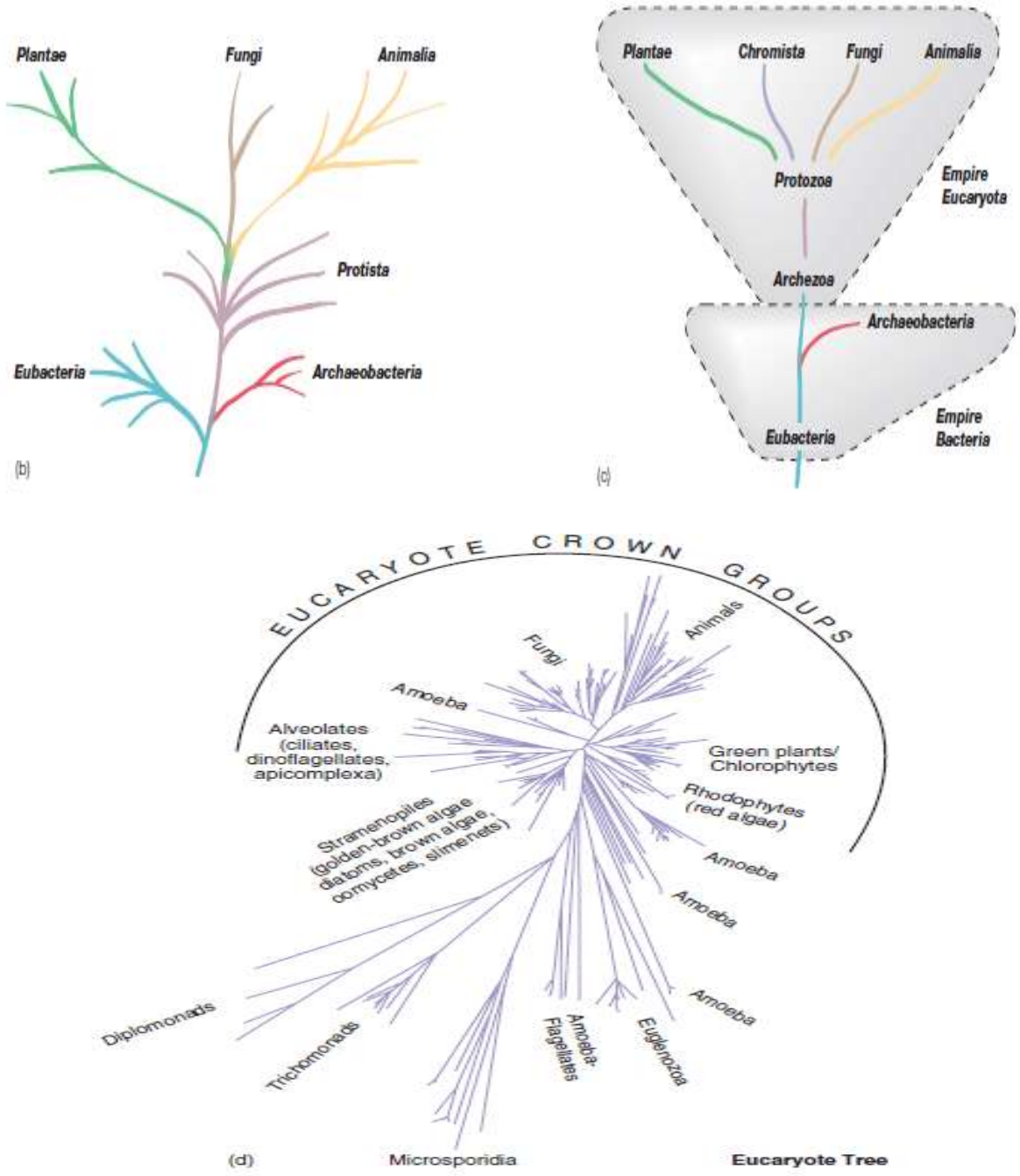


Figure 1.8 Systems of Eucaryotic and Procaryotic Phylogeny. Simplified schematic diagrams of the (**a**) five-kingdom system (Whittaker), (**b**) six-kingdom system, (**c**) eight-kingdom system (Cavalier-Smith), and (**d**) the universal and eucaryotic trees according to Sogin.

The empire Bacteria contains two kingdoms, the Eubacteria and the Archaeobacteria. The second empire, the Eucaryota, contains six kingdoms of eucaryotic organisms. There are two new kingdoms of eucaryotes. The Archezoa are primitive eucaryotic unicellular organisms such as Giardia that have 70S ribosomes and lack Golgi apparatuses, mitochondria, chloroplasts, and peroxisomes. The kingdom Chromista contains mainly photosynthetic organisms that have their chloroplasts within the lumen of the rough endoplasmic reticulum rather than in the cytoplasmic matrix (as is the case in the kingdom Plantae). Diatoms, brown algae, cryptomonads, and oomycetes are all placed in the Chromista. The boundaries of the remaining four kingdoms—Plantae, Fungi, Animalia, and Protozoa—have been adjusted to better define each kingdom and distinguish it from the others. Sogin and his coworkers do not cluster the eucaryotes into a few major divisions, but rather consider them to be a single domain or empire composed of a collection of independently evolved lineages (figure 1.8d). In this scheme the protists do not comprise a separate kingdom, but simply represent a level of organization with many separate lineages and tremendous diversity.

KARPAGAM ACADEMY OF HIGHER EDUCATION
FUNDAMENTALS OF MICROBIOLOGY AND CLASSIFICATION
(17MBP101)

Unit I Question	**Opt 1**
Who first described microorganisms such as bacteria?	Louis Pasteur
Who first developed the process of colony purification on solid media?	Louis Pasteur
What was the first successful solid medium for colony purification of bacter	Agar
Who first suggested using the thickening agent most commonly used for col	Louis Pasteur
The petridish was invented by	Richard Petri
Credit for the first vaccine for the prevention of human disease is generally	Edward Jenner
____ in 1836, passed air through strong sulphuric acid solution and then to l	John Needham
_____ disease is otherwise known as hydrophobia.	Chicken pox
What was the first bacterium shown to cause human disease?	*Anthrax*
The role of antibodies in fighting disease was first demonstrated by	vaccination
The role of blood cells in fighting disease was first demonstrated by	Pasteur
The first observation that bacteria-like organisms could be found in normal	Louis Pasteur
The first physician to make practical application of the germ theory of disea	Louis Pasteur
_____ in 1862, demonstrated the disease of tobacco plant known as mos	Louis Pasteur
Which of the following discoveries is not attributed to Sergei Winogradsky	Colony isolation
Anthrax is caused by a spore forming bacterium was first shown by	Robert Koch
Which one of the following can be employed to sterilize heat labile laborato	Autoclaving
Robert Koch name is associated with	Agar as a solidify
Tyndallisation is the process of _____	Continuous heati
The Swan necked flask was introduced by ____	Spallan Zani
Which of the following areas of microbiology was not a major research inte	Fermentation
Stains useful for identifying fungus include	haematoxylin and
Who is considered as the "natural philosopher"	Anton van Leeuw
The father of microscopy is ____	Anton van Leeuw
Anton van Leeuwenhoek devised compound microscope. His work publishe	Nature
Abiogenesis is otherwise known as ___	Spontaneous Ger
_____ was the first to observe and report microorganisms.	Watson, Crick
_____ provide the germ theory of fermentation	Fanny Hesse
Who defined numerical taxonomy	Carl linnaeus
How many characters should consider for accurate classification?	10 to 20
Proportion of characters that match regardless whether the attributes is	Sj
Which one is calculated by ignoring any character that both organism	Sj
Organism with great similarity are separated from similar organism and	phylum
Which defenetion is not yet accepted in numerical taxonomy	genus
Five Kingdom concept was devised by_____	Carl linnaeus
Example for morphological features	cell shape
Example for physiological character	cell shape
plasmid are very importance in taxonomy ,that carry gene coding for_____	phenotypic traits
On five kingdom classification, the organisms are based on	Pigmentation

Protists are _____	Prokaryotes with
A major problem in five kingdom concept is _____	Lack of distinctic
The kingdom fungi contains	Prokaryotes with
The kingdom chromista contains _____ organism	Halophilic
Diatoms, brownalgae, cryptomonads & oomycetes are placed in _____ k	Protista
The manual for classifying bacteria was first published in the year	1920
The manual for classifying bacteria was first published by	Antony von Leu
When the first edition of Bergey's manual was updated?	1923
Bergey's manual has been updated in the year 1984 and it was renamed as	Bergey's manual
Which cell lack a true membrane delimited nucleus	Prokaryotes
Which cell have a membrane enclosed Nucleus	Prokaryotes
Algae , fungi, protozoa and higher plants are placed in _____	Eukaryotes
In six kingdom system ,it divides _____ into two kingdom	monera
Eight kingdom system suggested by_____	Whittaker
Who first described universal tree concept	Whittaker
Who framed Eukaryotic tree	Whittaker
Who described Three domain concept	Whittaker
According to whom the protist simply represent level of organization but n	Whittaker
what is the term used for taxonomy	Somatic
Carl woese described	three domain con

Opt 2	Opt 3	Opt 4	Opt 5	Opt 6
Robert Koch	Fannie Hesse	Anton von Leeuwenhoek		
Robert Koch	Fannie Hesse	Anton von Leeuwenhoek		
Potato	Gelatin	Meat		
Robert Koch	Fannie Hesse	Richard Petri		
Hook	Pasteur	Anton von Leeuwenhoek		
Louis Pasteur	Iwanoski	Robert Koch		
Schulze	Edward Jenner	Louis Pasteur		
Anthrax	Rabies	Cholera		
Mycobacterium	*Diphtheria*	*Streptococcus*		
antitoxin	attenuation	phagocytosis		
Koch	Metchnikoff	Chamberland		
Robert Koch	Fannie Hesse	Anton von Leeuwenhoek		
Joseph Lister	Fannie Hesse	Anton von Leeuwenhoek		
Demetri Iwanosky	Fannie Hesse	Anton von Leeuwenhoek		
Colony enrichment on s	Bacteria oxidation of iro	CO2 fixation by non-photosynthe		
Debary	Louis Pastuer	Adolf Meyer		
Hot air oven	Disinfection	Ultrafilteration		
Pour plate method	Enrichment culture	Selective media		
Discontinuous heating	Semidry heating	High temperature heating		
Francois Appert	Louis Pasteur	Robert Koch		
vaccine production	nitrogen fixation	sterilisation		
Crystal violet	Grams iodine	Safarinin		
Francois Appert	Louis Pasteur	Robert Koch		
Francois Appert	Louis Pasteur	Robert Koch		
Biology	Journal of microscopy	Micrography		
Biogenesis	Vaccination	Pasteurisation		
Wasserman	Leeuwen hoek	Robert hook		
Jaco Henle	Pasteur	Leeuwen hoek		
Sneath & Sokal	Robert koch	Robert koch & Hooke		
20 to 30	50 to 70	60 to 70		
Ssm	Sn	Scx		
Ssm	Sn	Scx		
phlogenetic	Phytic	phenons		
Kingdom	family	Order		
Carl woose	Whittaker	Charles		
cytoplasm	mitochondria	ribosomes		
cellwall constituents	cell size	motility		
phylogenetic traits	genetic traits	molecular traits		
Environment	Nutrient Type	Temperature		

Eukaryotes with multice	Prokaryotes with multice	Eukaryotes with unicellular		
Lack of distinction betw	Lack of genomic variatic	Lack of cell type variation		
Eukaryotes with multice	Prokaryotes with multice	Eukaryotes with unicellular		
Photosynthetic	Non Photosynthetic	Thermophilic		
Fungi	Monera	Chromista		
1923	1940	1929		
David Friefelder	David Bergey	Benjamin		
1952	1929	974		
Bergey's manual of class	Bergey's manual of pigm	Bergey's manual of genomic bacte		
Eukaryotes	Archae	Plantae		
Eukaryotes	Archae	Plantae		
Prokaryotes	Archae	animalia		
protesta	chromista	Plantae		
Sogin	Cavalier smith	Carl woese		
Sogin	Cavalier smith	Carl woese		
Sogin	Cavalier smith	Carl woese		
Sogin	Carl woese	Cavalier smith		
Sogin	Carl woese	Cavalier smith		
Classification	Variation	Systematic		
five domineconcept	nodomine concept	two domineconcept		

Answer
Anton von Leeuwenhoek
Robert Koch
Agar
Fannie Hesse
Richard Petri
Edward Jenner
Schulze
Rabies
Anthrax
antitoxin
Metchnikoff
Louis Pasteur
Joseph Lister
Demetri Iwanosky
Colony isolation on solid phase medium
Robert Koch
Ultrafilteration
Pour plate method
Discontinuous heating
Louis Pasteur
nitrogen fixation
haematoxylin and eosin
Anton van Leeuwenhoek
Anton van Leeuwenhoek
Micrography
Spontaneous Generation
Leeuwen hoek
Pasteur
Sneath & Sokal
50 to 70
Ssm
Sj
phenons
genus
Whittaker
cell shape
cellwall constituents
phenotypic traits
Nutrient Type

Eukaryotes with unicellular
Lack of distinction between archae & bacteria
Eukaryotes with multicellular
Photosynthetic
Chromista
1923
David Bergey
974
Bergey's manual of systemic bacterialogy
Prokaryotes
Eukaryotes
Eukaryotes
monera
Cavalier smith
Carl woese
Carl woese
Carl woese
Sogin
Systematic
three domain concept

DEPARTMENT OF MICROBIOLOGY
KARPAGAM ACADEMY OF HIGHER EDUCATION
(Established Under Section 3 of UGC Act, 1956)
Eachanari Post, Coimbatore, Tamil Nadu, India – 641 021

I M.Sc MICROBIOLOGY - 2017 – 2019 Batch

FUNDAMENTALS OF MICROBIOLOGY AND CLASSIFICATION (17MBP101)

SEMESTER – I **4H – 4C**

POSSIBLE QUESTION

Unit I

TWO MARK

1. Define Microbiology.
2. Define taxonomy.
3. Define classification.
4. Define nomenclature.
5. Define identification.
6. Define systematic.
7. What is microbial taxonomy?
8. Give the Historical events
    a. Leeuwenhoek b. Joseph lister
9. What are Koch postulates?
10. Explain the term vaccination.
11. Define Mycology
12. Define Phycology

EIGHT MARKS

13. Outline the Whittakers kingdom of classification.
14. Discuss about cow pox vaccination.
15. Contribution of Semmelweiss to micrbiology.
16. Comments on Francesco redis experiment.
17. Write about the Scope of microbiology?
18. Outline the History of Microbiology.
19. Comment on Discovery of Microorganism.

20. Give the recent development and golden age of microbiology.
21. Discuss about the Modern microbiology.
22. Write a detailed note on branches of microbiology?
23. Explain about the antiseptic surgery.
24. Explain in detail about Koch postulates and his findings.
25. Give the theory of .spontaneous generation.
26. Describe the role of microbe in disease.
27. Why taxanomy is important give the reason?
28. Give the outline of microbial evolution and diversity?
29. Explain phenotic classification and phylogenetic classification?
30. Neatly sketch the universal phylogenitic tree?
31. Discuss about the domain, archea?
32. Discuss about the bacteria, eucarya?
33. Describe and detailed the universal phylogenitic tree?
34. Give the hieracheal arrangement taxonomy?
35. Explain about taxonomy ranks?

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LECTURE PLAN

Unit II

S.No	Duration	Topic	Reference
1	2	Microscopy, Simple, Compound	R1: pgs ;18 -21, R3:2.1-3, W4
2	2	Dark-field, Phase contrast	R1: pgs ;21 –25, R3:2.5,W4
3	2	Fluorescent microscopes	R1: pgs; 25 - 27, R3:2.7-9,W4
4	2	Electron microscopes(SEM), (TEM)	R1: pgs; 30-36, R3:2.9-2.13,W4, W5
5	2	Confocal microscopy – Principles and their application	W5
6	1	Stains and Staining techniques	R1:pgs; 27
7	1	Simple, Differential staining methods	R1:pgs 28-29
8	1	Recapitulation and discussion of question	
	Total Hrs: 13		

R3: Sharma,D.K,2013.Microbiology.1st edition, Narosa publishing house, NewDelhi.

W4: www.britannica.com

W5: www.bruker-axs.se/spectral.nsf

UNIT – II

Microscope

Microscope is an instrument that produces enlarged images of small objects, allowing the observer an exceedingly close view of minute structures at a scale convenient for examination and analysis. Although optical microscopes are the subject of this article, an image may also be enlarged by many other wave forms, including acoustic, X-ray, or electron beam, and be received by direct or digital imaging or by a combination of these methods. The microscope may provide a dynamic image (as with conventional optical instruments) or one that is static (as with conventional scanning electron microscopes).The magnifying power of a microscope is an expression of the number of times the object being examined appears to be enlarged and is a dimensionless ratio. It is usually expressed in the form 10× (for an image magnified 10-fold), sometimes wrongly spoken as "ten eks" as though the × were an algebraic symbol rather than the correct form, "ten times." The resolution of a microscope is a measure of the smallest detail of the object that can be observed. Resolution is expressed in linear units, usually micrometers (μm).

The most familiar type of microscope is the optical, or light, microscope, in which glass lenses are used to form the image. Optical microscopes can be simple, consisting of a single lens, or compound, consisting of several optical components in line. The hand magnifying glass can magnify about 3 to 20×. Single-lensed simple microscopes can magnify up to 300× and they are capable of revealing bacteria while compound microscopes can magnify up to 2,000×. A simple microscope can resolve below 1 micrometre (μm; one millionth of a metre); a compound microscope can resolve down to about 0.2 μm.

Images of interest can be captured by photography through a microscope, a technique known as **photomicrography**. From the 19th century this was done with film, but digital imaging is now extensively used instead. Some digital microscopes have dispensed with an eyepiece and provide images directly on the computer screen. This has given rise to a new series of low-cost digital microscopes with a wide range of imaging possibilities, including time-lapse micrography, which has brought previously complex and costly tasks within reach of the young or amateur microscopist.

Other types of microscopes use the wave nature of various physical processes. The most important is the electron microscope, which uses a beam of electrons in its image formation. The Transmission Electron Microscope (TEM) has magnifying powers of more than 1,000,000×. TEMs form images of thin specimens, typically sections, in a near vacuum. A Scanning Electron Microscope (SEM), which creates a reflected image of relief in a contoured specimen, usually has a lower resolution than a TEM but can show solid surfaces in a way that the conventional electron microscope cannot. There are also microscopes that use lasers, sound, or X-rays. The Scanning Tunneling Microscope (STM), which can create images of atoms, and the Environmental Scanning Electron Microscope (ESEM), which generates images using electrons of specimens in a gaseous environment, uses other physical effects that further extend the types of objects that can be examined.

The simple microscope

The simple microscope consists of a single lens traditionally called a loupe. The most familiar present-day example is a reading or magnifying glass. Present-day higher-magnification lenses are often made with two glass elements that produce a colour-corrected image. They can be worn around the neck packaged in a cylindrical form that can be held in place immediately in front of the eye. These are generally referred to as eye loupes or jewelers' lenses. The traditional simple microscope was made with a single magnifying lens, which was often of sufficient optical quality to allow the study of microscopical organisms including *Hydra* and protists.

Magnification

It is instinctive, when one wishes to examine the details of an object, to bring it as near as possible to the eye. The closer the object is to the eye, the larger the angle that it subtends at the eye, and thus the larger the object appears. If an object is brought too close, however, the eye can no longer form a clear image. The use of the magnifying lens between the observer and the object enables the formation of a "virtual image" that can be viewed in comfort.

To obtain the best possible image, the magnifier should be placed directly in front of the eye. The object of interest is then brought toward the eye until a clear image of the object is seen.

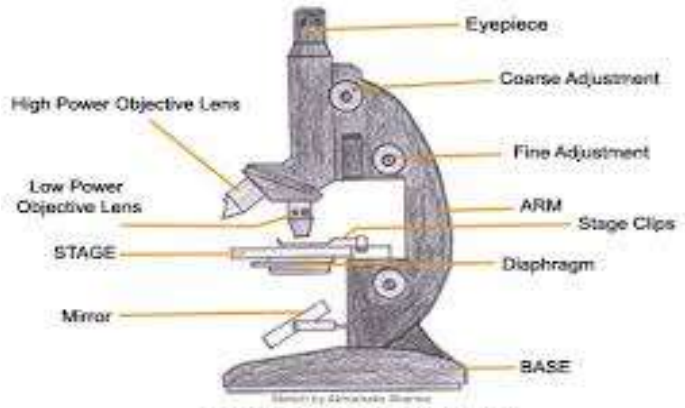
Without lenses, the highest possible magnification is when the object is brought to the closest position at which a clear virtual image is observed. For many people, this image distance is about 25 cm (10 inches). As people age, the nearest point of distinct vision recedes to greater distances, thus making a magnifier a useful adjunct to vision for older people.The magnifying power, or extent to which the object being viewed appears enlarged, and the field of view, or size of the object that can be viewed, are related by the geometry of the optical system. A working value for the magnifying power of a lens can be found by dividing the least distance of distinct vision by the lens' focal length, which is the distance from the lens to the plane at which the incoming light is focused. Thus, for example, a lens with a least distance of distinct vision of 25 cm and a focal length of 5 cm (2 inches) will have a magnifying power of about 5×.

If the diameter of the magnifying lens is sufficient to fill or exceed the diameter of the pupil of the eye, the virtual image that is viewed will appear to be of substantially the same brightness as the original object. The field of view of the magnifier will be determined by the extent to which the magnifying lens exceeds this working diameter and also by the distance separating the lens from the eye. The clarity of the magnified virtual image will depend upon the aberrations present in the lens, its contour, and the manner in which it is used.The limitations on resolution (and therefore magnifying power) imposed by the constraints of a simple microscope can be overcome by the use of a compound microscope, in which the image is relayed by two lens arrays. One of them, the objective, has a short focal length and is placed close to the object being examined. It is used to form a real image in the front focal plane of the second lens, the eyepiece or ocular. The eyepiece forms an enlarged virtual image that can be viewed by the observer. The magnifying power of the compound microscope is the product of the magnification of the objective lens and that of the eyepiece

Compound microscope

In addition to these two lens arrays, a compound microscope consists of a body tube, in which the lenses can be housed and kept an appropriate distance apart; a condenser lens that lies beneath the specimen stage and focuses light upon the specimen; and an illumination system, which either transmits light through or reflects light from the object being examined. A method

for focusing the microscope, usually with coarse and fine focusing controls, must also be provided.



Labeled Microscope Diagram

The basic form of a compound microscope is monocular: a single tube is used, with the objective at one end and a single eyepiece at the other. In order to permit viewing with two eyes and thereby increase comfort and acuity, a single objective can be employed in a binocular tube fitted with a matched pair of eyepieces; beam-splitting prisms are used to send half of the light from the image formed by the objective to each eye. These prisms are mounted in a rotating mechanical assembly so that the separation between the eyepieces can be made to match the required interpupillary distance for the observer. A true stereoscopic microscope is configured by using two objectives and two eyepieces, enabling each eye to view the object separately, making it appear three-dimensional.

Microscopy

Purposes

1. To become familiar with the history and diversity of microscope instruments.
2. To understand the components, use, and care of the compound brightfield microscope.
3. To learn the correct use of the microscope for observation and measurement of microorganisms.

Brightfield Microscope

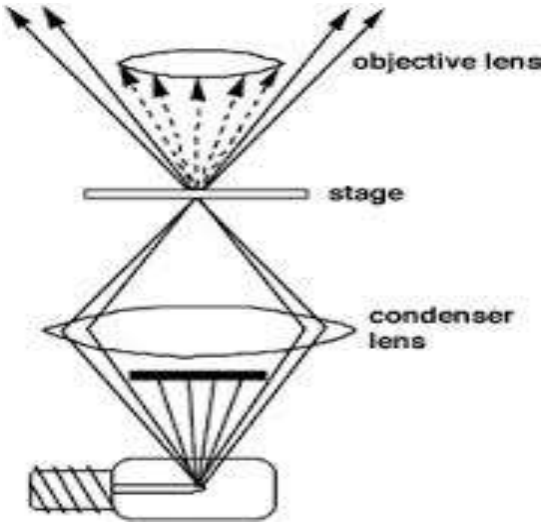
This instrument contains two lens systems for magnifying specimens: the ocular lens in the eyepiece and the objective lens located in the nose-piece. The specimen is illuminated by a beam

of tungsten light focused on it by a sub-stage lens called a condenser, and the result is that the specimen appears dark against a bright background. A major limitation of this system is the absence of contrast between the specimen and the surrounding medium, which makes it difficult to observe living cells. Therefore, most brightfield observations are performed on nonviable, stained preparations.

Dark-Field Microscope

This is similar to the ordinary light microscope; however, the condenser system is modified so that the specimen is not illuminated directly. The con-denser directs the light obliquely so that the light is deflected or scattered from the spec-imen, which then appears bright against a dark background. Living specimens may be observed more readily with darkfield than with brightfield microscopy.

Living, unstained cells and organisms can be observed by simply changing the way in which they are illuminated. A hollow cone of light is focused on the specimen in such a way that unreflected and unrefracted rays do not enter the objective. Only light that has been reflected or refracted by the specimen forms an image (figure 2.7). The field surrounding a specimen appears black, while the object itself is brightly illuminated because the background is dark, this type of microscopy is called dark-field microscopy. Considerable internal structure is often visible in larger eucaryotic microorganisms. The dark-field microscope is used to identify bacteria like the thin and distinctively shaped Treponema pallidum (figure 2.8a), the causative agent of syphilis.



Phase-Contrast Microscope

Observation of microorganisms in an unstained state is possible with this microscope. Its optics includes special objectives and a condenser that make visible cellular components that differ only slightly in their refractive indexes. As light is transmitted through a specimen with a refractive index different from that of the surrounding medium, a portion of the light is refracted (bent) due to slight varia-tions in density and thickness of the cellular components. The special optics convert the difference between transmitted light and refracted rays, resulting in a significant vari-ation in the intensity of light and thereby producing a discernible image of the struc-ture under study. The image appears dark against a light background.

The Phase-Contrast Microscope

Unpigmented living cells are not clearly visible in the bright field microscope because there is little difference in contrast between the cells and water. Thus microorganisms often must be fixed and stained before observation to increase contrast and create variations in color between cell structures. A phase-contrast microscope converts slight differences in refractive index and cell density into easily detected variations in light intensity and is an excellent way to observe living cells .

The condenser of a phase-contrast microscope has an annular stop, an opaque disk with a thin transparent ring, which produces a hollow cone of light (figure 2.9). As this cone passes through a cell, some light rays are bent due to variations in density and refractive index within the specimen and are retarded by about 1 /4 wavelength. The deviated light is focused to form an image of the object. Undeviated light rays strike a phase ring in the phase plate, a special optical disk located in the objective, while the deviated rays miss the ring and pass through the rest of the plate. If the phase ring is constructed in such a way that the undeviated light passing through it is advanced by 1/4 wavelength, the deviated and undeviated waves will be about 1 /2 wavelength out of phase and will cancel each other when they come together to form an image. The background, formed by undeviated light, is bright, while the unstained object appears dark and well-defined. This type of microscopy is called dark-phase-contrast microscopy. Color filters often are used to improve the image.

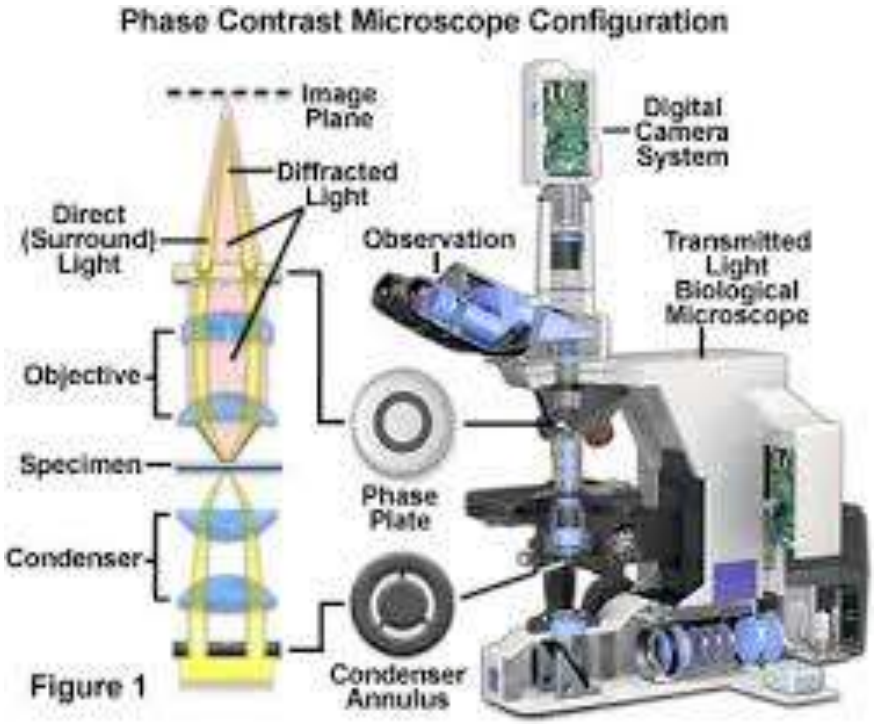


Figure 1

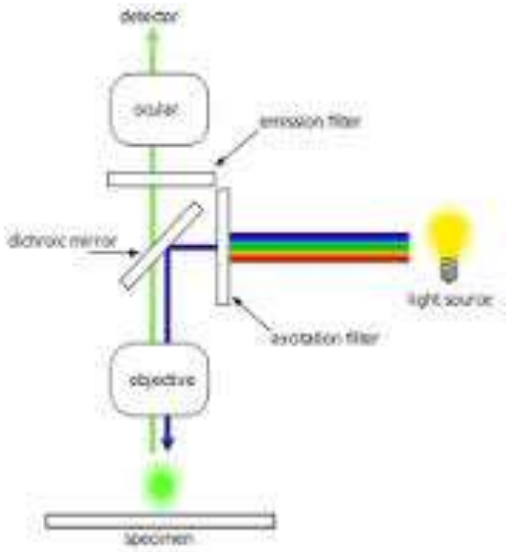
Phase-contrast microscopy is especially useful for studying microbial motility, determining the shape of living cells,and detecting bacterial components such as endospores and inclusion bodies that contain poly-hydroxybutyrate, polymetaphosphate, sulfur, or other substances. These are clearly visible because they have refractive indexes markedly different from that of water. Phasecontrast microscopes also are widely used in studying eucaryotic cells.

Fluorescence Microscope

This microscope is used most frequently to visualize speci-mens that are chemically tagged with a fluorescent dye. The source of illumination is an ultraviolet (UV) light obtained from a high-pressure mercury lamp or hydrogen quartz lamp. The ocular lens is fitted with a filter that permits the longer ultraviolet wavelengths to pass, while the shorter wavelengths are blocked or eliminated. Ultraviolet radiations are absorbed by the fluorescent label and the energy is re-emitted in the form of a different wavelength in the visible light range. The fluorescent dyes absorb at wavelengths between 230 and 350 nanometers (nm) and emit orange, yellow, or greenish light. This microscope is used primarily for the detection of antigen-antibody reactions. Antibodies are conjugated with a fluorescent dye that becomes excited in the presence of

ultraviolet light, and the fluorescent portion of the dye becomes visible against a black background.

The microscopes thus far considered produce an image from light that passes through a specimen. An object also can be seen because it actually emits light, and this is the basis of fluorescence microscopy. When some molecules absorb radiant energy, they become excited and later release much of their trapped energy as light. Any light emitted by an excited molecule will have a longer wavelength (or be of lower energy) than the radiation originally absorbed. Fluorescent light is emitted very quickly by the excited molecule as it gives up its trapped energy and returns to a more stable state. The fluorescence microscope exposes a specimen to ultraviolet, violet, or blue light and forms an image of the object with the resulting fluorescent light. A mercury vapor arc lamp or other source produces an intense beam, and heat transfer is limited by a special infrared filter. The light passes through an exciter filter that transmits only the desired wavelength. A dark- field condenser provides a black background against which the fluorescent objects glow. Usually the specimens have been stained with dye molecules, called fluorochromes, that fluoresce brightly upon exposure to light of a specific wavelength, but some microorganisms are autofluorescing. The microscope forms an image of the fluorochrome-labeled microorganisms from the light emitted when they fluoresce. A barrier filter positioned after the objective lenses removes any remaining ultraviolet light, which could damage the viewer's eyes, or blue and violet light, which would reduce the image's contrast.



The fluorescence microscope has become an essential tool in medical microbiology and microbial ecology. Bacterial pathogens (e.g., Mycobacterium tuberculosis, the cause of tuberculosis) can be identified after staining them with fluorochromes or specifically labeling them with fluorescent anti- bodies using immunofluorescence procedures. In ecological studies the fluorescence microscope is used to observe microorganisms stained with fluorochrome-labeled probes or fluorochromes such as acridine orange and DAPI (diamidino-2- phenylindole, a DNA-specific stain). The stained organisms will fluoresce orange or green and can be detected even in the midst of other particulate material. It is even possible to distinguish live bacteria from dead bacteria by the color they fluoresce after treatment with a special mixture of stains. Thus the microorganisms can be viewed and directly counted in a relatively undisturbed ecological niche.

Electron Microscopy

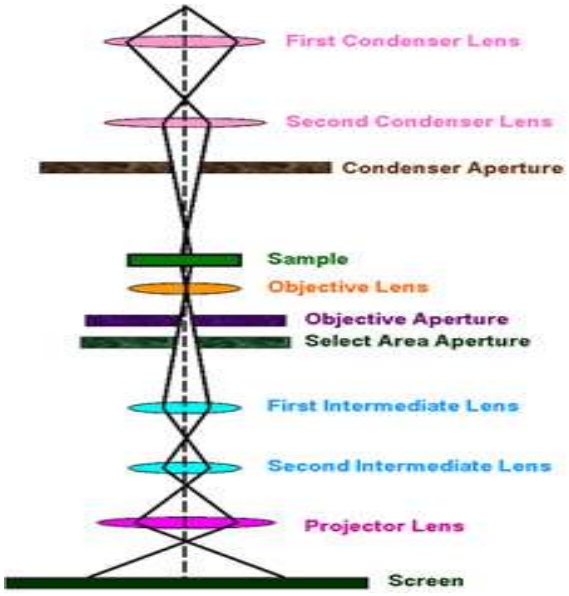
This instrument provides a revolutionary method of microscopy, with magnifications up to one million. This permits visualization of submicroscopic cel-lular particles as well as viral agents. In the electron microscope, the specimen is illu-minated by a beam of electrons rather than light, and the focusing is carried out by elec-tromagnets instead of a set of optics. These components are sealed in a tube in which a complete vacuum is established. Transmission electron microscopes require speci-mens that are thinly prepared, fixed, and dehydrated for the electron beam to pass freely through them. As the electrons pass through the specimen, images are formed by direct-ing the electrons onto photographic film, thus making internal cellular structures visi-ble. Scanning electron microscopes are used for visualizing surface characteristics rather than intracellular structures A narrow beam of electrons scans back and forth, producing a three-dimensional image as the electrons are reflected off the specimen's surface.

While scientists have a variety of optical instruments with which to perform routine laboratory procedures and sophisticated research, the compound brightfield micro-scope is the "workhorse" and is commonly found in all biological laboratories. Although you should be familiar with the basic principles of microscopy, you probably have not been exposed to this diverse array of complex and expensive equipment. Therefore, only the compound brightfield microscope will be discussed in depth and used to examine specimens.

Transmission Electron Microscope

The very best light microscope has a resolution limit of about 0.2 µm. Because bacteria usually are around 1 µm in diameter, only their general shape and major morphological features are visible in the light microscope. The detailed internal structure of larger microorganisms also cannot be effectively studied by light microscopy. These limitations arise from the nature of visible light waves, not from any inadequacy of the light microscope itself.

Recall that the resolution of a light microscope increases with a decrease in the wavelength of the light it uses for illumination. Electron beams behave like radiation and can be focused much as light is in a light microscope. If electrons illuminate the specimen, the microscope's resolution is enormously increased because the wavelength of the radiation is around 0.005 nm, approximately 100,000 times shorter than that of visible light. The transmission electron microscope has a practical resolution roughly 1,000 times better than the light microscope; with many electron microscopes, points closer than 5 Å or 0.5 nm can be distinguished, and the useful magnification is well over 100,000ɤ. microbial morphology can now be studied in great detail.



A modern transmission electron microscope (TEM) is complex and sophisticated , but the basic principles behind its operation can be understood readily. A heated tungsten filament in the electron gun generates a beam of electrons that is then focused on the specimen by the condenser. Since electrons cannot pass through a glass lens, doughnut-shaped electromagnets

called magnetic lenses are used to focus the beam. The column containing the lenses and specimen must be under high vacuum to obtain a clear image because electrons are deflected by collisions with air molecules. The specimen scatters electrons passing through it, and the beam is focused by magnetic lenses to form an enlarged, visible image of the specimen on a fluorescent screen. A denser region in the specimen scatters more electrons and therefore appears darker in the image since fewer electrons strike that area of the screen. In contrast, electron-transparent regions are brighter. The screen can also be moved aside and the image captured on photographic film as a permanent record.

Specimen Preparation

The distinctive features of the TEM place harsh restrictions on the nature of samples that can be viewed and the means by which those samples must be prepared. Since electrons are quite easily absorbed and scattered by solid matter, only extremely thin slices of a microbial specimen can be viewed in the average TEM. The specimen must be around 20 to 100 nm thick, about 1⁄50 to 1⁄10 the diameter of a typical bacterium, and able to maintain its structure when bombarded with electrons under a high vacuum! Such a thin slice cannot be cut unless the specimen has support of some kind; the necessary support is provided by plastic. After fixation with chemicals like glutaraldehyde or osmium tetroxide to stabilize cell structure, the specimen is dehydrated with organic solvents (e.g., acetone or ethanol).

Complete dehydration is essential because most plastics used for embedding are not water soluble. Next the specimen is soaked in unpolymerized, liquid epoxy plastic until it is completely permeated, and then the plastic is hardened to form a solid block. Thin sections are cut from this block with a glass or diamond knife using a special instrument called an ultramicrotome. Cells usually must be stained before they can be seen clearly in the bright-field microscope; the same is true for observations with the TEM. The probability of electron scattering is determined by the density (atomic number) of the specimen atoms. Biological molecules are composed primarily of atoms with low atomic numbers (H, C, N, and O), and electron scattering is fairly constant throughout the unstained cell.

Therefore specimens are prepared for observation by soaking thin sections with solutions of heavy metal salts like lead citrate and uranyl acetate. The lead and uranium ions bind to cell

structures and make them more electron opaque, thus increasing contrast in the material. Heavy osmium atoms from the osmium tetroxide fixative also "stain" cells and increase their contrast. The stained thin sections are then mounted on tiny copper grids and viewed. Although specimens normally are embedded in plastic and thin sectioned to reveal the internal structure of the smallest cell, there are other ways in which microorganisms and smaller objects can be readied for viewing. One very useful technique is negative staining.

The specimen is spread out in a thin film with either phosphotungstic acid or uranyl acetate. Just as in negative staining for light microscopy, heavy metals do not penetrate the specimen but render the background dark, whereas the specimen appears bright in photographs. Negative staining is an excellent way to study the structure of viruses, bacterial gas vacuoles, and other similar material.

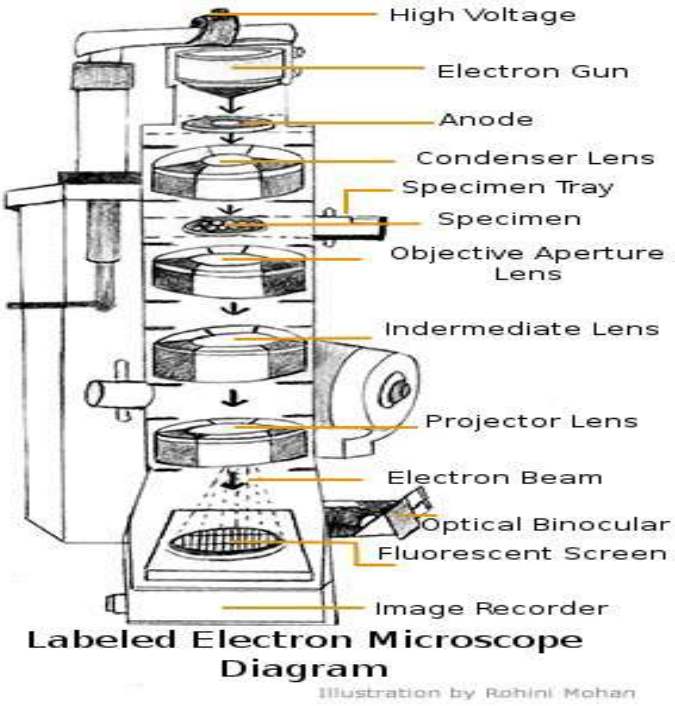
A microorganism also can be viewed after shadowing with metal. It is coated with a thin film of platinum or other heavy metal by evaporation at an angle of about 45° from horizontal so that the metal strikes the microorganism on only one side. The area coated with metal scatters electrons and appears light in photographs, whereas the uncoated side and the shadow region created by the object is dark. The specimen looks much as it would if light were shining on it to cast a shadow. This technique is particularly useful in studying virus morphology, bacterial flagella, and plasmids.

The TEM will also disclose the shape of organelles within microorganisms if specimens are prepared by the freeze-etching procedure. Cells are rapidly frozen in liquid nitrogen and then warmed to 100°C in a vacuum chamber. Next a knife that has been precooled with liquid nitrogen (196°C) fractures the frozen cells, which are very brittle and break along lines of greatest weakness, usually down the middle of internal membranes. The specimen is left in the high vacuum for a minute or more so that some of the ice can sublimate away and uncover more structural detail (sometimes this etching step is eliminated). Finally, the exposed surfaces are shadowed and coated with layers of platinum and carbon to form a replica of the surface. After the specimen has been removed chemically, this replica is studied in the TEM and provides a detailed, three-dimensional view of intracellular structure. An advantage of freeze-etching is that

it minimizes the danger of artifacts because the cells are frozen quickly rather than being subjected to chemical fixation, dehydration, and plastic embedding.

Scanning Electron Microscope

More recently the scanning electron microscope (SEM) has been used to examine the surfaces of microorganisms in great detail; many instruments have a resolution of 7 nm or less. The SEM differs from other electron microscopes in producing an image from electrons emitted by an object's surface rather than from transmitted electrons. Specimen preparation is easy, and in some cases air-dried material can be examined directly. Most often, however, microorganisms must first be fixed, dehydrated, and dried to preserve surface structure and prevent collapse of the cells when they are exposed to the SEM's high vacuum. Before viewing, dried samples are mounted and coated with a thin layer of metal to prevent the buildup of an electrical charge on the surface and to give a better image.



The SEM scans a narrow, tapered electron beam back and forth over the specimen (figure 2.27). When the beam strikes a particular area, surface atoms discharge a tiny shower of electrons called secondary electrons, and these are trapped by a special detector. Secondary electrons entering the detector strike a scintillator causing it to emit light flashes that a photomultiplier

converts to an electrical current and amplifies. The signal is sent to a cathode-ray tube and produces an image like a television picture, which can be viewed or photographed.

The number of secondary electrons reaching the detector depends on the nature of the specimen's surface. When the electron beam strikes a raised area, a large number of secondary electrons enter the detector; in contrast, fewer electrons escape a depression in the surface and reach the detector. Thus raised areas appear lighter on the screen and depressions are darker. A realistic three-dimensional image of the microorganism's surface with great depth of focus results. The actual in situ location of microorganisms in ecological niches such as the human skin and the lining of the gut also can be examined.

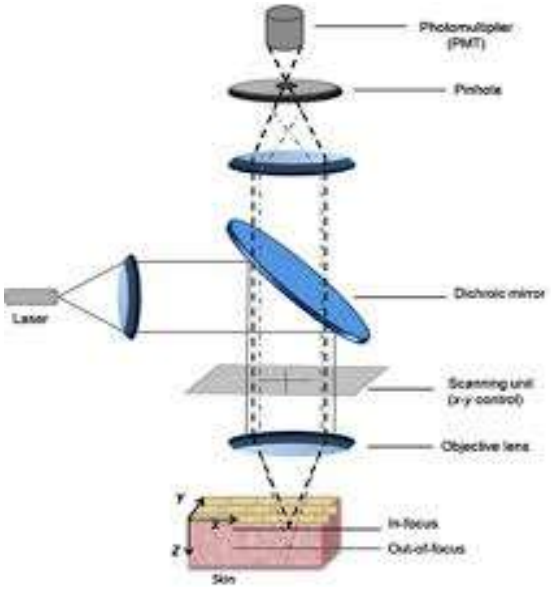
Confocal Microscopy

A conventional light microscope, which uses a mixed wavelength light source and illuminates a large area of the specimen, will have a relatively great depth of field. Even if not in focus, images of bacteria from all levels within the field will be visible. These will include cells above, in, and below the plane of focus.

As a result the image can be murky, fuzzy, and crowded.The solution to this problem is the confocal scanning laser microscope (CSLM) or confocal microscope. Fluorescently stained specimens are usually examined. A focused laser beam strikes a point in the specimen (figure 2.30). Light from the illuminated spot is focused by an objective lens onto a plane above the objective. An aperture above the objective lens blocks out stray light from parts of the specimen that lie above and below the plane of focus. The laser is scanned over a plane in the specimen (beam scanning) or the stage is moved (stage scanning) and a detector measures the illumination from each point to produce an image of the optical section. When many optical sections are scanned, a computer can combine them to form a three-dimensional image from the digitized signals. This image can be measured and analyzed quantitatively.

The confocal microscope improves images in two ways. First, illumination of one spot at a time reduces interference from light scattering by the rest of the specimen. Second, the aperture above the objective lens blocks out stray light as previously mentioned. Consequently the image has excellent contrast and resolution. A depth of 1 □m or less in a thick preparation can be directly

observed. Special computer software is used to create high-resolution, three-dimensional images of cell structures and complex specimens such as biofilms.



Scanning Transmission Electron Microscope (STEM)

The STEM was invented in the 1930s along with the transmission electron microscope (TEM) and offers imaging modes and enhanced microanalysis capabilities not available with a TEM. The STEM also has several similarities to the scanning electron microscope (SEM). One such similarity is that the STEM uses a relatively low electron accelerating voltage of approximately 30 keV. Only thin specimens may be imaged with a STEM; a highly focused beam of electrons is scanned over the specimen and electrons that pass through the sample are collected to fabricate transmission images. In this way, a STEM is very similar to a SEM. Backscattered electrons and x-rays are produced during a STEM scan, which is a similarity to TEM scans. A STEM can be created by adding transmission detectors to an SEM or by adding scanning coils to a TEM. The latter option is usually not employed because the minimum probe diameter is large and the resolution of microanalysis is limited.

One common imaging mode for the STEM is known as bright field imaging. In both a TEM and STEM, transmitted electrons are collected on the axis to create the bright field. By using a large detector and angle of travel, a STEM can be used to image much thicker samples than a TEM is

capable of imaging. The reason for this is because there is no objective lens below the sample to cause chromatic aberration as there is in a TEM. For example, a STEM can image a sample up to a few microns thick at 200 keV using the bright field imaging technique, while a TEM can only image an object about 0.5 microns thick using the same energy.

Another advantage of the STEM is its ability to execute High Angle Annular Dark Field (HAADF) imaging. The images collected from this technique are solely from elastically scattered electrons which are repelled by the nuclei of the sample. This is achieved by setting the inner angle of the annular dark field detector to a large value of approximately 30 milliradians so that no Bragg diffracted electrons are collected. With this technique, high resolution is possible without the undesirable diffraction contrast which can mask important structural information. The probe diameter is the primary factor that determines the resolution of the dark field. HAADF is very useful for the imaging of inorganic and organic solids in addition to both crystalline and amorphous materials. Another important advantage of the STEM over the TEM is its ability to collect secondary electrons in the same way as a SEM. This ability makes it possible to use a STEM to correlate surface information with bulk information from the STEM mode and image samples which are too thick for STEM observation.

A major advantage of the STEM over a standard SEM is that an ultra-high resolution can be produced with a STEM because of the comparatively high accelerating voltage used. Overall, a STEM is a very valuable piece of equipment to the nanotechnologist and materials scientist because it blends the advantages of both a TEM and SEM into a single, versatile piece of imaging equipment.

Staining Techniques

Because microbial cytoplasm is usually transparent, it is necessary to stain microorganisms before they can be viewed with the light microscope. In some cases, staining is unnecessary, for example when microorganisms are very large or when motility is to be studied, and a drop of the microorganisms can be placed directly on the slide and observed. A preparation such as this is called a **wet mount.** A wet mount can also be prepared by placing a drop of culture on a cover-slip (a glass cover for a slide) and then inverting it over a hollowed-out slide. This

procedure is called the **hanging drop.**In preparation for staining, a small sample of microorganisms is placed on a slide and permitted to air dry. The smear is heat fixed by quickly passing it over a flame. **Heat fixing** kills the organisms, makes them adhere to the slide, and permits them to accept the stain.

Simple staining technique.

Staining can be performed with basic dyes such as crystal violet or methylene blue, positively charged dyes that are attracted to the negatively charged materials of the microbial cytoplasm. Such a procedure is the **simple stain procedure.** An alternative is to use a dye such as nigrosin or Congo red, acidic, negatively charged dyes. They are repelled by the negatively charged cytoplasm and gather around the cells, leaving the cells clear and unstained. This technique is called the **negative stain technique.**

Differential staining technique.

The **differential stain technique** distinguishes two kinds of organisms. An example is the **Gram stain technique.** This differential technique separates bacteria into two groups, Gram-positive bacteria and Gram-negative bacteria. Crystal violet is first applied, followed by the mordant iodine, which fixes the stain. Another differential stain technique is the **acid-fast technique.** This technique differentiates species of *Mycobacterium* from other bacteria. Heat or a lipid solvent is used to carry the first stain, carbolfuchsin, into the cells. Then the cells are washed with a dilute acid-alcohol solution. *Mycobacterium* species resist the effect of the acid-alcohol and retain the carbolfuchsin stain (bright red). Other bacteria lose the stain and take on the subsequent methylene blue stain (blue). Thus, the acid-fast bacteria appear bright red, while the nonacid-fast bacteria appear blue when observed under oil-immersion microscopy. Other stain techniques seek to identify various bacterial structures of importance. For instance, a special stain technique highlights the **flagella** of bacteria by coating the flagella with dyes or metals to increase their width. Flagella so stained can then be observed.

A special stain technique is used to examine bacterial **spores.** Malachite green is used with heat to force the stain into the cells and give them color. A counterstain, safranin, is then used to give color to the nonsporeforming bacteria. At the end of the procedure, spores stain green and other

cells stain red. Other stain techniques seek to identify various bacterial structures of importance. For instance, a special stain technique highlights the flagella of bacteria by coating the flagella with dyes or metals to increase their width. Flagella so stained can then be observed.

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KARPAGAM ACADEMY OF HIGHER EDUCATION FUNDAMENTALS OF MICROBIOLOGY AND CLASSIFICATION (17MBP101)	
Unit II Questions	**Opt 1**
One angstrom is equal to _____.	One tenth of a mi
With the increase in the value of numerical aperture of an objective lens the	Remains the sam
Endospores and inclusion bodies can be best detected with the help of ____	Fluorescence mic
Treponema pallidum can be best identified using _____.	Fluorescence mic
The generally preferred method of observing wet mounts of bacteria is ____	Phase contrast m
Best resolution attained by an electron microscope is _____.	02.nm
Best resolution attained by an electron microscope is _____.	0.2nm
In which type of microscopy the microorganism is likely to show varying d	Dark-field
Which of the following is a supravital fluorescent stain?	Acridine orange
Which of the following is not used as a fixative?	Ethanol
Magnification of the microscope depends on which of the following?	light source
Fixation means _____	Preserves interna
The most commonly used fixation in light microscopy for observing microo	Osmic acid
Stains are used to _____.	Store cultures
Which is the coloring agent used in biological?	Stain
Dye is an organic compound containing _____.	Iodophore and ch
Basic stains are absorbed at _____.	pH values higher
Which of the following is not a basic stain?	Crystal violet
Which of the following is a neutral stain?	Picric acid
Rhodamin is a _____	Dye
Which of the following is not a mordant?	Tannic acid
Which of the following is a differential staining?	Gram staining
Spore staining involves the use of _____.	Malachite green a
The maximum useful magnification of bright field microscope is _____	1000
Red light has the wavelength of about____	640nm
Blue light has a wavelength of about _____	440nm
Ultra violet light has shorter wavelenth of _____	100-200 nm
Light may pass through a substance, a phenomenon known as _____	Diffraction
Substance that absorbs light of one wavelength will emit light of different w	Diffraction
The _____ is the ratio of the speed of light in a given medium to the s	Refractive index
Distortion based on the shape of the lens is called _____	Chromatic aberra
Distortion based on the color of light is called _____	Chromatic aberra
Modern microscopic lenses called_____	Convex lenses
Enlargement of the image of an object is called _____	Resolution
Resolving power(R) = _____	0.4 l/NA
Air has refractive index of _____	0
Commonly used stains include_____	Crystal violet
Which of the following used in negative staining _____	Crystal violet
A substance that fixes the primary stain in the bacterial cells is known as a	Primary agent

The 10x objective is the use the total magnification is _____	100x
Fluorescent dye binds to the _____ of the cell walls of mycobacteria.	Mycolic acid
The resolution of bright field microscope for viewing bacteria is _____.	300nm
Fluorescent dye _____ is illuliminated with blue light, it emits green ligh	Nigrosin
The darkfield _____ lenses focus light on the specimen at an oblique an	Concave lens
Triponema pallidum that causes syphilis is viewed by _____ microscop	Light
If the 10x objective is in use the total magnification is _____.	10X
Bacteria are measured in units called _____.	mm
The ocular has a magnification of _____ x.	100X
When observing a specimen using the power or oil immersion objective one	Fine adjustment
The foundation of the microscope is _____.	Arm
If the specimen is in focus using one object and requires very little adjustm	Par focal
_____ microscope that utilizes electrons to illuminate the specimen bei	Dark field
_____ microscope that illuminate the specimen in a blackened field.	Dark field
The _____ knob is used first when focusing with scanning or low power ob	Fine adjustment
The part of the microscope that is also called the eye piece.	Ocular
Immersion oil prevents the _____ of light.	Refraction
The low power objective has a magnification of _____x.	10X
The high power objective has a magnification of _____x.	10X
The source of electron is a hot _____ filament in an electron gun.	Copper
In scanning electron microscopy the emitted light is converted to a _____	Heat
_____Used as an indicator in Mcintosh anaerobic jar for preparation of ana	Methylene blue
_____ is a simple media	Blood agar
_____ media contain additives that enhance the growth of the desired o	Simple
When *S.typhi* is grown in Wilson and Blair medium, containing sulphite, th	Black
Mac Conkey's agar is a _____ medium	Selective
_____ is a transport medium	Mac Conkey
In Mac conkey's Medium, lactose fermenters produce _____ colonie	Pink
Assay medium is also known as _____	Selective media

Opt 2	**Opt 3**	**Opt 4**	**Opt 5**	**Opt 6**
One thousandth of a mic	One thousand of a nanor	One tenth of a nanometer		
Increases indefinitely	Decreases	Increases up to a limit		
Dark field microscope	Dark field microscope	Bright field microscope		
Dark field microscope	Bright field microscope	Phase contrast microscope		
Interference microscopy	Dark field microscopy	Fluorescence microscopy		
0.5nm	0.2 mm	2nm		
0.5nm	0.5 mm	0.2 mm		
Bright field	Phase contrast	Electron		
Congored	Neutral red	Safarinin		
Glacial acetic acid	Formaldehyde	Conc.HCI		
Magnifying power of the	Wire	body tube		
Colouration	Modent	smearing		
Aldehydes	Heat	Chloroform		
To reveal their shape and	pathogen	Antiseptic		
Dye	Chromophore	Auxochrome		
Chromophore and chron	Chromophore and auxoc	Auxochrome and ionophore		
Isolelectric point	pH values lower than the	Very low pH		
Methyl violet	Safranin	Nigrosin		
Giemsa	Neutral red	Malachite green		
Stain	Decolariser	dehydration		
Salts of aluminium	Salts of chromium	Salts of sodium		
Nuclear staining	Spore staining	Capsule staining		
Lactophenol cotton blue	Crystal violet and safran	Methylene blue and carbol fuchsi		
1, 500	0, 0001	100000		
680 nm	620nm	600nm		
480nm	20nm	480nm		
100-300nm	100-400nm	100-300nm		
Transmission	Fluorescence	Absorption		
Transmission	Fluorescence	Absorption		
Diffraction	Transmission rate	reflection		
Spherical aberration	reflection	refraction		
Spherical aberration	reflection	refraction		
Concave lenses	Flat field lenses	Lenses		
Resolving power	Magnification	Reflection		
0.3 l/NA	0.5 l/NA	0.2 l/NA		
1	2	3		
Nigrosin	Methylene blue	Saffranin		
Nigrosin	Methylene blue	Saffranin		
Mordant	Colourant	Coolant		

10x	1000x	1400x		
Dipimelic acid	Dipicolinic acid	Chromogen		
20nm	400nm	250nm		
Methylene blue	Flurescein isothiocyanat	Safranin		
Convex lens	Convex-convex lens	Condensor lens		
Bright field	Dark field	Electron		
100X	1000X	5X		
mm	ml	cm		
10x	1000X	1,400X		
Coarse adjustment	Mechanical stage	Rotator		
Lens	Base	Condenser		
Focus	Wet mount	Scanning		
Bright field	Electron	Flurescence.		
Electron	Bright field	Simple		
Coarse adjustment	Mechanical stage	nobe		
Objective	Arm	Diapharm		
Absorption	Transmission	reflection		
40X	1000X	100 X		
40X	100X	1000X		
Tungsten	zinc	mercury		
Electrical current	Sound	light		
Phenol red	Bromothymol blue	Neutral red		
Robertson’s medium	Mac conkey’s agar	Nutrient agar		
Transport	Selective	Indicator		
Green	Pink	Yellow		
Transport	Simple	Enrichment		
Blood agar	Stuart’s Media	Nutrient agar		
Yellow	Black	Green		
Complex media	Production media	Indicator media		

Answer
One tenth of a micrometer
Increases indefinitely
Bright field microscope
Dark field microscope
Phase contrast microscopy
0.2 mm
0.2 mm
Electron
Acridine orange
Conc.HCI
Magnifying power of the eyepiece

Preserves internal and external structures of microorganisms

Heat
To reveal their shape and size
Chromophore
Chromophore and auxochrome

pH values higher than the isocletric point

Nigrosin
Neutral red
Dye
Salts of aluminium
Gram staining
Malachite green and safranin
1000
620nm
b480nm
100-300nm
Diffraction
Fluorescence
Diffraction
Chromatic aberration
Chromatic aberration
Convex lenses
Resolution
0.3 l/NA
0
Saffranin
Crystal violet
Mordant

1000x
Mycolic acid
20nm
Flurescein isothiocyanate
Condensor lens
Dark field
1000X
mm
10X
Coarse adjustment
Lens
Par focal
Electron
Dark field
Fine adjustment
Ocular
Refraction
10X
100X
Tungsten
Electrical current
Methylene blue
Nutrient agar
Selective
Black
Selective
Stuart’s Media
Pink
Production media

DEPARTMENT OF MICROBIOLOGY
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Eachanari Post, Coimbatore, Tamil Nadu, India – 641 021

I M.Sc MICROBIOLOGY - 2017 – 2019 Batch

FUNDAMENTALS OF MICROBIOLOGY AND CLASSIFICATION (17MBP101)

SEMESTER – I **4H – 4C**

POSSIBLE QUESTION

Unit II

TWO MARK

1. Define Microscope.
2. Define resolution power.
3. Comment TEM &SEM.
4. Define STEM.
5. Define magnification.
6. What is focal length?
7. Uses of phase contrast microscope.
8. Define phase contrast microscope and compound microscope.
9. Define magnifying power.

EIGHT MARKS

10. Explain the principles of fluorescence microscope.
11. Explain the principles of scanning electron microscope.
12. Define confocal microscope.
13. What are the components of SEM?
14. Give the applications of SEM
15. What are the principles of electron microscope?
16. Explain Dark field microscope with a diagram.
17. Explain in details about the microscope and its parts.
18. Comment on types of microscope.
19. Write the working principles of phase contrast microscope.
20. Explain specimen preparation for TEM.

21. Give the working principle and applications of SEM.
22. Explain in detail about the sample collection and preparation of SEM.
23. Explain in detail about fluorescence microscope.
24. Discuss the working principles and magnification of simple microscope.
25. Explain in detail about STEM.
26. Differentiate between Bright field and Dark field microscope.
27. Give the application of confocal microscope.

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LECTURE PLAN

Unit III

S.No	Duration	Topic	Reference
1	2	Classification of bacteria- Bergey's manual and its importance	R1: pgs ;440, - 446,T1: pgs; 24-28
2	2	Classification of algae – Chlamydomonas, Volvox,	R1: pgs ;571 - 573, T1: pgs; 28 - 29
3	2	Diatoms, Red algae and Brown algae	R1: pgs ;578 - 579 W6
4	1	Classification of Virus – DNA, RNA Viruses	W7,T2 555
5	1	Classification and Taxonomy of fungi-Alexopolous	R1:553- 554,W8
6	2	Economical importance of Fungi, Classification, of protozoa	W9, W10
7	2	*Entamoeba histolytica, Giardia, Trichomonas, Plasmodium*	R1: 584-592, W11,12,13
8	1	Recapitulation and discussion of question	
	Total Hrs: 13		

T1: Dubey, R.C. and D.K. Maheswari, 2010. A Text book of Microbiology. 3rd Edition, S. Chand and Company, New Delhi.

T2: Ananthanarayanan, R. and C.K.J. Panicker, 2005. Text book of Microbiology. 7th Edition. Orient Longman. New Delhi.

W6: www.wikipedia.org (fmp.conncoll.edu)

W7: www.en.wikipedia.org

W8: www.britanica.com

W9: www.heebnotes.com

W10: www.standardnotes.blogspot.in

W11: www.en.wikipedia.org

W12: https://web.stanford.edu

W13: web.stanford.edu

UNIT – III

Classifcation of Bacteria (Bergey's Manual)

In 1923, David Bergey, professor of bacteriology at the University of Pennsylvania, and four colleagues published a classification of bacteria that could be used for identification of bacterial species, the *Bergey's Manual of Determinative Bacteriology.* This manual is now in its ninth edition. The first edition of *Bergey's Manual of Systematic Bacteriology*, a more detailed work that contains descriptions of all procaryotic species currently identified, also is available (**Box 19.1**). The first volume of the second edition has been published recently. This section briefly describes the current edition of *Bergey's Manual of Systematic Bacteriology* (or *Bergey's Manual*) and then discusses at more length the new second edition.

The First Edition of *Bergey's Manual of Systematic Bacteriology*

Because it has not been possible in the past to classify prokaryotes satisfactorily based on phylogenetic relationships, the system given in the first edition of *Bergey's Manual of Systematic Bacteriology* is primarily phenetic. Each of the 33 sections in the four volumes contains procaryotes that share a few easily determined characteristics and bears a title that either describes these properties or provides the vernacular names of the procaryotes included. The characteristics used to define sections are normally features such as general shape and morphology, Gram-staining properties, oxygen relationship, motility, the presence of endospores, the mode of energy production, and so forth. Procaryotic groups are divided among the four volumes in the following manner:

(1) Gram-negative bacteria of general, medical, or industrial importance;
(2) Gram-positive bacteria other than actinomycetes;
(3) Gram-negative bacteria with distinctive properties, cyanobacteria, and archaea; and
(4) Actinomycetes (gram-positive filamentous bacteria).

Gram-staining properties play a singularly important role in this phenetic classification; they even determine the volume into which a species is placed. There are good reasons for this significance. As noted in chapter 3, Gram staining usually reflects fundamental differences in

bacterial wall structure. Gram-staining properties also are correlated with many other properties of bacteria. Typical gram-negative bacteria, gram-positive bacteria, and mycoplasmas (bacteria lacking walls) differ in many characteristics, as can be seen in **table 19.9.** For these and other reasons, bacteria traditionally have been classified as gram positive or gram negative. This approach is retained to some extent in more phylogenetic classifications and is a useful way to think about bacterial diversity.

The Second Edition of *Bergey's Manual of Systematic Bacteriology*

There has been enormous progress in procaryotic taxonomy since 1984, the year the first volume of *Bergey's Manual of Systematic Bacteriology* was published. In particular, the sequencing of rRNA, DNA, and proteins has made phylogenetic analysis of prokaryotes feasible. As a consequence, the second edition of *Bergey's Manual* will be largely phylogenetic rather than phonetic and thus quite different from the first edition. Although the new edition will not be completed for some time, it is so important that its general features will be described here. Undoubtedly the details will change as work progresses, but the general organization of the new *Bergey's Manual* can be summarized.

The second edition will be published in five volumes. It will have more ecological information about individual taxa. The second edition will not group all the clinically important prokaryotes together as the first edition did. Instead, pathogenic species will be placed phylogenetically and thus scattered throughout the following five volumes.

Volume 1—*The Archaea, and the Deeply Branching and Phototrophic Bacteria*

Volume 2—*The Proteobacteria*

Volume 3—*The Low G + C Gram-Positive Bacteria*

Volume 4—*The High G + C Gram-Positive Bacteria*

Volume 5—*The Planctomycetes, Spirochaetes, Fibrobacteres, Bacteroidetes,* and *Fusobacteria* (Volume 5 also will contain a section that updates descriptions and phylogenetic arrangements that have been revised since publication of volume 1).

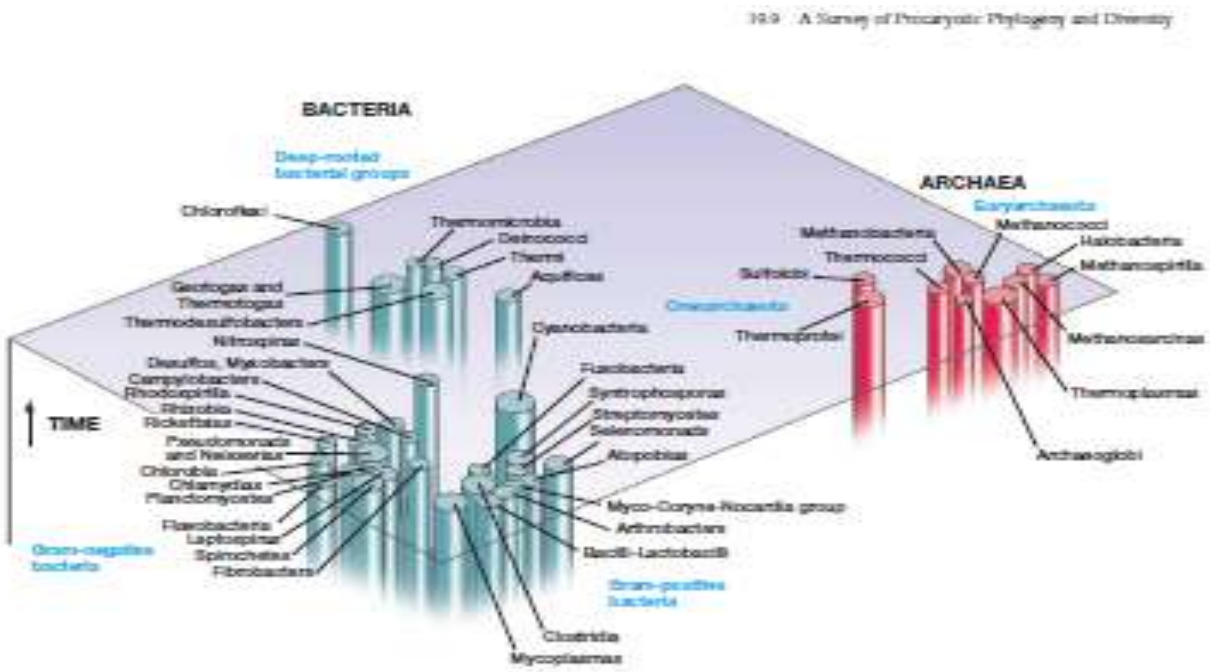


Figure 19.13 **Major Procaryotic Groups and Their Relatedness.** Disk size is roughly proportional to the relative number of sequenced procaryotes in each group. Closely related procaryotic groups are clustered together. Note that the two procaryotic domains (Bacteria and Archaea) are clearly separate. The cylinders fade out to indicate that the antiquity of these groups is uncertain.

The second edition's five volumes will have a different organization than the first edition. The greatest change in organization of the volumes will be with respect to the gram-negative bacteria. The first edition describes all gram-negative bacteria in two volumes. Volume 1 contains the gram-negative bacteria of general, medicalor industrial importance; volume 3 describes the archaea, cyanobacteria, and remaining gram-negative groups. The second edition describes the gram-negative bacteria in three volumes, with volume 2 reserved for the proteobacteria. The two editions treat the gram-positive bacteria more similarly. Although volume 2 of the first edition does have some high G + C bacteria, much of its coverage is equivalent to the new volume 3. Volume 4 of the first edition describes the actinomycetes and is similar to volume 4 of the second edition (high G + C gram-positive bacteria), although the new volume 4 will have broader coverage. For example, *Micrococcus* and *Corynebacterium* are in volume 2 of the first edition and will be in volume 4 of the second edition. **Table 19.10** summarizes the planned organization of the second edition and indicates where the discussion of a particular group may be found in this textbook. **Figure 19.13** depicts the major groups and their relatedness to each other.

Table 19.10 Organization of *Bergey's Manual of Systematic Bacteriology*

Taxonomic Rank	Representative Genera	Textbook Coverage
Volume 1. *The Archaea and the Deeply Branching and Phototrophic Bacteria*		
Domain *Archaea*		
Phylum *Crenarchaeota*	*Thermoproteus, Pyrodictium, Sulfolobus*	pp. 456–58
Phylum *Euryarchaeota*		
Class I. *Methanobacteria*	*Methanobacterium*	pp. 458–61
Class II. *Methanococci*	*Methanococcus*	
Class III. *Halobacteria*	*Halobacterium, Halococcus*	pp. 461–63
Class IV. *Thermoplasmata*	*Thermoplasma, Picrophilus*	p. 463
Class V. *Thermococci*	*Thermococcus, Pyrococcus*	p. 463
Class VI. *Archaeoglobi*	*Archaeoglobus*	p. 463
Class VII. *Methanopyri*	*Methanopyrus*	p. 458
Domain *Bacteria*		
Phylum *Aquificae*	*Aquifex, Hydrogenobacter*	p. 467
Phylum *Thermotogae*	*Thermotoga, Geotoga*	pp. 467–68
Phylum *Thermodesulfobacteria*	*Thermodesulfobacterium*	
Phylum "Deinococcus-Thermus"	*Deinococcus, Thermus*	p. 468
Phylum *Chrysiogenetes*	*Chrysogenes*	
Phylum *Chloroflexi*	*Chloroflexus, Herpetosiphon*	p. 470
Phylum *Thermomicrobia*	*Thermomicrobium*	
Phylum *Nitrospira*	*Nitrospira*	
Phylum *Deferribacteres*	*Geovibrio*	
Phylum *Cyanobacteria*	*Prochloron, Synechococcus, Pleurocapsa, Oscillatoria, Anabaena, Nostoc, Stigonema*	pp. 471–76
Phylum *Chlorobi*	*Chlorobium, Pelodictyon*	pp. 470–71
Volume 2. *The Proteobacteria*		
Phylum *Proteobacteria*		
Class I. Alphaproteobacteria	*Rhodospirillum, Rickettsia, Caulobacter, Rhizobium, Brucella, Nitrobacter, Methylobacterium, Beijerinckia, Hyphomicrobium*	pp. 487–95
Class II. Betaproteobacteria	*Neisseria, Burkholderia, Alcaligenes, Comamonas, Nitrosomonas, Methylophilus, Thiobacillus*	pp. 495–98
Class III. Gammaproteobacteria	*Chromatium, Leucothrix, Legionella, Pseudomonas, Azotobacter, Vibrio, Escherichia, Klebsiella, Proteus, Salmonella, Shigella, Yersinia, Haemophilus*	pp. 498–507
Class IV. Deltaproteobacteria	*Desulfovibrio, Bdellovibrio, Myxococcus, Polyangium*	pp. 507–13
Class V. Epsilonproteobacteria	*Campylobacter, Helicobacter*	p. 514
Volume 3. *The Low G + C Gram-Positive Bacteria*		
Phylum *Firmicutes*		
Class I. Clostridia	*Clostridium, Peptostreptococcus, Eubacterium, Desulfotomaculum, Heliobacterium, Veillonella*	pp. 523–25
Class II. *Mollicutes*	*Mycoplasma, ureaplasma, Spiroplasma, Acholeplasma*	pp. 518–21
Class III. Bacilli	*Bacillus, Caryophanon, Paenibacillus, Thermoactinomyces, Lactobacillus, Streptococcus, Enterococcus, Listeria, Leuconostoc, Staphylococcus*	pp. 525–33
Volume 4. *The High G + C Gram-Positive Bacteria*		
Phylum *Actinobacteria*		
Class *Actinobacteria*	*Actinomyces, Micrococcus, Arthrobacter, Corynebacterium, Mycobacterium, Nocardia, Actinoplanes, Propionibacterium, Streptomyces, Thermomonospora, Frankia, Actinomadura, Bifidobacterium*	pp. 539–49
Volume 5. *The Planctomycetes, Spirochaetes, Fibrobacteres, Bacteriodetes, and Fusobacteria*		
Phylum *Planctomycetes*	*Planctomyces, Gemmata*	p. 477
Phylum *Chlamydiae*	*Chlamydia*	pp. 477–78
Phylum *Spirochaetes*	*Spirochaeta, Borrelia, Treponema, Leptospira*	pp. 479–81
Phylum *Fibrobacteres*	*Fibrobacter*	
Phylum *Acidobacteria*	*Acidobacterium*	
Phylum *Bacteroidetes*	*Bacteroides, Porphyromonas, Prevotella, Flavobacterium, Sphingobacterium, Flexibacter, Cytophaga*	pp. 481–83
Phylum *Fusobacteria*	*Fusobacterium, Streptobacillus*	
Phylum *Verrucomicrobia*	*Verrucomicrobium*	
Phylum *Dictyoglomi*	*Dictyoglomus*	

A Survey of Procaryotic Phylogeny and Diversity

Before beginning a detailed introduction to procaryotic diversity, it might be best to very briefly survey the major groups in the order they are discussed in the second edition of *Bergey's Manual.* This overview is meant only as a general survey of procaryotic diversity. The second edition places procaryotes into 25 phyla, only some of which will be mentioned here. Many of these groups willbe discussed in much more detail in chapters 20 through 24. Recall that all organisms may be placed in one of three domains or empires as depicted in the universal phylogenetic tree (figure 19.3). We are concerned only with the procaryotic domains, *Archaea* and *Bacteria,* in this survey.

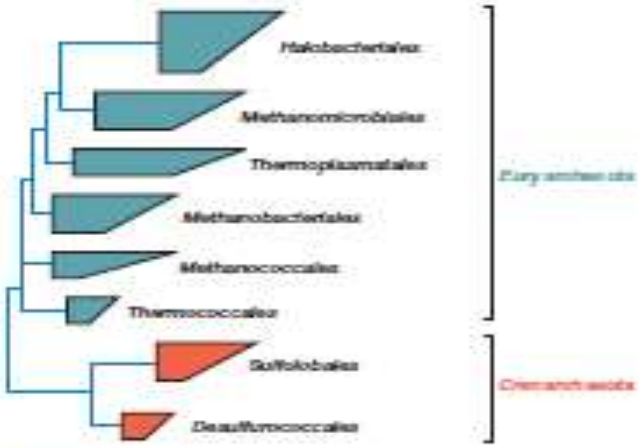


Figure 19.14 **Phylogeny of the Archaea.** The tree is based on 16S rRNA data and shows relationships between the better-studied orders. Each tetrahedron represents a group of related organisms; its horizontal edges indicate the shortest and longest branches in the group. See text for discussion.

Volume 1 contains a wide diversity of procaryotes in two domains: the *Archaea* and the *Bacteria.* The *Archaea* differ from *Bacteria* in many ways as summarized in table 19.8. At present, they are divided into two phyla based on rRNA sequences (**figure 19.14**). The phylum *Crenarchaeota* contains thermophilic and hyperthermophylic sulfur-metabolizing organisms of the orders *Thermoproteales, Desulfurococcales,* and *Sulfolobales.* However, recently many other *Crenarchaeota* have been discovered. Some are inhibited by sulfur; others grow in the oceans at low temperatures as picoplankton. The phylum clearly is more diverse than first thought. The second phylum, the *Euryarchaeota,* contains primarily methanogenic prokaryotes and halophilic procaryotes; thermophilic, sulfur-reducing organisms (the thermoplasmas and thermococci) also

are in this phylum. The two phyla are divided into eight classes and 12 orders. The bacteria are an extraordinarily diverse assemblage of procaryotes that have been divided into 23 phyla (**figure 19.15**).

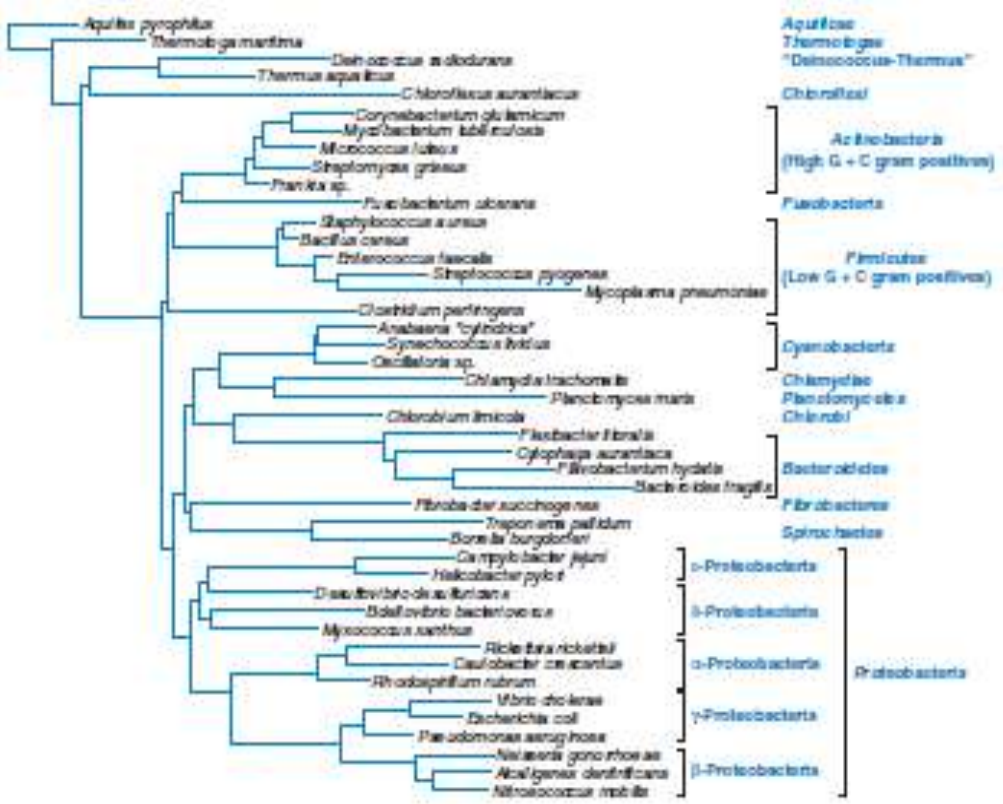


Figure 19.15 **Phylogeny of the Bacteria.** The tree is based on 16S rRNA comparisons. See text for discussion. *Source: The Ribosomal Database Project.*

In volume 1 are placed deeply branching bacterial groups and phototrophic bacteria. The more important phyla are described in the following sections.

1. **Phylum *Aquificae*.** The phylum *Aquificae* contains autotrophic bacteria such as *Aquifex* and *Hydrogenobacter* that can use hydrogen for energy production. *Aquifex* (meaning "water maker") actually produces water by using hydrogen to reduce oxygen. This group contains some of the most thermophilic organisms known and is the deepest or earliest branch of the bacteria.
2. **Phylum *Thermotogae*.** This phylum is composed of one class and five genera. *Thermotoga* and other members of the class *Thermotogae* are anaerobic, thermophilic,

fermentative, gram-negative bacteria that have unusual fatty acids and resemble *Aquifex* with respect to their etherlinked lipids.

3. **Phylum "Deinococcus Thermus**." The order *Deinococcales* contains bacteria that are extraordinarily radiation resistant. The genus *Deinococcus* is gram positive. It has high concentrations of carotenoid pigments, which may protect it from radiation, and unique lipids.
4. **Phylum *Chloroflexi***: The phylum *Chloroflexi* has one class and two orders. Many members of this gram-negative group are called green nonsulfur bacteria. *Chloroflexus* carries out anoxygenic photosynthesis and is a gliding bacterium; in contrast, *Herpetosiphon* is a nonphotosynthetic, respiratory gliding bacterium. Both genera have unusual peptidoglycans and lack lipopolysaccharides in their outer membranes.
5. **Phylum *Cyanobacteria***. The oxygenic photosynthetic bacteria are placed in the phylum *Cyanobacteria,* which contains the class *Cyanobacteria* and five subsections. Cyanobacteria have chlorophyll *a* and almost all species possess phycobilins. These bacteria can be unicellular or filamentous, either branched or unbranched. Thecyanobacteria in the subsections differ from each other in general morphological characteristics and reproduction. Cyanobacteria incorporate CO2 photosynthetically through use of the Calvin cycle just like plants and many purple photosynthetic bacteria.
6. **Phylum *Chlorobi***. The phylum *Chlorobi* contains anoxygenic photosynthetic bacteria known as the green sulfur bacteria. They can incorporate CO2 through the reductive tricarboxylic acid cycle rather than the Calvin cycle and oxidize sulfide to sulfur granules, which accumulate outside the cell.

Volume 2 of the second edition is devoted completely to the gram-negative proteobacteria, often called the purple bacteria. The phylum *Proteobacteria* is a large and extremely complex group that currently contains over 1,300 species in 384 genera. Even though they are all related, the group is quite diverse in morphology, physiology, and life-style. All major nutritional typesare represented: phototrophy, heterotrophy, and chemolithotrophy of several varieties. Many species important in medicine, industry, and biological research are proteobacteria. Obvious examples are the genera *Escherichia, Neisseria, Pseudomonas, Rhizobium, Rickettsia, Salmonella,* and *Vibrio.* The phylum is divided into five classes based on

rRNA data. Because photosyntheticbacteria are found in the _, , and _ classes of the proteobacteria, many believe that the whole phylum arose from a photosynthetic ancestor. Presumably many strains lost photosynthesis when adapting metabolically to new ecological niches.

1. **Class I – *Alphaproteobacteria:*** The _-proteobacteria include most of the oligotrophic forms (those capable of growing at low nutrient levels). *Rhodospirillum* and other purple nonsulfur bacteria are photosynthetic. Some genera have unusual metabolic modes: methylotrophy (e.g., *Methylobacterium*), chemolithotrophy (*Nitrobacter*), and nitrogen fixation (*Rhizobium*). *Rickettsia* and *Brucella* are important pathogens. About half of the microbes in this group have distinctive morphology such as prosthecae (*Caulobacter*, *Hyphomicrobium*).
2. **Class II** – ***Betaproteobacteria****:* The -proteobacteria overlap the _ subdivision metabolically. However the proteobacteria tend to use substances that diffuse from organic decomposition in the anaerobic zone of habitats. Some of these bacteria use such substances as hydrogen (*Alcaligenes*), ammonia (*Nitrosomonas*), methane (*Methylobacillus*), or volatile fatty acids (*Burkholderia*).
3. **Class III** – ***Gammaproteobacteria:*** The _-proteobacteria compose a large and complex group of thirteen orders and 20 families. They often are chemoorganotrophic, facultatively anaerobic, and fermentative. However, there is considerable diversity among the _-proteobacteria with respect to energy metabolism. Some important families such as *Enterobacteriaceae, Vibrionaceae,* and *Pasteurellaceae* use the Embden-Meyerhof pathway and the pentose phosphate pathway. Others such as the *Pseudomonadaceae* and *Azotobacteriaceae* are aerobes and have the Entner-Doudoroff and pentose phosphate pathways. A few are photosynthetic (e.g., *Chromatium* and *Ectothiorhodospira*), methylotrophic (*Methylococcus*), or sulfur-oxidizing (*Beggiatoa*).
4. **Class IV** – ***Deltaproteobacteria:*** The -proteobacteria contain seven orders, and 17 families. Many of these bacteria can be placed in one of three groups. Some are predators on other bacteria as the class name implies (e.g., *Bdellovibrio*). The order *Myxococcales* contains the fruiting myxobacteria such as *Myxococcus, Stigmatella,* and *Polyangium.* The myxobacteria often also prey on other bacteria. Finally, the class has a variety of

anaerobes that generate sulfide from sulfate and sulfur while oxidizing organic nutrients (*Desulfovibrio*).

5. **Class V** – ***Epsilonproteobacteria:*** This section is composed of only one order, *Campylobacterales,* and two families. Despite its small size two important pathogenic genera are _-proteobacteria: *Campylobacter* and *Helicobacter.*

Volume 3 of *Bergey's Manual* surveys the gram-positive bacteria with low G + C content in their DNA, which are members of the phylum *Firmicutes.* The dividing line is about 50% G _C; bacteria with a mol% lower than this value are in volume 3. Most of these bacteria are gram positive and heterotrophic. However because of their close relationship to low G + C gram-positive bacteria, the mycoplasmas are placed here even though they lack cell walls and stain gram negative. There is considerable variation in morphology: some are rods, others are cocci, and mycoplasmas are pleomorphic. Endospores may be present. The phylum contains three classes.

1. **Class I – *Clostridia*:** This class contains three orders and 11 families. Although they vary in morphology and size, the members tend to be anaerobic. Genera such as *Clostridium, Desulfotomaculum,* and *Sporohalobacter* form truebacterial endospores; many others do not. *Clostridium* is one of the largest bacterial genera.
2. **Class II – *Mollicutes:*** The class *Mollicutes* contains five orders and six families. Members of the class often are called mycoplasmas. These bacteria lack cell walls and cannot make peptidoglycan or its precursors. Because mycoplasmas are bounded by the plasma membrane, they are pleomorphic and vary in shape from cocci to helical or branched filaments. They are normally nonmotile and stain gram negative because of the absence of a cell wall. In contrast with almost all other bacteria, most species require sterols for growth. The genera *Mycoplasma* and *Spiroplasma* contain several important animal and plant pathogens.
3. **Class III – *Bacilli*:** This large class comprises a wide variety of gram positive, aerobic or facultatively anaerobic, rods and cocci. The class Bacilli has two orders, *Bacillales* and *Lactobacillales,* and 16 families. As with the members of the class Clostridia, some genera (e.g., *Bacillus, Sporosarcina, Paenibacillus,* and *Sporolactobacillus*) form true

endospores. The section contains many medically and industrially important genera: *Bacillus, Lactobacillus, treptococcus, Lactococcus, Enterococcus, Listeria,* and *Staphylococcus.*

Volume 4 is devoted to the high G _C gram positives, those bacteria with mol% values above 50 to 55%. All bacteria in this volume are placed in the phylum *Actinobacteria* and class *Actinobacteria.* There is enormous morphological variety among these procaryotes. Some are cocci, others are regular or irregular rods. High G _C gram positives called actinomycetes often form complex branching hyphae. Although none of these bacteria produce true endospores, many genera do form a variety of asexual spores and some have complex life cycles. There is considerable variety in cell wall chemistry among the high G + C gram positives. For example, the composition of peptidoglycan varies greatly. Mycobacteria produce large mycolic acids that distinguish their walls from those of other bacteria. The taxonomy of these bacteria is very complex. There are five subclasses, six orders, 14 suborders, and 40 families. Genera such as *Actinomyces, Arthrobacter, Corynebacterium, Micrococcus, Mycobacterium,* and *Propionibacterium* were placed in volume 2 of the first edition. They are now in the new volume 4 within the suborders *Actinomycineae, Micrococcineae, Corynebacterineae,* and *Propionibacterineae* because rRNA studies have shown them to be actinobacteria. The largest and most complex genus is *Streptomyces,* which contains over 500 species.

Volume 5 describes an assortment of nine phyla that are located here for convenience. The inclusion of these groups in volume 5 does not imply that they are directly related. Although they are all gram-negative bacteria, there is considerable variation in morphology, physiology, and life cycle pattern. Several genera are of considerable biological or medical importance. We will briefly consider four of the nine phyla.

1. **Phylum *Planctomycetes*:** The planctomycetes are related to the chlamydias according to their rRNA sequences. The phylum contains only one order, one family, and four genera. Planctomycetes are coccoid to ovoid or pearshaped cells that lack peptidoglycan. Some have a membrane-enclosed nucleoid. Although they are normally unicellular, the genus *Isosphaera* will form chains. They divide by budding and may produce nonprosthecate

appendages called stalks. Planctomycetes grow in aquatic habitats, and many move by flagella or gliding motility.

2. Phylum *Chlamydiae:* This small phylum contains one class, one order, and four families. The genus *Chlamydia* is by far the most important genus. *Chlamydia* is an obligately intracellular parasite with a unique life cycle involving two distinctive stages: elementary bodies and reticulate bodies. These bacteria do resemble planctomycetes in lacking peptidoglycan. They are small coccoid organisms with no appendages. Chlamydias are important pathogens and cause many human diseases.
3. **Phylum *Spirochaetes:*** This phylum contains helically shaped, motile, gram-negative bacteria characterized by a unique morphology and motility mechanism. The exterior boundary is a special outer membrane that surrounds the protoplasmic cylinder, which contains the cytoplasm and nucleoid. Periplasmic flagella lie between the protoplasmic cylinder and the outer membrane. The flagella rotate and move the cell even though they do not directly contact the external environment. These chemoheterotrophs can be free living, symbiotic, or parasitic. For example, the genera *Treponema* and *Borrelia* contain several important human pathogens. The phylum has one class, *Spirochaetes,* three families, and 13 genera.
4. **Phylum *Bacteroidetes:*** This phylum has three classes (*Bacteroides, Flavobacteria,* and *Sphingobacteria*), three orders, and 12 families. Some of the better-known gener are *Bacteroides, Flavobacterium, Flexibacter,* and *Cytophaga.* The gliding bacteria *Flexibacter* and *Cytophaga* are ecologically significant and will be discussed later.

The classification in the first and second editions of *Bergey's Manual* are so different that two appendixes are provided to help in the transition. Appendix III gives the classification of prokaryotes according to the first edition. Appendix IV describes the classification system the second edition of *Bergey's Manual* will employ. Finally, it must be emphasized that procaryotic nomenclature is as much in flux as classification. The names of families and genera are fairly well established and stable in the new system (at least in the absence of future discoveries); in fact, many family and genus names remain unchanged in the second edition of *Bergey's Manual.* In contrast, the names of orders and higher taxa are not always completely settled in the second edition.

The phylogenetic tree and prokaryotic species lists provide a current view of Bergey's phylogenetic procaryotic classification. Because the names of kingdoms, classes, and orders are still changing, their use will be kept to the minimum necessary for consistency with the use of taxa by the first edition, ease of communication, and student comprehension. Some higher taxonomic names may well change in the next few years, but we will still employ them here for the above reasons.

ALGAE:

The term algae means different things to different people, and even the professional botanist and biologist find algae embarrassingly elusive of definition.Thus, laymen have given such names as "pond scums,""frog spittle,""water mosses," and "seaweeds," while some professionals shrink from defining them.—Harold C. Bold and Michael J.Wynne

This unit presents some general features of algae. Becauseb18S rRNA analysis has shown that these organisms arose independently on different occasions; the algae do not represent a monophyletic group. Accordingly, the taxa "algae" should not be used in molecular taxonomy schemes. The term algae can still be used (as it is in this chapter) to denote a group of related eucaryotic organisms that share some morphological, reproductive, ecological, and biochemical characteristics.

Phycology or **algology**

It is the study of algae. The word phycology is derived from the Greek *phykos,* meaning seaweed. The term **algae** [s., alga] were originally used to define simple "aquatic plants." As noted above, it no longer has any formal significance in classification schemes. Instead the algae can be described as eucaryotic organisms that have chlorophyll *a* and carry out oxygen-producing photosynthesis. They differ from other photosynthetic eucaryotes in lacking a well-organized vascular conducting system and in having very simple reproductive structures. In sexual reproduction the whole organism may serve as a gamete; unicellular structures (gametangia) may produce gametes; or gametes can be formed by multicellular gametangia in which every cell is fertile. Unlike the case with plants, algal gametangia do not have nonfertile cells.

Distribution of Algae

Algae most commonly occur in water (fresh, marine, or brackish) in which they may be suspended (**planktonic**) or attached and living on the bottom (**benthic**). A few algae live at the water-atmosphere interface and are termed **neustonic. Plankton** [Greek *plankos,* wandering] consists of free-floating, mostly microscopic aquatic organisms. **Phytoplankton** is made up of algae and small plants, whereas **zooplankton** consists of animals and nonphotosynthetic protists. Some algae grow on moist rocks, wood, trees, and on the surface of moist soil. Algae also live as endosymbionts in various protozoa, mollusks, worms, and corals. Several algae grow as endosymbionts within plants, some are attached to the surface of various structures, and a few lead a parasitic existence. Algae also associate with fungi to form lichens.

Classification of Algae

According to the five-kingdom system of Whittaker, the algae belong to seven divisions distributed between two different kingdoms (**table 3.1**). This classical classification is based on cellular, not organismal, properties. Some more important propertiesinclude:

(1) Cell wall (if present) chemistry and morphology;
(2) Form in which food or assimilatory products of photosynthesis are stored;
(3) Chlorophyll molecules and accessory pigments that contribute to photosynthesis;
(4) Flagella number and the location of their insertion in motile cells;
(5) Morphology of the cells and/or body (thallus);
(6) Habitat;
(7) Reproductive structures; and
(8) Life history patterns.

Based on these properties the algae are arranged by divisions in **table 3.2,** which summarizes their more significant characteristics.

Table 3.1 Classical Classification of Algaea

Division (Common Name)	Kingdom
Chrysophyta (yellow-green and golden-brown algae; diatoms)	*Protista* (single cell or colonial; eucaryotic)
Euglenophyta (photosynthetic euglenoid flagellates)	*Protista*
Pyrrhophyta (dinoflagellates)	*Protista*
Charophyta (stoneworts)	*Protista*
Chlorophyta (green algae)	*Protista*
Phaeophyta (brown algae)	*Plantae* (multicellular; eucaryotic)
Rhodophyta (red algae)	*Plantae*

[a]Five-kingdom system.

Molecular systems have placed some of the classical algae with plants (green algae); some as a separate lineage (red algae); some with the stramenopiles (goldenbrown and yellow-green algae, brown algae, and diatoms); some with the alveolates (dinoflagellates); and still others with some protozoa (euglenoids). Two of these groups, the alveolates and stramenopiles, have been created recently as a result of rRNA comparisons and ultrastructural studies. The alveolates have mitochondria with tubular cristae and subsurface alveoli or sacs that abut the surface. Dinoflagellates, ciliate protozoa, and the apicomplexan protozoa are alvolates . The stramenopiles have mitochondria with tubular cristae and hollow hairs that give rise to a small number of fine hairs (tripartite tubular hairs). These hairs are usually on their flagella. Photosynthetic forms often have chlorophylls a and c. Some common stramenopiles are the opalinid protozoa, oomycetes, diatoms, brown algae or phaeophytes, chrysophytes, and xanthophytes. Although a few groups such as the diatoms have lost their hairs, they are still considered stramenopiles based on the rRNA data, mitochondrial characteristics, and other properties.

Table 3.2 Comparative Summary of Some Algal Characteristics

Division	Approximate Number of Species	Common Name and Representative	Pigments: Chlorophylls	Pigments: Phycobilins (Phycobiliproteins)	Pigments: Carotenoids	Thylakoids per Stack in Chloroplast
Chlorophyta	7,500	Green algae *(Chlamydomonas)*	*a, b*	–	β-carotene, ± α-carotene, xanthophylls	3–6
Charophyta	250	Stoneworts or brittleworts *(Chara)*	*a, b*	–	α-, β-, τ-carotene, xanthophylls	Many
Euglenophyta	700	Euglenoids *(Euglena)*	*a, b*	–	β-carotene, xanthophylls, ± τ-carotene	3
Chrysophyta	6,000	Golden-brown, yellow-green algae; diatoms, *(Cyclotella)*	*a*, c_1/c_2, rarely *d*	–	α-, ß-, ε-carotene, fucoxanthin, xanthophylls	3
Phaeophyta	1,500	Brown algae *(Sargassum)*	*a, c*	–	β-carotene, fucoxanthin, xanthophylls	3
Rhodophyta	3,900	Red algae *(Corallina)*	*a*, rarely *d*	C-phycocyanin, allophycocyanin, phycoerythrin	Xanthophylls (β-carotene, zeaxanthine, ± α-carotene)	1
Pyrrhophyta	1,100	Dinoflagellates *(Gymnodinium)*	*a*, c_1, c_2	–	β-carotene, fucoxanthin, peridinin, dinoxanthin	3

[a] Refers specifically to the vegetative cells. Spores, akinetes, zygotes contain waxes, nonsaponifiable polymers, and phenolic substances.
[b] The following abbreviations are used: fresh water (fw), brackish water (bw), salt water (sw), terrestrial (t).

Ultrastructure of the Algal Cell

The eucaryotic algal cell (**figure 3.1**) is surrounded by a thin, rigid cell wall. Some algae have an outer matrix lying outside the cell wall. This usually is flexible and gelatinous, similar to bacterial capsules. When present, the flagella are the locomotor organelles. The nucleus has a typical nuclear envelope with pores; within the nucleus are a nucleolus, chromatin, and karyolymph.

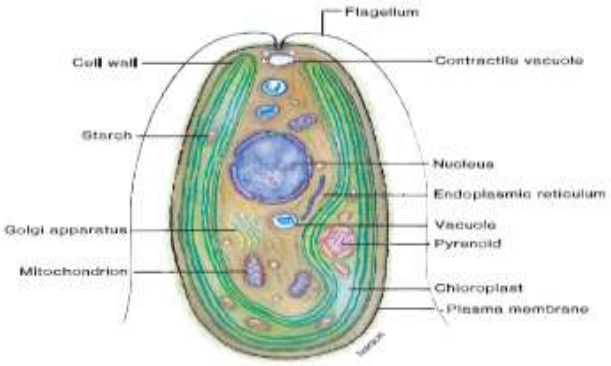


Figure 3.1 Algal Morphology. Schematic drawing of a typical eucaryotic algal cell showing some of its organelles and other structures.

The chloroplasts have membrane- bound sacs called thylakoids that carry out the light reactions of photosynthesis. These organelles are embedded in the stroma where the dark reactions of carbon dioxide fixation take place. A dense proteinaceous area, the **pyrenoid** that is associated with synthesis and storage of starch may be present in the chloroplasts. Mitochondrial structure varies greatly in the algae. Some algae (euglenoids) have discoid cristae; some, lamellar cristae (green and red algae); and the remaining, (golden-brown and yellow- green, brown, and diatoms) have tubular cristae.

Algal Nutrition

Algae can be either autotrophic or heterotrophic. Most are photoautotrophic; they require only light and CO2 as their principal source of energy and carbon. Chemoheterotrophic algae require external organic compounds as carbon and energy sources.

Structure of the Algal Thallus (Vegetative Form)

The vegetative body of algae is called the **thallus** [pl., thalli]. It varies from the relative simplicity of a single cell to the more striking complexity of multicellular forms, such as the giant kelps. Single-celled algae may be as small as bacteria, whereas kelp can attain a size over

75 m in length. Algae are unicellular (**figure 3.2*a,b,g***), colonial (figure 3.2*c*), filamentous (figure 3.2*d*), membranous and bladelike (figure 3.2*e*), or tubular (figure 3.2*f*).

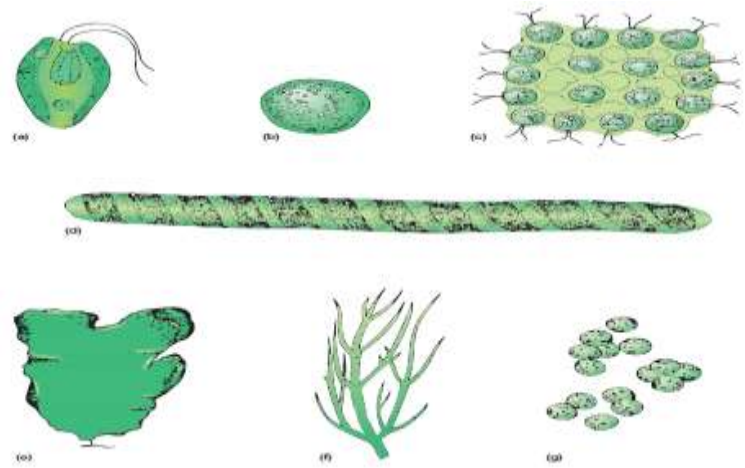


Figure 3.2 Diagrammatic Algal Bodies: (**a**) unicellular, motile, *Cryptomonas;* (**b**) unicellular, nonmotile, *Palmellopsis;* (**c**) colonial, *Gonium;* (**d**) filamentous, *Spirotaenia;* (**e**) bladelike kelp, *Monostroma;* (**f**) leafytubular axis, branched tufts or plumes, *Stigeoclonium;* (**g**) unicellular, nonmotile, *Chrysocapsa.*

Algal Reproduction

Some unicellular algae reproduce asexually. In this kind of reproduction, gametes do not fuse to form a zygote. There are three basic types of asexual reproduction: fragmentation, spores, and binary fission. In **fragmentation** the thallus breaks up and each fragmented part grows to form a new thallus. **Spores** can be formed in ordinary vegetative cells or in specialized structures termed sporangia [s., sporangium; Greek *spora,* seed, and *angeion,* vessel]. Flagellated motile spores are called **zoospores.** Nonmotile spores produced by sporangia are termed **aplanospores.** In some unicellular algae **binary fission** occurs (nuclear division followed by division of the cytoplasm). Other algae reproduce sexually. Eggs are formed within relatively unmodified vegetative cells called **oogonia** [s., oogonium] that function as female structures. Sperm are produced in special male reproductive structures called **antheridia** [s., antheridium]. In sexual reproduction these gametes fuse to produce a diploid **zygote.**

CHLAMYDOMONAS (Green Algae)

The *Chlorophyta* or green algae [Greek *chloros,* green] are an extremely varied division. They grow in fresh and salt water, in soil, on other organisms, and within other organisms. The *Chlorophyta* have chlorophylls a and b along with specific carotenoids, and they store carbohydrates as starch. Many have cell walls of cellulose. They exhibit a wide diversity of body forms, ranging from unicellular to colonial, filamentous, membranous or sheetlike, and tubular types (**figure 3.3**). Some species have a holdfast structure that anchors them to the substratum. Both asexual and sexual reproduction occurs in green algae. In molecular classification schemes, green algae are associated with the land plants and have mitochondria with lamellar cristae.

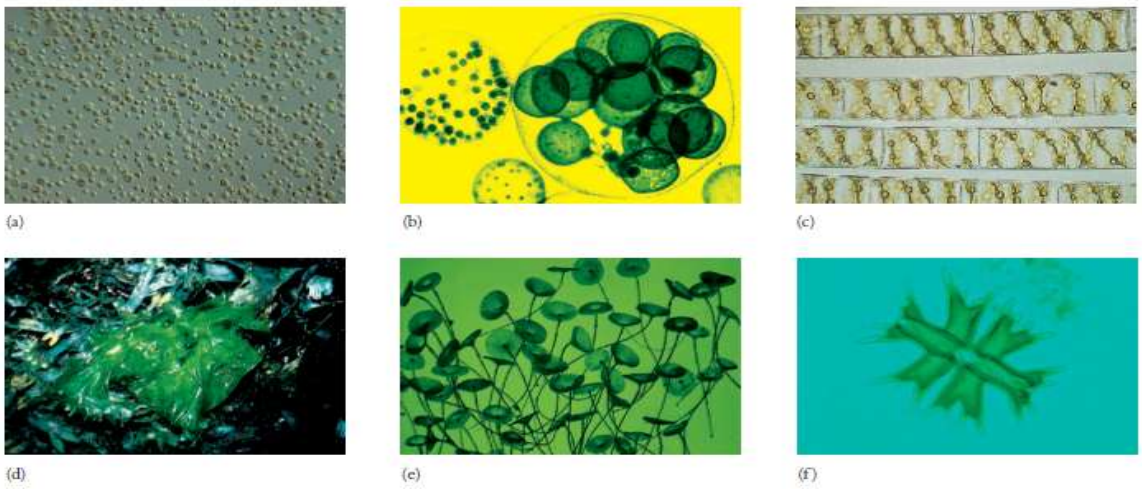


Figure 3.3 ***Chlorophyta*** **(Green Algae); Light Micrographs. (a)** *Chlorella,* a unicellular nonmotile green alga (_160). (**b**) *Volvox,* a typical green algal colony (_450). (**c**) *Spirogyra,* a filamentous green alga (_100). Four filaments are shown. Note the ribbonlike, spiral chloroplasts within each filament. (**d**) *Ulva,* commonly called sea lettuce, has a leafy appearance. (**e**) *Acetabularia,* the mermaid's wine goblet. (**f**) *Micrasterias,* a large desmid (_150).

Chlamydomonas is a representative unicellular green alga (**figure3.4**). Individuals have two flagella of equal length at the anterior end by which they move rapidly in water. Each cell has a single haploid nucleus, a large chloroplast, a conspicuous pyrenoid, and a **stigma (eyespot**) that aids the cell in phototactic responses. Two small contractile vacuoles at the base of the flagella

function as osmoregulatory organelles that continuously remove water. *Chlamydomonas* reproduces asexually by producing zoospores through cell division. The alga also reproduces sexually when some products of cell division act as gametes and fuse to form a four flagellated diploid zygote that ultimately loses its flagella and enters a resting phase. Meiosis occurs at the end of this resting phase and produces four haploid cells that give rise to adults. From 0rganisms like *Chlamydomonas,* several distinct lines of evolutionary specialization have evolved in the green algae. The first line contains nonmotile unicellular green algae, such as *Chlorella. Chlorella* (figure 26.3*a*) is widespread both in fresh and salt water and also in soil. It only reproduces asexually and lacks flagella, eyespots, and contractile vacuoles; the nucleus is very small. Motile, colonial organisms such as *Volvox* represent a second major line of evolutionary specialization.

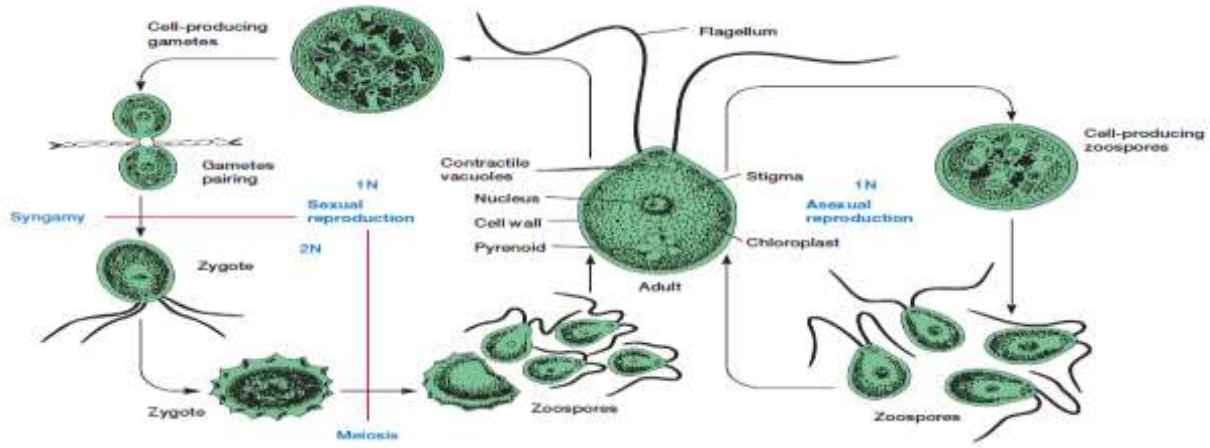


Figure 3.4 ***Chlamydomonas:*** **The Structure and Life Cycle of This Motile Green Alga.** During asexual reproduction, all structures are haploid; during sexual reproduction, only the zygote is diploid.

A *Volvox* colony (figure26.3*b; see also figure 2.8*b) is a hollow sphere made up of a single layer of 500 to 60,000 individual cells, each containing two flagella and resembling a *Chlamydomonas* cell. The flagella of all the cells beat in a coordinated way to rotate the colony in a clockwise direction as it moves through the water. Only a few cells are reproductive, and these are located at the posterior end of the colony. Some divide asexually and produce new colonies. Others produce gametes. After fertilization, the zygote divides to form a daughter colony. In both cases

the daughter colonies stay within the parental colony until it ruptures. A green alga, *Prototheca moriformis*, causes the disease **protothecosis** in humans and animals. *Prototheca* cells are fairly common in the soil, and it is from this site that most infections occur. Severe systemic infections, such as massive invasion of the bloodstream, have been reported in animals. More common in humans is the subcutaneous type of infection. It starts as a small lesion and spreads slowly through the lymph glands, covering large areas of the body.

Volvox

Volvox is a type of green algae or chlorophyte. Amongst the genera of green algae that form spherical colonies, volvox are the most developed. Their colonies contain flagellate cells that can be 50,000 in number and are embedded in coenobium or hollow muscilagenous spheres. These hollow spheres are formed of extracellular matrix which contains gelatinous glycoprotein. The cytoplasmic strands of volvox which connect the cells together make it greenish in color. Freshwater ditches, ponds, and shallow puddles are the places where these organisms thrive. The different volvox characteristics and facts makes this organism a model for studying fundamental questions pertaining to evolution. Let us delve into different volvox facts through the information presented in this write-up.

Characteristics

- The volvox grows well in eutrophic water bodies. Lakes that are rich in nutrients allow a prolific and healthy growth of this algae.
- Every single ovoid or spherical cell in volvox colonies possess two flagella. A pair of contractile vacuoles, along with single cup-shaped chloroplasts, are present at the base of these flagella.
- The cells of volvox can be single or biflagellate.
- Individual algal cells of volvox are attached to each other by means of cytoplasmic strands. These individual cells are characterized by the presence of red eye spots on their surface.
- Flagellar movement of cells present in volvox colonies is used for swimming (rolling motion) and also in changing the direction.

- The muscilage produced by every individual cell in the colony can be distinct or inconspicuous.
- In a particular colony of volvox, cells at the anterior possess phototactic abilities, which are attributed to larger eyespots.
- Posterior cells of the volvox colonies are more into other functions, like reproduction.
- Volvox is a polyphyletic organism, which means that it has multiple ancestral lineages. Different species of volvox have evolved from at least four different ancestral lineages.
- The size of volvox colonies can range from 100-6000 microns.
- Most of the volvox species are microscopic organisms, and therefore we cannot see them with naked eyes. However, few colonies are as big as 1 mm in diameter, making them easily visible.
- In the different stages of development of volvox, one can get to see their daughter cells and in few cases, even grand-daughter cells.
- One can find parasites feeding on cells of volvox in some colonies. A rotifer called Proales parasita thrives by feeding on cells of volvox.

Reproduction

The volvox regenerates both by sexual and asexual reproduction. The asexual cells, called gonidia, present at the posterior of volvox colonies contribute in asexual reproduction through repeated division. Growth of the gonads (daughter cells) takes place from cells that are present around the equator of the colony. The somatic/vegetative cells do not divide and are therefore unable to take part in reproduction. Sexual reproduction takes place by the fusion of sperms and egg cells. It is necessary to note that volvox can be monoecious or dioecious. The fertilization of male and female gametes in sexual reproduction leads to the formation of zygotes. The species called Volvox aureus has a smooth surface. Volvox globator, on the other hand, has zygotes that are star-shaped.

The different characteristics of volvox presented in the article should help in understanding more about these wonderful aquatic creatures. Their highly organized structure and way of functioning makes volvox an interesting topic of study.

***Rhodophyta* (Red Algae)**

The division Rhodophyta, the red algae [Greek *rhodon,* rose], includes most of the seaweeds (**figure 3.5**). A few reds are unicellular but most are filamentous and multicellular. Some red algae are up to 1 m long. The stored food is the carbohydrate called florideanstarch (composed of _-1,4 and _-1,6 linked glucose residues). The red algae contain the red pigment phycoerythrin, one of the two types of phycobilins that they possess. The other accessory pigment is the blue pigment phycocyanin. The presence of these pigments explains how the red algae can live at depths of 100 m or more. The wavelengths of light (green, violet, and blue) that penetrate these depths are not absorbed by chlorophyll *a* but instead by these phycobilins. Not surprisingly the concentrations of these pigments often increases with depth as light intensity decreases. The phycobilins, after absorbing the light energy, pass it on to chlorophyll *a.* The algae appear decidedly red when phycoerythrin predominates over the other pigments. When phycoerythrin un- dergoes photodestruction in bright light, other pigments predominate and the algae take on shades of blue, brown, and dark green. The cell walls of most red algae include a rigid inner part composed of microfibrils and a mucilaginous matrix. The matrix is composed of sulfated polymers of galactose called agar, funori, porphysan, and carrageenan. These four polymers give the red algae their flexible, slippery texture. Agar is used extensively in the laboratory as a culture medium component. Many red algae also deposit calcium carbonate in their cell walls and play an important role in building coral reefs.

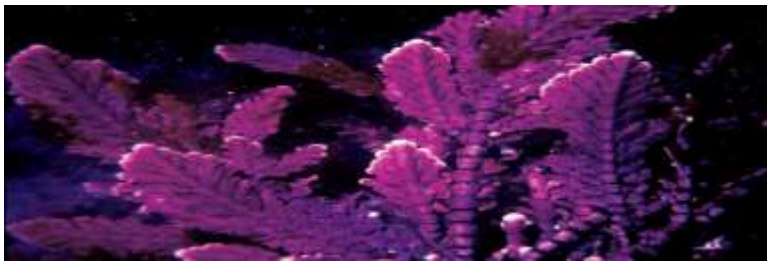


Figure 3.5 ***Rhodophyta* (Red Algae).** These algae (e.g., *Corallina gracilis*) are much smaller and more delicate than the brown algae. Most red algae have a filamentous, branched morphology as seen here.

***Chrysophyta* (Golden-Brown and Yellow-Green Algae; Diatoms)**

The division *Chrysophyta* is quite diversified with respect to pigment composition, cell wall, and type of flagellated cells. In molecular classification schemes, these algae are associated with the stramenopiles and have mitochondria with tubular cristae. The division is divided into three major classes: golden-brown algae [Greek *chrysos,* gold], yellow-green algae, and diatoms. The major photosynthetic pigments are usually chlorophylls *a* and *c*1/*c*2, and the carotenoid fucoxanthin. When fucoxanthin is the dominant pigment, the cells have agolden-brown color. The major carbohydrate reserve in the *Chrysophyta* is **chrysolaminarin**. Some *Chrysophyta* lack cell walls; others have intricately patterned coverings external to the plasma membrane, such as **scales (figure 3.6*a*),** walls, and plates. Diatoms have a distinctivetwo-piece wall of silica, called a **frustule.** Two anteriorly attached flagella of unequal length are common among *Chrysophyta*(figure3.5*b*), but some species have no flagella, andothers have either one flagellum or two that are of equal length. Most *Chrysophyta* are unicellular or colonial. Reproduction usually is asexual but occasionally sexual. Although some marine forms are known, most of the yellow-green and golden-brown algae live in fresh water. Blooms of some species produce unpleasant odors and tastes in drinking water. The **diatoms** (figure 3.6*c,d*) are photosynthetic, circular or oblong chrysophyte cells with frustules composed of two halves or thecae that overlap like a petri dish [therefore their name is from the Greek *diatomsos,* cut in two]. The larger half is the **epitheca,** and the smaller half is the **hypotheca.** Diatoms grow in freshwater, salt water, and moist soil and comprise a large part of the phytoplankton. The chloroplasts of these chrysophytes contain chlorophylls *a* and *c* as well as carotenoids. Some diatoms are facultative heterotrophs and can absorb carbon containing molecules through the holes in their walls. The vegetative cells of diatoms are diploid; exist as unicellular, colonial, or filamentous shapes; lack flagella; and have a single large nucleus and smaller plastids. Reproduction consists of the organism dividing asexually, with each half then constructing a new theca within the old one. Because of this mode of reproduction, diatoms get smaller with each reproductive cycle. However, when they diminish to about 30% of their original size, sexual reproduction usually occurs. The diploid vegetative cells undergo meiosis to form gametes, which then fuse to produce a zygote. The zygote develops into and auxospore, which increases in size again and forms a new wall. The mature auxospore eventually divides mitotically to produce vegetative cells with normal frustules. Diatom frustules are composed of crystallized silica [Si(OH)4] with very fine markings (figure

3.6*c,d*). They have distinctive, and often exceptionally beautiful, patterns that are different for eachspecies. Frustule morphology is very useful in diatom identification.

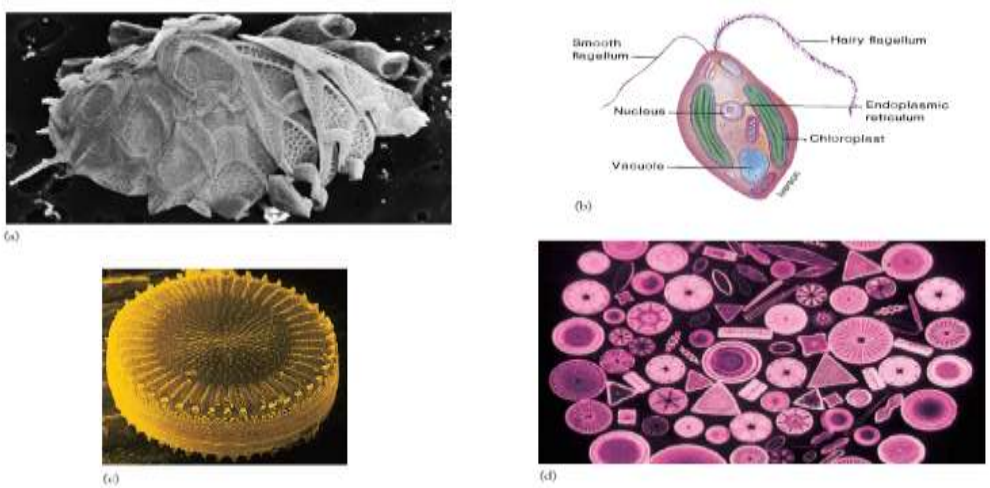


Figure 3.6 ***Chrysophyta*** **(Yellow-Green and Golden-Brown Algae; Diatoms). (a**) Scanning electron micrograph of *Mallomonas,* a chrysophyte, showing its silica scales. The scales are embedded in the pectin wall but synthesized within the Golgi apparatus and transported to the cell surface in vesicles (_9,000). (**b**) *Ochromonas,* a unicellular chrysophyte. Diagram showing typical cell structure. (**c**) Scanning electron micrograph of a diatom, *Cyclotella meneghiniana* (_750). (**d**) Assorted diatoms as arranged by a light microscopist (_900).

Phaeophyta **(Brown Algae)**

The *Phaeophyta* or brown algae [Greek *phaeo,* brown] consist of multicellular organisms that occur almost exclusively in the sea. Some species have the largest linear dimensions (length) known in the eucaryotic world (chapter opening figure). Since the brown algae have tubular cristae, they are associated with stramenopiles in molecular classification schemes. Most of the conspicuous seaweeds that are brown to olive green in color are assigned to this division. The simplest brown algae consist of small openly branched filaments; the larger, more advanced species have a complex arrangement. Some large **kelps** are conspicuously differentiated into flattened blades, stalks, and holdfast organs that anchor them to rocks (**figure 3.7**). Some, such as *Sargassum,* form huge floating masses that dominate the Sargasso Sea. The color of these algae reflects the presence of the brown pigment fucoxanthin, in addition to chlorophylls *a* and *c,* _-

carotene, and violaxanthin. The main storage product is **laminarin,** which is quite similar in structure to chrysolaminarin.

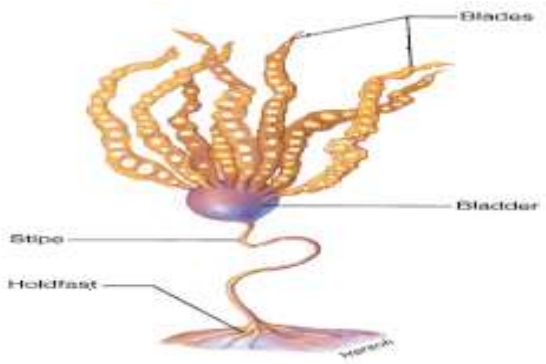


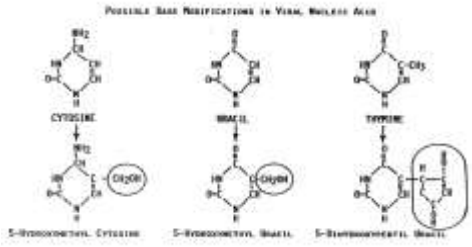
Figure 3.7 ***Phaeophyta*** **(Brown Algae).**

General Properties of Viruses

1. Viruses contain either DNA or RNA as their genetic material, but not both. This nucleic acid usually has unique chemical and/or physical features which makes it distinguishable from human nucleic acid.
2. Viral nucleic acid is enclosed in a capsid made up of protein subunits called protomeres.
3. Some species of viruses have a membrane, the envelope, surrounding the capsid; other species do not have an envelope, i.e., they are naked. Enveloped viruses have glyco-protein spikes arising from their envelope. These spikes have enzymatic, absorptive, hemagglutinating and/or antigenic activity.
4. The morphology of a virus is determined by the arrangement of the protomeres. When protomeres aggregate into units of five or six (capsomeres) and then condense to form a geometric figure having 20 equal triangular faces and 12 apices, the virus is said to have icosahedral (cubic) morphology. When protomeres aggregate to form a capped tube, they are said to have helical morphology. Any other arrangement of the protomeres results in a complex morphology.
5. All viruses undergo a replication cycle in their human host cell consisting of adsorption, penetration, uncoating, nucleic acid replication, maturation and release stages.

6. During the viral replication cycle, an accumulation of mature viruses, incomplete viruses and viral parts occurs within the cell.This accumulation is the inclusion body. The size, shape, location and chemical properties of the inclusion body are used by the pathologist to diagnose viral infectious disease.
7. A virally-infected cell generally presents three signals that it is infected. The first is the production of double-stranded RNA, which induces interferon; the second is the expression of viral protein on the surface of the plasma membrane, thus causing activation of cytotoxic T-cells, natural killer cells and sometimes induction of antibody synthesis. The third is the formation of an inclusion body either within the cytoplasm or the nucleus or very rarely within both the cytoplasm and nucleus.
8. In general, all DNA-containing viruses replicate in the host cell nucleus. The exceptions to the rule are the poxviruses.
9. In general, all RNA-containing viruses replicate in the host cell cytoplasm. The exceptions to the rule are the retroviruses and the orthomyxoviruses.

Structure



1. **Nucleic acid** -contains 3-400 genes

   Deoxyribonucleic Acid (DNA) -unique features

   a. Single and/or double stranded
   b. Glycosylated and/or methylated
   c. Gaps present in double stranded molecule
   d. Circular or linear
   e. Bound protein molecules
   f. Unique purine and/or pyrimidine bases present
   g. Ribonucleotides present

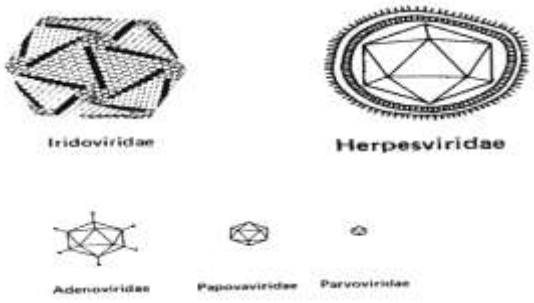
Ribonucleic Acid (RNA) - Unique features

h. Single or double stranded
i. Segmented or unsegmented
j. Bound protein molecules
k. Unique purine and/or pyrimidine bases present
l. Folding pattern

2. **Capsid**: The capsid accounts for most of the virion mass. It is the protein coat of the virus. It is a complex and highly organized entity which gives form to the virus. Subunits called protomeres aggregate to form capsomeres which in turn aggregate to form the capsid.
3. **Envelope**: this is an amorphous structure composed of lipid, protein and carbohydrate which lies to the outside of the capsid. It contains a mosaic of antigens from the host and the virus. A naked virus is one without an envelope.
4. **Spikes**: These are glycoprotein projections which have enzymatic and/or adsorption and/or hemagglutinating activity. They arise from the envelope and are highly antigenic.

Morphology (Symmetry)

Icosahedral: The protomeres aggregate in groups of five or six to form the capsomere. In electron micrographs, capsomeres are recognized as regularly spaced rings with a central hole. The shape and dimensions of the icosahedron depends on characteristics of its protomeres. All icosahedral capsids have 12 corners each occupied by a penton capsomere and 20 triangular faces, each containing the same number of hexon capsomeres. Icosahedral symmetry is identical to cubic symmetry.



Helical: The protomeres are not grouped in capsomeres, but are bound to each other so as to form a ribbon-like structure. This structure folds into a helix because the protomeres are thicker at one end than at the other. The diameter of the helical capsid is determined by characteristics of its protomeres, while its length is determined by the length of the nucleic acid it encloses.

Complex: e.g., that exhibited by poxvirus and rhabdovirus. This group comprises all those viruses which do not fit into either of the above two groups.

Replication Cycle

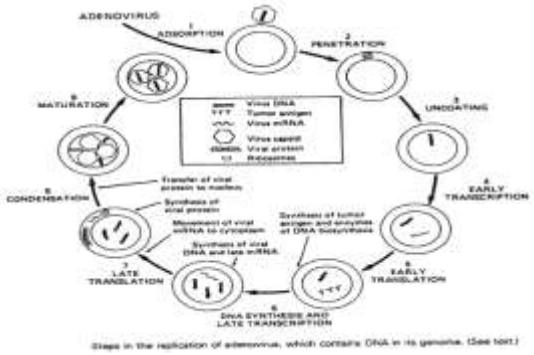
1. **Adsorption** -Viruses can enter cells via phagocytosis, viropexis or adsorption. Adsorption is the most common process and the most highly specific process. It requires the interaction of a unique protein on the surface of the virus with a highly specific receptor site on the surface of the cell.

2. **Penetration** -This occurs by one or more processes.

- Enveloped viruses fuse their envelope with the membrane of the host cell. This involves local digestion of the viral and cellular membranes, fusion of the membranes and concomitant release of the nucleocapsid into the cytoplasm.
- Naked viruses bind to receptor sites on the cellular membrane, digest the membrane and enter into the cytoplasm intact.
- Both naked and enveloped viruses can be ingested by phagocytic cells. However, in this process they enter the cytoplasm enclosed in a cytoplasmic membrane derived from the phagocytic cell.

3. **Uncoating** -During this stage cellular proteolytic enzymes digest the capsid away from the nucleic acid. This always occurs in the cytoplasm of the host cell. The period of the replication cycle between the end of the uncoating stage and maturation of new viral particles is termed the eclipse. Thus during the eclipse stage, no complete viral particles can be viewed within the cell.

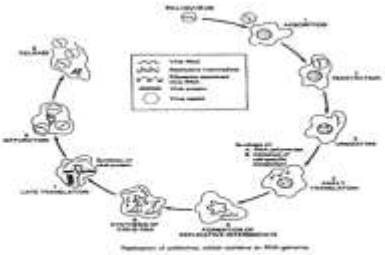
4. **Replication** of nucleic acid. Replication of viral nucleic acid is a complex and variable process. The specific process depends on the nucleic acid type.



NOTE: Symmetrical transcription of DNA gives rise to double-stranded RNA.

DNA virus replication -<u>with the exception of the poxviruses, all DNA viruses replicate in the nucleus</u>. In some cases one of the DNA strands is transcribed (in others both strands of a small part of the DNA may be transcribed) (step 4) into specific mRNA, which in turn is translated (step 5) to synthesize virus-specific proteins such as tumor antigen and enzymes necessary for biosynthesis of virus DNA. This period encompasses the early virus functions. Host cell DNA synthesis is temporarily elevated and is then suppressed as the cell shifts over to the manufacture of viral DNA (step 6). As the viral DNA continues to be transcribed, late virus functions become apparent. Messenger RNA transcribed during the later phase of infection (step 6) migrates to the cytoplasm and is translated (step 7). Proteins for virus capsids are synthesized and are transported to the nucleus to be incorporated into the complete virion (step 8).Assembly of the protein subunits around the viral DNA results in the formation of complete virions (step 9), which are released after cell lysis.The single-stranded DNA viruses first form a double stranded

DNA, utilizing a host DNA-dependent DNA polymerase. They then undergo a typical replication cycle.



RNA virus replication -with the exception of the orthomyxoviruses and retroviruses, all RNA viruses replicate in the cytoplasm of the host cell. The exact process varies with the species of virus. The single-stranded RNA that is released after uncoating will act as either: (a) the mRNA to synthesize viral-coded proteins; or (b) a template to synthesize mRNA; or (c) a template to synthesize double stranded RNA, which is then used as a template to synthesize mRNA; or (d) a template to synthesize double-stranded DNA, which is then utilized as a template to synthesize mRNA. This latter process occurs only with the retroviruses (oncornaviruses).

The replication of poliovirus, which contains a single-stranded RNA as its genome, provides a useful example. All of the steps are independent of host DNA and occur in the cell cytoplasm. Polioviruses absorb to cells at specific cell receptor sites (step 1), losing in the process one virus polypeptide. The sites are specific for virus coat-cell interactions. After attachment, the virus particles are taken into the cell by viropexis (similar to pinocytosis) (step 2), and the viral RNA is uncoated (step 3). The single-stranded RNA then serves as its own messenger RNA. This messenger RNA is translated (step 4), resulting in the formation of an RNA-dependent RNA polymerase that catalyzes the production of a replication intermediate (RI), a partially double-stranded molecule consisting of a complete RNA strand and numerous partially completed strands (step 5). At the same time, inhibitors of cellular RNA and protein synthesis are produced. Synthesis of (+) and (-) strands of RNA occurs by similar mechanisms. The RI consists of one complete (-) strand and many small pieces of newly synthesized (+) strand RNA (step 6). The replicative form (RF) consists of two complete RNA strands, one (+) and one (-).

The single (+) strand RNA is made in large amounts and may perform any one of three functions: (a) serve as messenger RNA for synthesis of structural proteins; b) serve as template for continued RNA replication; or (c) become encapsulated, resulting in mature progeny virions. The synthesis of viral capsid proteins (step 7) is initiated at about the same time as RNA synthesis.

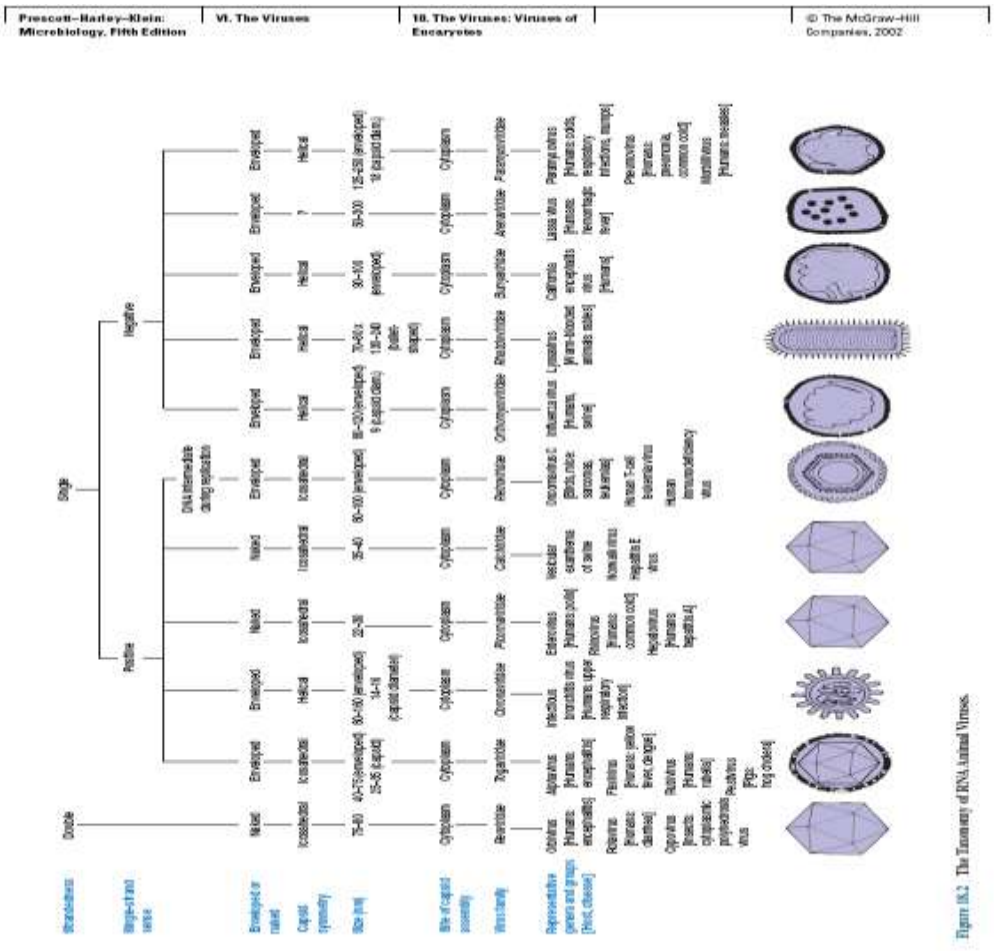
The entire poliovirus genome acts as its own mRNA, forming a polysome of approximately 350S, and is translated to form a single large polypeptide that is subsequently cleaved to produce the various viral capsid polypeptides. Thus, the poliovirus genome serves as a polycistronic messenger molecule. Poliovirus contains four polypeptides.

5. **Maturation and Release**

- **Naked viruses** -Maturation consists of two main processes: the assembly of the capsid, and its association with the nucleic acid. Maturation occurs at the site of nucleic acid replication. After they are assembled into mature viruses, naked virions may become concentrated in large numbers at the site of maturation, forming inclusion bodies. Naked virions are released in different ways, which depend on the virus and the cell type. Generally, RNA-containing naked viruses are released rapidly after maturation and there is little intracellular accumulation; therefore, these viruses do not form predominant inclusion bodies. On the other hand, DNA-containing naked icosahedral viruses that mature in the nucleus do not reach the cell surface as rapidly, and are released when the cells undergo autolysis or in some cases are extruded without lysis. In either case they tend to accumulate within the infected cells over a long period of time. Thus, they generally produce highly visible inclusion bodies.
- **Enveloped viruses** -In the maturation of enveloped viruses, a capsid must first be assembled around the nucleic acid to form the nucleocapsid, which is then surrounded by the envelope. During the assembly of the nucleocapsid, virus-coded envelope proteins are also synthesized. These migrate to the plasma membrane (if assembly occurs in the cytoplasm) or to the nuclear membrane (if assembly occurs in the nucleus) and become incorporated into that membrane. Envelopes are formed around the nucleocapsids by budding of cellular membranes. NOTE: Enveloped viruses will have an antigenic

mosaicism characteristic of the virus and the host cell. Viruses are slowly and continuously released by the budding process with the results that: (a) the cell is not lysed; and (b) little intracellular accumulation of virus occurs; and (c) inclusion bodies are not as evident as with naked viruses.

- **Complex viruses** -These viruses, of which the poxvirus is a good example, begin the maturation process by forming multilayered membranes around the DNA. These layers differentiate into two membranes: The inner one contains the characteristic nucleoid, while the external one acquires the characteristic pattern of the surface of the virion.



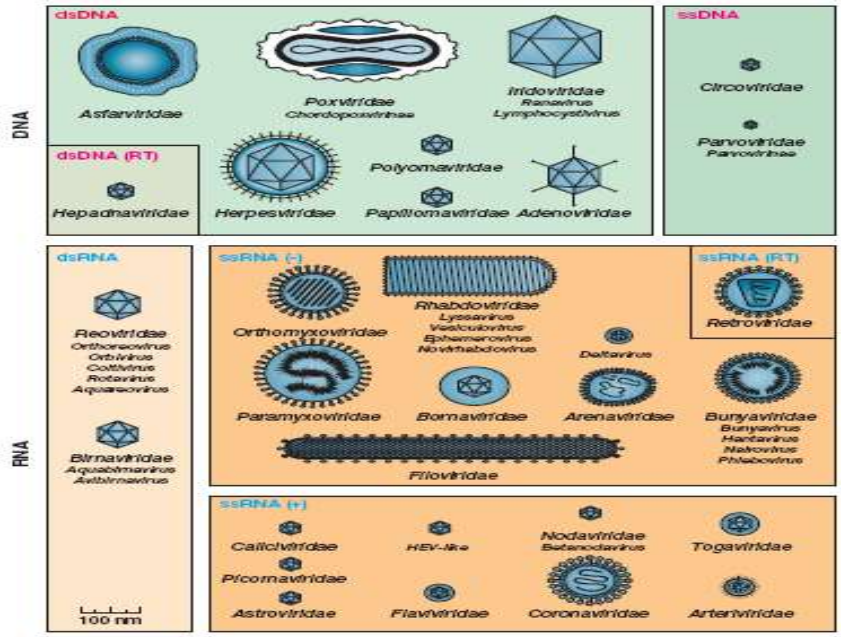


Figure 18.3 A Diagrammatic Description of the Families and Genera of Viruses That Infect Vertebrates. RT stands for reverse transcriptase.

Classification of fungi

Since the 1990s, dramatic changes have occurred in the classification of fungi. Improved understanding of relationships of fungi traditionally placed in the phyla Chytridiomycota and Zygomycota has resulted in the dissolution of outmoded taxons and the generation of new taxons. The Chytridiomycota is retained but in a restricted sense. One of Chytridiomycota's traditional orders, the Blastocladiales, has been raised to phylum status as the Blastocladiomycota. Similarly, the group of anaerobic rumen chytrids, previously known as order Neocallimastigales, has been recognized as a distinct phylum, the Neocallimastigomycota. The phylum Zygomycota is not accepted in the phylogenetic classification of fungi because of remaining doubts about relationships between the groups that have traditionally been placed in this phylum. The consequences of this decision are the recognition of the phylum Glomeromycota and of four subphyla incertae sedis (Latin for "of uncertain position"): Mucoromycotina, Kickxellomycotina, Zoopagomycotina, and Entomophthoromycotina**.**

The true fungi, which make up the monophyletic clade called kingdom Fungi, comprise seven phyla: Chytridiomycota, Blastocladiomycota, Neocallimastigomycota, Microsporidia, Glomeromycota, Ascomycota, and Basidiomycota (the latter two being combined in the subkingdom Dikarya). The group of ancestral fungi is thought to be represented by the present-day Chytridiomycota, or water molds, although the Microsporidia may be an equally ancient sister group. The first major steps in the evolution of higher fungi were the loss of the chytrid flagellum and the development of branching, aseptate fungal filaments, which occurred as terrestrial fungi diverged from water molds 600 to 800 million years ago. Septate filaments evolved as the Glomeromycota diverged from a combined clade of pre-basidiomycota and pre-ascomycota fungi about 500 million years ago. Hyphae with the characteristic appearance of modern Basidiomycota can be seen in some of the earliest known specimens of plant fossils. Therefore, Ascomycota and Basidiomycota probably diverged as so-called sister groups, which are placed together in subkingdom Dikarya, about 300 million years ago. The easily recognizable mushroom fungi probably diversified 130 to 200 million years ago, soon after flowering plants became an important part of the flora and well before the age of dinosaurs. A relatively recent evolutionary radiation, perhaps 60 to 80 million years ago, of anaerobic Chytridiomycota occurred as grasses and grazing mammals became more abundant; the chytrid fungi serve as symbionts within the rumen of such animals, thereby enabling the grazing mammals to digest grasses.

Distinguishing taxonomic features The following classification is adapted from Ainsworth & Bisby's Dictionary of the Fungi, 9th ed. (2001), and has been amended to adopt the phylogenetic arrangement from the Assembling the Fungal Tree of Life (AFTOL) project funded by the U.S. National Science Foundation. AFTOL is a work in progress, and uncertainties remain about the exact relationships of many groups. These uncertain groups are indicated in the annotated classification below by the term incertae sedis, meaning "of uncertain position," the standard term for a taxonomic group of unknown or undefined relationship. The phylogenetic classification of fungi divides the kingdom into 7 phyla, 10 subphyla, 35 classes, 12 subclasses, and 129 orders. Fungus, plural fungi, any of about 80,000 known species of organisms of the kingdom Fungi, which includes the yeasts, rusts, smuts, mildews, molds, mushrooms, and toadstools. There are also many funguslike organisms, including slime molds and oomycetes that

do not belong to kingdom Fungi but are often called fungi. Many of these funguslike organisms are included in the kingdom Chromista. Fungi are among the most widely distributed organisms on Earth and are of great environmental and medical importance. Many fungi are free-living in soil or water; others form parasitic or symbiotic relationships with plants or animals.The fungi are eukaryotic organisms; i.e., their cells contain membrane-bound organelles and clearly defined nuclei. Historically, the fungi were included in the plant kingdom; however, because fungi lack chlorophyll and are distinguished by unique structural and physiological features (i.e., components of the cell wall and cell membrane), they have been separated from plants. In addition, the fungi are clearly distinguished from all other living organisms, including animals, by their principal modes of vegetative growth and nutrient intake. Fungi grow from the tips of filaments (hyphae) that make up the bodies of the organisms (mycelia), and they digest organic matter externally before absorbing it into their mycelia.While mushrooms and toadstools (poisonous mushrooms) are by no means the most numerous or economically significant fungi, they are the most easily recognized fungi. The Latin word for mushroom, fungus (plural fungi), has come to stand for the whole group. Similarly, the study of fungi is known as mycology—a broad application of the Greek word for mushroom, mykēs. Fungi other than mushrooms are sometimes collectively called molds, although this term is better restricted to fungi of the sort represented by bread mold. (For information about slime molds, which exhibit features of both the animal and the fungal worlds, see protist)

Importance of fungi

Humans have been indirectly aware of fungi since the first loaf of leavened bread was baked and the first tub of grape must was turned into wine. Ancient peoples were familiar with the ravages of fungi in agriculture but attributed these diseases to the wrath of the gods. The Romans designated a particular deity, Robigus, as the god of rust and, in an effort to appease him, organized an annual festival, the Robigalia, in his honour.

Fungi are everywhere in very large numbers—in the soil and the air, in lakes, rivers, and seas, on and within plants and animals, in food and clothing, and in the human body. Together with bacteria, fungi are responsible for breaking down organic matter and releasing carbon, oxygen, nitrogen, and phosphorus into the soil and the atmosphere. Fungi are essential to many household

and industrial processes, notably the making of bread, wine, beer, and certain cheeses. Fungi are also used as food; for example, some mushrooms, morels, and truffles are epicurean delicacies, and mycoproteins (fungal proteins), derived from the mycelia of certain species of fungi, are used to make foods that are high in protein.

Studies of fungi have greatly contributed to the accumulation of fundamental knowledge in biology. For example, studies of ordinary baker's or brewer's yeast (Saccharomyces cerevisiae) led to discoveries of basic cellular biochemistry and metabolism. Some of these pioneering discoveries were made at the end of the 19th century and continued during the first half of the 20th century. From 1920 through the 1940s, geneticists and biochemists who studied mutants of the red bread mold, Neurospora, established the one-gene–one-enzyme theory, thus contributing to the foundation of modern genetics. Fungi continue to be useful for studying cell and molecular biology, genetic engineering, and other basic disciplines of biology.

The medical relevance of fungi was discovered in 1928, when Scottish bacteriologist Alexander Fleming noticed the green mold Penicillium notatum growing in a culture dish of Staphylococcus bacteria. Around the spot of mold was a clear ring in which no bacteria grew. Fleming successfully isolated the substance from the mold that inhibited the growth of bacteria. In 1929 he published a scientific report announcing the discovery of penicillin, the first of a series of antibiotics—many of them derived from fungi—that have revolutionized medical practice.

Another medically important fungus is Claviceps purpurea, which is commonly called ergot and causes a plant disease of the same name. The disease is characterized by a growth that develops on grasses, especially on rye. Ergot is a source of several chemicals used in drugs that induce labour in pregnant women and that control hemorrhage after birth. Ergot is also the source of lysergic acid, the active principle of the psychedelic drug lysergic acid diethylamide (LSD). Other species of fungi contain chemicals that are extracted and used to produce drugs known as statins, which control cholesterol levels and ward off coronary heart disease. Fungi are also used in the production of a number of organic acids, enzymes, and vitamins.

Form and function of fungi

Size range

The mushrooms, because of their size, are easily seen in fields and forests and consequently were the only fungi known before the invention of the microscope in the 17th century. The microscope made it possible to recognize and identify the great variety of fungal species living on dead or live organic matter. The part of a fungus that is generally visible is the fruiting body, or sporophore. Sporophores vary greatly in size, shape, colour, and longevity. Some are microscopic and completely invisible to the unaided eye; others are no larger than a pin head; still others are gigantic structures. Among the largest sporophores are those of mushrooms, bracket fungi, and puffballs. Some mushrooms reach a diameter of 20 to 25 cm (8 to 10 inches) and a height of 25 to 30 cm (10 to 12 inches). Bracket, or shelf, fungi can reach 40 cm (16 inches) or more in diameter. A specimen of the bracket fungus Fomitiporia ellipsoidea discovered in 2010 on Hainan Island in southern China had a fruiting body measuring 10.8 metres (35.4 feet) in length and 82–88 cm (2.7–2.9 feet) in width. It may have held some 450 million spores and weighed an estimated 400–500 kg (882–1,102 pounds), at the time making it the largest fungal fruiting body ever documented. Puffballs also can grow to impressive sizes. The largest puffballs on record measured 150 cm (5 feet) in diameter. The number of spores within such giants reaches several trillion.

Distribution and abundance

Fungi are either terrestrial or aquatic, the latter living in freshwater or marine environments. Freshwater species are usually found in clean, cool water because they do not tolerate high degrees of salinity. However, some species are found in slightly brackish water, and a few thrive in highly polluted streams. Soil that is rich in organic matter furnishes an ideal habitat for a large number of species; only a small number of species are found in drier areas or in habitats with little or no organic matter. Fungi are found in all temperate and tropical regions of the world where there is sufficient moisture to enable them to grow. A few species of fungi live in the Arctic and Antarctic regions, although they are rare and are more often found living in symbiosis with algae in the form of lichens (see below Lichens). About 80,000 species of fungi have been

identified and described, but mycologists estimate that there may be as many as 1.5 million total species.

Basic morphology

A typical fungus consists of a mass of branched, tubular filaments enclosed by a rigid cell wall. The filaments, called hyphae (singular hypha), branch repeatedly into a complicated, radially expanding network called the mycelium, which makes up the thallus, or undifferentiated body, of the typical fungus. The mycelium grows by utilizing nutrients from the environment and, upon reaching a certain stage of maturity, forms—either directly or in special fruiting bodies—reproductive cells called spores. The spores are released and dispersed by a wide variety of passive or active mechanisms; upon reaching a suitable substrate, the spores germinate and develop hyphae that grow, branch repeatedly, and become the mycelium of the new individual. Fungal growth is mainly confined to the tips of the hyphae, and all fungal structures are therefore made up of hyphae or portions of hyphae.Some fungi, notably the yeasts, do not form a mycelium but grow as individual cells that multiply by budding or, in certain species, by fission. In addition, the so-called cryptomycota, a primitive group of microscopic fungi, diverge significantly from the standard body plan of other fungi in that their cell walls lack the rigid polymer known as chitin. These microscopic fungi also possess a whiplike flagellum.

Structure of the thallus

In almost all fungi the hyphae that make up the thallus have cell walls. (The thalli of the true slime molds lack cell walls and, for this and other reasons, are classified as protists rather than fungi.) A hypha is a multibranched tubular cell filled with cytoplasm. The tube itself may be either continuous throughout or divided into compartments, or cells, by cross walls called septa (singular septum). In nonseptate (i.e., coenocytic) hyphae the nuclei are scattered throughout the cytoplasm. In septate hyphae each cell may contain one to many nuclei, depending on the type of fungus or the stage of hyphal development. The cells of fungi are similar in structure to those of many other organisms. The minute nucleus, readily seen only in young portions of the hypha, is surrounded by a double membrane and typically contains one nucleolus. In addition to the

nucleus, various organelles—such as the endoplasmic reticulum, Golgi apparatus, ribosomes, and liposomes—are scattered throughout the cytoplasm.

Hyphae usually are either nonseptate (generally in the more primitive fungi) or incompletely septate (meaning that the septa are perforated). This permits the movement of cytoplasm (cytoplasmic streaming) from one cell to the next. In fungi with perforated septa, various molecules are able to move rapidly between hyphal cells, but the movement of larger organelles, such as mitochondria and nuclei, is prevented. In the absence of septa, both mitochondria and nuclei can be readily translocated along hyphae. In mating interactions between filamentous Basidiomycota, the nuclei of one parent often invade the hyphae of the other parent, because the septa are degraded ahead of the incoming nuclei to allow their passage through the existing hyphae. Once the incoming nuclei are established, septa are re-formed.

Variations in the structure of septa are numerous in the fungi. Some fungi have sievelike septa called pseudosepta, whereas fungi in other groups have septa with one to few pores that are small enough in size to prevent the movement of nuclei to adjacent cells. Basidiomycota have a septal structure called a dolipore septum that is composed of a pore cap surrounding a septal swelling and septal pore. This organization permits cytoplasm and small organelles to pass through but restricts the movement of nuclei to varying degrees.

The wall of the hypha is complex in both composition and structure. Its exact chemical composition varies in different fungal groups. In some funguslike organisms the wall contains considerable quantities of cellulose, a complex carbohydrate that is the chief constituent of the cell walls of plants. In most fungi, however, two other polymers—chitin and glucan (a polymer of glucose linked at the third carbon and branched at the sixth), which forms an α-glucan layer and a special β-1,3-1,6-glucan layer—form the main structural components of the wall. Among the many other chemical substances in the walls of fungi are some that may thicken or toughen the wall of tissues, thus imparting rigidity and strength. The chemical composition of the wall of a particular fungus may vary at different stages of the organism's growth—a possible indication that the wall plays some part in determining the form of the fungus. In some fungi, carbohydrates are stored in the wall at one stage of development and are removed and utilized at a later stage.

In some yeasts, fusion of sexually functioning cells is brought about by the interaction of specific chemical substances on the walls of two compatible mating types.

When the mycelium grows in or on a surface, such as in the soil, on a log, or in culture medium, it appears as a mass of loose, cottony threads. The richer the composition of the growth medium, the more profuse the threads and the more feltlike the mass. On the sugar-rich growth substances used in laboratories, the assimilative (somatic) hyphae are so interwoven as to form a thick, almost leathery colony. On the soil, inside a leaf, in the skin of animals, or in other parasitized plant or animal tissues, the hyphae are usually spread in a loose network. The mycelia of the so-called higher fungi does, however, become organized at times into compact masses of different sizes that serve various functions. Some of these masses, called sclerotia, become extremely hard and serve to carry the fungus over periods of adverse conditions of temperature and moisture. One example of a fungus that forms sclerotia is Claviceps purpurea, which causes ergot, a disease of cereal grasses such as rye. The underground sclerotia of Poria cocos, an edible pore fungus also known as tuckahoe, may reach a diameter of 20 to 25 cm.

Various other tissues are also produced by the interweaving of the assimilative hyphae of some fungi. Stromata (singular stroma) are cushionlike tissues that bear spores in various ways. Rhizomorphs are long strands of parallel hyphae cemented together. Those of the honey mushroom (Armillaria mellea), which are black and resemble shoestrings, are intricately constructed and are differentiated to conduct water and food materials from one part of the thallus to another.

Sporophores and spores

When the mycelium of a fungus reaches a certain stage of growth, it begins to produce spores either directly on the somatic hyphae or, more often, on special sporiferous (spore-producing) hyphae, which may be loosely arranged or grouped into intricate structures called fruiting bodies, or sporophores

The more primitive fungi produce spores in sporangia, which are saclike sporophores whose entire cytoplasmic contents cleave into spores, called sporangiospores. Thus, they differ from more advanced fungi in that their asexual spores are endogenous. Sporangiospores are both

naked and flagellated (zoospores) or walled and nonmotile (aplanospores). The more primitive aquatic and terrestrial fungi tend to produce zoospores. The zoospores of aquatic fungi and funguslike organisms swim in the surrounding water by means of one or two variously located flagella (whiplike organs of locomotion). Zoospores produced by terrestrial fungi are released after a rain from the sporangia in which they are borne and swim for a time in the rainwater between soil particles or on the wet surfaces of plants, where the sporangia are formed by parasitic fungi. After some time, the zoospores lose their flagella, surround themselves with walls, and encyst. Each cyst germinates by producing a germ tube. The germ tube may develop a mycelium or a reproductive structure, depending on the species and on the environmental conditions. The bread molds, which are the most advanced of the primitive fungi, produce only aplanospores (nonmotile spores) in their sporangia.

The more advanced fungi do not produce motile spores of any kind, even though some of them are aquatic in fresh or marine waters. In these fungi, asexually produced spores (usually called conidia) are produced exogenously and are typically formed terminally or laterally on special spore-producing hyphae called conidiophores. Conidiophores may be arranged singly on the hyphae or may be grouped in special asexual fruiting bodies, such as flask-shaped pycnidia, mattresslike acervuli, cushion-shaped sporodochia, or sheaflike synnemata.

Sexually produced spores of the higher fungi result from meiosis and are formed either in saclike structures (asci) typical of the Ascomycota or on the surface of club-shaped structures (basidia) typical of the Basidiomycota. Asci and basidia may be borne naked, directly on the hyphae, or in various types of sporophores, called ascocarps (also known as ascomata) or basidiocarps (also known as basidiomata), depending on whether they bear asci or basidia, respectively. Well-known examples of ascocarps are the morels, the cup fungi, and the truffles. Commonly encountered basidiocarps are mushrooms, brackets, puffballs, stinkhorns, and bird's-nest fungi.

Growth

Under favourable environmental conditions, fungal spores germinate and form hyphae. During this process, the spore absorbs water through its wall, the cytoplasm becomes activated, nuclear division takes place, and more cytoplasm is synthesized. The wall initially grows as a spherical

structure. Once polarity is established, a hyphal apex forms, and from the wall of the spore a germ tube bulges out, enveloped by a wall of its own that is formed as the germ tube grows.

The hypha may be roughly divided into three regions: (1) the apical zone about 5–10 micrometres (0.0002–0.0004 inch) in length, (2) the subapical region, extending about 40 micrometres back of the apical zone, which is rich in cytoplasmic components, such as nuclei, Golgi apparatus, ribosomes, mitochondria, the endoplasmic reticulum, and vesicles, but is devoid of vacuoles, and (3) the zone of vacuolation, which is characterized by the presence of many vacuoles and the accumulation of lipids.

Growth of hyphae in most fungi takes place almost exclusively in the apical zone (i.e., at the very tip). This is the region where the cell wall extends continuously to produce a long hyphal tube. The cytoplasm within the apical zone is filled with numerous vesicles. These bubblelike structures are usually too small to be seen with an ordinary microscope but are clearly evident under the electron microscope. In higher fungi the apical vesicles can be detected with an ordinary microscope equipped with phase-contrast optics as a round spot with a somewhat diffuse boundary. This body is universally known by its German name, the Spitzenkörper, and its position determines the direction of growth of a hypha.

The growing tip eventually gives rise to a branch. This is the beginning of the branched mycelium. Growing tips that come in contact with neighbouring hyphae often fuse with them to form a hyphal net. In such a vigorously growing system, the cytoplasm is in constant motion, streaming toward the growing tips. Eventually, the older hyphae become highly vacuolated and may be stripped of most of their cytoplasm. All living portions of a thallus are potentially capable of growth. If a small piece of mycelium is placed under conditions favourable for growth, it develops into a new thallus, even if no growing tips are included in the severed portion.

Growth of a septate mycelium (i.e., with cross walls between adjacent cells) entails the formation of new septa in the young hyphae. Septa are formed by ringlike growth from the wall of the hypha toward the centre until the septa are complete. In the higher fungi the septum stops growing before it is complete; the result is a central pore through which the cytoplasm flows,

thus establishing organic connection throughout the thallus. In contrast to plants, in which the position of the septum separating two daughter cells determines the formation of tissues, the fungal septum is always formed at right angles to the axis of growth. As a result, in fungal tissue formation, the creation of parallel hyphae cannot result from longitudinal septum formation but only from outgrowth of a new branch. In fungi, therefore, the mechanism that determines the point of origin and subsequent direction of growth of hyphal branches is the determining factor in developmental morphogenesis.The individual fungus is potentially immortal, because it continues to grow at the hyphal tips as long as conditions remain favourable. It is possible that, in undisturbed places, mycelia exist that have grown continuously for many thousands of years. The older parts of the hyphae die and decompose, releasing nitrogen and other nutrients into the soil.

Some species of endophytic fungi, such as Neotyphodium and Epichloë, which invade the seeds of grasses (e.g., ryegrass and fescue) and grow within the plant, grow not through extension of the hyphal tips but by intercalary growth, in which the hyphae attach to the growing cells of the plant. This type of growth enables the hyphae of the fungus to grow at the same rate that the plant grows. Intercalary growth of endophytic fungi was discovered in 2007, although for many years scientists suspected that these fungi possessed unique adaptations that allow them to grow as if they were natural parts of their hosts.

The underground network of hyphae of a mushroom can grow and spread over a very large area, often several metres (yards) in diameter. The underground hyphae obtain food from organic matter in the substratum and grow outward. The hyphal branches at the edge of the mycelium become organized at intervals into elaborate tissues that develop aboveground into mushrooms. Such a circle of mushrooms is known as a fairy ring, because in the Middle Ages it was believed to represent the path of dancing fairies. The ring marks the periphery of an enormous fungus colony, which, if undisturbed, continues to produce ever wider fairy rings year after year. Fungi can grow into enormous colonies. Some thalli of Armillaria species, which are pathogens of forest trees, are among the largest and oldest organisms on Earth.

Nutrition

Unlike plants, which use carbon dioxide and light as sources of carbon and energy, respectively, fungi meet these two requirements by assimilating preformed organic matter; carbohydrates are generally the preferred carbon source. Fungi can readily absorb and metabolize a variety of soluble carbohydrates, such as glucose, xylose, sucrose, and fructose. Fungi are also characteristically well equipped to use insoluble carbohydrates such as starches, cellulose, and hemicelluloses, as well as very complex hydrocarbons such as lignin. Many fungi can also use proteins as a source of carbon and nitrogen. To use insoluble carbohydrates and proteins, fungi must first digest these polymers extracellularly. Saprobic fungi obtain their food from dead organic material; parasitic fungi do so by feeding on living organisms (usually plants), thus causing disease.

Fungi secure food through the action of enzymes (biological catalysts) secreted into the surface on which they are growing; the enzymes digest the food, which then is absorbed directly through the hyphal walls. Food must be in solution in order to enter the hyphae, and the entire mycelial surface of a fungus is capable of absorbing materials dissolved in water. The rotting of fruits, such as peaches and citrus fruits in storage, demonstrates this phenomenon, in which the infected parts are softened by the action of the fungal enzymes. In brown rot of peaches, the softened area is somewhat larger than the actual area invaded by the hyphae: the periphery of the brown spot has been softened by enzymes that act ahead of the invading mycelium. Cheeses such as Brie and Camembert are matured by enzymes produced by the fungus Penicillium camemberti, which grows on the outer surface of some cheeses. Some fungi produce special rootlike hyphae, called rhizoids, which anchor the thallus to the growth surface and probably also absorb food. Many parasitic fungi are even more specialized in this respect, producing special absorptive organs called haustoria.

SAPROBIOSIS

Together with bacteria, saprobic fungi are to a large extent responsible for the decomposition of organic matter. They are also responsible for the decay and decomposition of foodstuffs. Among other destructive saprobes are fungi that destroy timber and timber products as their mycelia

invade and digest the wood; many of these fungi produce their spores in large, woody, fruiting bodies—e.g., bracket or shelf fungi. Paper, textiles, and leather are often attacked and destroyed by fungi. This is particularly true in tropical regions, where temperature and humidity are often very high.

The nutritional requirements of saprobes (and of some parasites that can be cultivated artificially) have been determined by growing fungi experimentally on various synthetic substances of known chemical composition. Fungi usually exhibit the same morphological characteristics in these culture media as they do in nature. Carbon is supplied in the form of sugars or starch; the majority of fungi thrive on such sugars as glucose, fructose, mannose, maltose, and, to a lesser extent, sucrose. Decomposition products of proteins, such as proteoses, peptones, and amino acids, can be used by most fungi as nitrogen sources; ammonium compounds and nitrates also serve as nutrients for many species. It is doubtful, however, that any fungus can combine, or fix, atmospheric nitrogen into usable compounds. Chemical elements such as phosphorus, sulfur, potassium, magnesium, and small quantities of iron, zinc, manganese, and copper are needed by most fungi for vigorous growth; elements such as calcium, molybdenum, and gallium are required by at least some species. Oxygen and hydrogen are absolute requirements; they are supplied in the form of water or are obtained from carbohydrates. Many fungi, deficient in thiamine and biotin, must obtain these vitamins from the environment; most fungi appear able to synthesize all other vitamins necessary for their growth and reproduction.As a rule, fungi are aerobic organisms, meaning they require free oxygen in order to live. Fermentations, however, take place under anaerobic conditions. Knowledge of the physiology of saprobic fungi has enabled industry to use several species for fermentation purposes. One of the most important groups of strictly anaerobic fungi are members of the genera Neocallimastix (phylum Neocallimastigomycota), which form a crucial component of the microbial population of the rumen of herbivorous mammals. These fungi are able to degrade plant cell wall components, such as cellulose and xylans, that the animals cannot otherwise digest.

Predation

A number of fungi have developed ingenious mechanisms for trapping microorganisms such as amoebas, roundworms (nematodes), and rotifers. After the prey is captured, the fungus uses hyphae to penetrate and quickly destroy the prey. Many of these fungi secrete adhesive substances over the surface of their hyphae, causing a passing animal that touches any portion of the mycelium to adhere firmly to the hyphae. For example, the mycelia of oyster mushrooms (genus Pleurotus) secrete adhesives onto their hyphae in order to catch nematodes. Once a passing animal is caught, a penetration tube grows out of a hypha and penetrates the host's soft body. This haustorium grows and branches and then secretes enzymes that quickly kill the animal, whose cytoplasm serves as food for the fungus.

Other fungi produce hyphal loops that ensnare small animals, thereby allowing the fungus to use its haustoria to penetrate and kill a trapped animal. Perhaps the most amazing of these fungal traps are the so-called constricting rings of some species of Arthrobotrys, Dactylella, and Dactylaria—soil-inhabiting fungi easily grown under laboratory conditions. In the presence of nematodes, the mycelium produces large numbers of rings through which the average nematode is barely able to pass. When a nematode rubs the inner wall of a ring, which usually consists of three cells with touch-sensitive inner surfaces, the cells of the ring swell rapidly, and the resulting constriction holds the worm tightly. All efforts of the nematode to free itself fail, and a hypha, which grows out of one of the swollen ring cells at its point of contact with the worm, penetrates and branches within the animal's body, thereby killing the animal. The dead animal is then used for food by the fungus. In the absence of nematodes, these fungi do not usually produce rings in appreciable quantities. A substance secreted by nematodes stimulates the fungus to form the mycelial rings.

Reproductive processes of fungi

Following a period of intensive growth, fungi enter a reproductive phase by forming and releasing vast quantities of spores. Spores are usually single cells produced by fragmentation of the mycelium or within specialized structures (sporangia, gametangia, sporophores, etc.). Spores may be produced either directly by asexual methods or indirectly by sexual reproduction. Sexual

reproduction in fungi, as in other living organisms, involves the fusion of two nuclei that are brought together when two sex cells (gametes) unite. Asexual reproduction, which is simpler and more direct, may be accomplished by various methods.

Asexual reproduction

Typically in asexual reproduction, a single individual gives rise to a genetic duplicate of the progenitor without a genetic contribution from another individual. Perhaps the simplest method of reproduction of fungi is by fragmentation of the thallus, the body of a fungus. Some yeasts, which are single-celled fungi, reproduce by simple cell division, or fission, in which one cell undergoes nuclear division and splits into two daughter cells; after some growth, these cells divide, and eventually a population of cells forms. In filamentous fungi the mycelium may fragment into a number of segments, each of which is capable of growing into a new individual. In the laboratory, fungi are commonly propagated on a layer of solid nutrient agar inoculated either with spores or with fragments of mycelium.

Budding, which is another method of asexual reproduction, occurs in most yeasts and in some filamentous fungi. In this process, a bud develops on the surface of either the yeast cell or the hypha, with the cytoplasm of the bud being continuous with that of the parent cell. The nucleus of the parent cell then divides; one of the daughter nuclei migrates into the bud, and the other remains in the parent cell. The parent cell is capable of producing many buds over its surface by continuous synthesis of cytoplasm and repeated nuclear divisions. After a bud develops to a certain point and even before it is severed from the parent cell, it is itself capable of budding by the same process. In this way, a chain of cells may be produced. Eventually, the individual buds pinch off the parent cell and become individual yeast cells. Buds that are pinched off a hypha of a filamentous fungus behave as spores; that is, they germinate, each giving rise to a structure called a germ tube, which develops into a new hypha. Although fragmentation, fission, and budding are methods of asexual reproduction in a number of fungi, the majority reproduce asexually by the formation of spores. Spores that are produced asexually are often termed mitospores, and such spores are produced in a variety of ways.

Sexual reproduction

Sexual reproduction, an important source of genetic variability, allows the fungus to adapt to new environments. The process of sexual reproduction among the fungi is in many ways unique. Whereas nuclear division in other eukaryotes, such as animals, plants, and protists, involves the dissolution and re-formation of the nuclear membrane, in fungi the nuclear membrane remains intact throughout the process, although gaps in its integrity are found in some species. The nucleus of the fungus becomes pinched at its midpoint, and the diploid chromosomes are pulled apart by spindle fibres formed within the intact nucleus. The nucleolus is usually also retained and divided between the daughter cells, although it may be expelled from the nucleus, or it may be dispersed within the nucleus but detectable

Sexual reproduction in the fungi consists of three sequential stages: plasmogamy, karyogamy, and meiosis. The diploid chromosomes are pulled apart into two daughter cells, each containing a single set of chromosomes (a haploid state). Plasmogamy, the fusion of two protoplasts (the contents of the two cells), brings together two compatible haploid nuclei. At this point, two nuclear types are present in the same cell, but the nuclei have not yet fused. Karyogamy results in the fusion of these haploid nuclei and the formation of a diploid nucleus (i.e., a nucleus containing two sets of chromosomes, one from each parent). The cell formed by karyogamy is called the zygote. In most fungi the zygote is the only cell in the entire life cycle that is diploid. The dikaryotic state that results from plasmogamy is often a prominent condition in fungi and may be prolonged over several generations. In the lower fungi, karyogamy usually follows plasmogamy almost immediately. In the more evolved fungi, however, karyogamy is separated from plasmogamy. Once karyogamy has occurred, meiosis (cell division that reduces the chromosome number to one set per cell) generally follows and restores the haploid phase. The haploid nuclei that result from meiosis are generally incorporated in spores called meiospores.

Fungi employ a variety of methods to bring together two compatible haploid nuclei (plasmogamy). Some produce specialized sex cells (gametes) that are released from differentiated sex organs called gametangia. In other fungi two gametangia come in contact, and nuclei pass from the male gametangium into the female, thus assuming the function of gametes. In still other fungi the gametangia themselves may fuse in order to bring their nuclei together.

Finally, some of the most advanced fungi produce no gametangia at all; the somatic (vegetative) hyphae take over the sexual function, come in contact, fuse, and exchange nuclei.

Fungi in which a single individual bears both male and female gametangia are hermaphroditic fungi. Rarely, gametangia of different sexes are produced by separate individuals, one a male, the other a female. Such species are termed dioecious. Dioecious species usually produce sex organs only in the presence of an individual of the opposite sex.

SEXUAL INCOMPATIBILITY

Many of the simpler fungi produce differentiated male and female organs on the same thallus but do not undergo self-fertilization because their sex organs are incompatible. Such fungi require the presence of thalli of different mating types in order for sexual fusion to take place. The simplest form of this mechanism occurs in fungi in which there are two mating types, often designated + and − (or A and a). Gametes produced by one type of thallus are compatible only with gametes produced by the other type. Such fungi are said to be heterothallic. Many fungi, however, are homothallic; i.e., sex organs produced by a single thallus are self-compatible, and a second thallus is unnecessary for sexual reproduction. Some of the most complex fungi (e.g., mushrooms) do not develop differentiated sex organs; rather, the sexual function is carried out by their somatic hyphae, which unite and bring together compatible nuclei in preparation for fusion. Homothallism and heterothallism are encountered in fungi that have not developed differentiated sex organs, as well as in fungi in which sex organs are easily distinguishable. Compatibility therefore refers to a physiological differentiation, and sex refers to a morphological (structural) one; the two phenomena, although related, are not synonymous.

SEXUAL PHEROMONES (HORMONES)

The formation of sex organs in fungi is often induced by specific organic substances. Although called sex hormones when first discovered, these organic substances are actually sex pheromones, chemicals produced by one partner to elicit a sexual response in the other. In Allomyces (order Blastocladiales) a pheromone named sirenin, secreted by the female gametes, attracts the male gametes, which swim toward the former and fuse with them. In Achlya (phylum Oomycota, kingdom Chromista) a sterol pheromone called antheridiol induces the formation of

gametangia and attracts the male to the female. In some simple fungi, which may have gametangia that are not differentiated structurally, a complex biochemical interplay between mating types produces trisporic acid, a pheromone that induces the formation of specialized aerial hyphae. Volatile intermediates in the trisporic acid synthetic pathway are interchanged between the tips of opposite mating aerial hyphae, causing the hyphae to grow toward each other and fuse together. In yeasts belonging to the phyla Ascomycota and Basidiomycota, the pheromones are small peptides. Several pheromone genes have been identified and characterized in filamentous ascomycetes and basidiomycetes.

Life cycle of fungi

In the life cycle of a sexually reproducing fungus, a haploid phase alternates with a diploid phase. The haploid phase ends with nuclear fusion, and the diploid phase begins with the formation of the zygote (the diploid cell resulting from fusion of two haploid sex cells). Meiosis (reduction division) restores the haploid number of chromosomes and initiates the haploid phase, which produces the gametes. In the majority of fungi, all structures are haploid except the zygote. Nuclear fusion takes place at the time of zygote formation, and meiosis follows immediately. Only in Allomyces and a few related genera and in some yeasts is alternation of a haploid thallus with a diploid thallus definitely known. The thallus is diploid in many members of the phylum Oomycota, and meiosis takes place just before the formation of the gametes.

In the higher fungi a third condition is interspersed between the haploid and diploid phases of the life cycle. In these fungi, plasmogamy (fusion of the cellular contents of two hyphae but not of the two haploid nuclei) results in dikaryotic hyphae in which each cell contains two haploid nuclei, one from each parent. Eventually, the nuclear pair fuses to form the diploid nucleus and thus the zygote. In the Basidiomycota, binucleate cells divide successively and give rise to a binucleate mycelium, which is the main assimilative phase of the life cycle. It is the binucleate mycelium that eventually forms the basidia—the stalked fruiting bodies in which nuclear fusion and meiosis take place prior to the formation of the basidiospores.

Fungi usually reproduce both sexually and asexually. The asexual cycle produces mitospores, and the sexual cycle produces meiospores. Even though both types of spores are produced by the

same mycelium, they are very different in form and easily distinguished (see above Sporophores and spores). The asexual phase usually precedes the sexual phase in the life cycle and may be repeated frequently before the sexual phase appears.

Some fungi differ from others in their lack of one or the other of the reproductive stages. For example, some fungi reproduce only sexually (except for fragmentation, which is common in most fungi), whereas others reproduce only asexually. A number of fungi exhibit the phenomenon of parasexuality, in which processes comparable to plasmogamy, karyogamy, and meiosis take place. However, these processes do not occur at a specified time or at specified points in the life cycle of the organism. As a result, parasexuality is characterized by the prevalence of heterokaryosis in a mycelium—i.e., the presence, side by side, of nuclei of different genetic composition.

Ecology of fungi

Relatively little is known of the effects of the environment on the distribution of fungi that utilize dead organic material as food (i.e., saprobic fungi; see above Nutrition). The availability of organic food is certainly one of the factors controlling such distribution. A great number of fungi appear able to utilize most types of organic materials, such as lignin, cellulose, or other polysaccharides, which have been added to soils or waters by dead vegetation. Most saprobic fungi are widely distributed throughout the world, only requiring that their habitats have sufficient organic content to support their growth. However, some saprobes are strictly tropical and others are strictly temperate-zone forms; fungi with specific nutritional requirements are even further localized.

Moisture and temperature are two additional ecological factors that are important in determining the distribution of fungi. Laboratory studies have shown that many, perhaps the majority, of fungi are mesophilic, meaning they have an optimum growth temperature of 20–30 °C (68–86 °F). Thermophilic species are able to grow at 50 °C (122 °F) or higher but are unable to grow below 30 °C. Although the optimum temperature for growth of most fungi lies at or above 20 °C, a large number of species are able to grow close to or below 0 °C (32 °F). The so-called snow molds and the fungi that cause spoilage of refrigerated foods are examples of this group.

Obviously, temperature relationships influence the distribution of various species. Certain other effects of temperature are also important factors in determining the habitats of fungi. Many coprophilous (dung-inhabiting) fungi, for example, although able to grow at a temperature of 20–30 °C, require a short period at 60 °C (140 °F) for their spores to germinate.

Economic Importance of Fungi

Fungi include many species which are of economic importance to man . We are harmed and benefited by Fungi directly or indirectly. Some account for beneficial and harmful activities of Fungi is as under:

Useful activities of Fungi:

(1) Destruction of organic waste: Saprophytic Fungi decompose plant and animal remains by acting as natural scavengers. Carbon dioxide released in the process is used by green plants. By some workers saprophytic Fungi have been designated as vegetative vultures.

(2) In Industry:

(A) Many fungi are used in the commercial preparation of many organic acids and some vitamin preparation. Aspergillus miger, A. glaucus, A. clavatus, citronyces citricus have been recommended for preparation citric acid. Many fungi also prepare gluconic acid lactic acid.

Aspergillus and Fusaruim are source riboflavin, a constituent of vitamin B. Yeasts are also rich in vitamin B.

(B) Recently Fungi have been founded to be the basis of entire alcoholic industry. The basis of alcoholic industry is production of ethyl alcohol by fermentation of sugar solutions by yeast. Yeasts are source of complex enzyme zymase which is responsible for the process of fermentation. Yeasts are used in making wines, bear and ciders.

(C) Certain yeasts Saccharamyces cerevisiaes form important basis of baking industries.

(D) Some fungi such as species of Penicillium are used in preparation of certain cheese.

(3) As food: Many fungi like mushroom (Agaricus), Puffballs (Lycoperdon), Morels (Morchella) are edible. They are important as protein sources. They are regarded as delicacies of table.

Large scale production of yeast is used in conversion of carbohydrates and in organic nitrogen salts in to edible and nutritional forms. Yeast food supplies a number of vitamins like thiamine, riboflavin, micotomic acid, Panthothenic acid, biotin etc. Yeast food is there fore supplement of human food requirements.

(4) In Medicines or Medicinal value: Recently many fungi have been founded to be responsible for producing certain antibiotic drugs which inhibit the growth of pathogenic micro-organisms. Some medicines formed from Fungi are Penicillin to kill bacteria that cause Pnemonia streptomycin. Auroemoycin, chloromycetin and Ephedrine from yeast.

(5) In plastic manufacture: Certain Fungi like odium lactis is widely used in plastic industry.

(6) Control and insect pest: Many Fungi like Ascherronia deyroides, Isaria ferinosa, Empusa sepulchralis help in controlling the infection of insect pests of the plants.

(7) Phytohormone or Auxins: Many growth promoting substances like Gibberellins are synthesized from the fungi like Fusarium maniforma and Dematium pullulans.

(8) Nutrition of plant: Many members of Phycomycetes, Ascomycetes, Basidiomycetes and fungi imerfecii are involved in the formation of mycorrhizae which are of fundamental importance in nutrition of tree like Cycas, Zamia and Pinus.

Harmful activities:

Fungi cause diseases to human, animals and plants. They cause destruction to clothes, paper, jute, leather, rubber, Paints, petroleum products, good grains and other bakery products. Harmful effects are:

(1) Many fungi cause very much loss to our timber trees by causing wood rot. Armillaria mellea, the honey mushroom causes red rot of apple and many forest trees. Many species of Polypores attack forest trees causing wood rot.

(2) Some would fungi like Rhizopus, Mucor, Aspergillus spoil our food. Their spores are always in the air and settle down on exposed jams, pickles, jelly, bread and fruits and develop mycelia and make food articles unfit for human use.

(3) Some parasitic fungi are causative agents of diseased of our cops, fruits and other economic plants. In them fungi like Puccimia and ustilago cause rusts and smuts. They are great enemies of crops and cause loss by reducing crop yield. The rusts reproduce yellow, orange or black pustules on the stem and leaves of cereal plants while smuts attack forests and produces a black powdery mass of smut **spores in the place of seed and** fruits. Damage caused to cereals (wheat, maize, oat and barley) by rust and smuts amounts to several hundred millions of rupees annually. Fungi diseases in plants are:

(i) While rust of crucifers by crystopus cindidus.

(ii) Powdery mildew by Erisiphae species.

(iii) Fruit root of apple by Rhizopus arrhizus.

(iv) Late blight of Potato by Phytopora infestans.

(v) Red rot of sugar came by colletorichum falcatum.

(vi) Some parasitic fungi cause diseases to animals.

Saprolegnia which occurs as saprophyte on dead fish or flies behave as facultative parasite producing serious diseases to crops and gold fishes.

(4) Some fungi are also cause some important diseases in human beings. Asperigillus as A.miger, A.fiavus, A.fumigatus are common human pathogens. Disease caused in aspergilloses of lungs and ears. Some parasitic fungi live in mucous membrane of throad, bronchii and lungs. Few fungi cause skin discoloration. A well known skin disease rung worm or "Dead" is also a fungus disease**.**

(5) Fungi have both positive ad negative roles in our daily life. So they are our friends as well as foes (enemy).

Beneficial Roles or Useful Activities.

i) Fungi are used as food. e.g. Mushrooms and Morels.

ii) Fungi are used in laboratory.

a) Baking Yeast (S. cerevisae)

b) Several alcoholic beverages such as wine, whiskey, beer, rum all are prepared by fermentation activity of sugar solution by wine yeast. (S. ellipsoidens) c) Some fungi are used in production of enzymes like amylase, pectimase

iii) Some fungi are used in production of several antibiotics and antibiotics and other useful medicine like penicillin, streptomycin, ergotine and ephedrine respectively.

iv) Several fungi are used in commercial production of different organic products like citric acid, fumaric, lactic and oxalic acid.

v) Fungi in agriculture:

a) Being saprophytes they decompose the organic matter and enhance the fertility of the soil.

b) Some fungi develop symbiotic relation with roots of higher plant like Pinus and help them in absorption of nutrients. Such fungi are known as mycorrhiza.

vi) Some fungi are used to produce hormone like Gibberellin.

Harmful Activities:

i) Food spoilage (destruction) caused by fungi like mucor and yeast.

ii) Some yeasts causes huge loss in silk industry to attack silk worms and kill the same.

iii) Several types of plant diseases caused by (different types of fungi) species of Nematospra they attack tomatoes, cotton and bean plants.

Similar disease like causal organisms

a. Stem rust of wheat – Pucvinia graministice

b. Early blight of potato – Alternaria solani

c. Late blight of potato – Phytiphtoria infestans

d. White rust of crucifer – Albugo candida

iv) Some fungi (*Cryptococcus neoformans*) may cause human disease like meningitis and brain tumor.

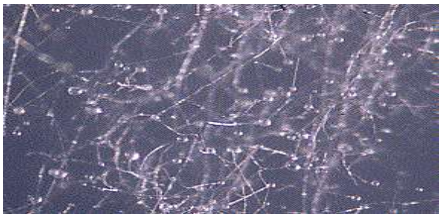
- Torula and other yeasts produce small nodules on the skin and lesions in the viscera and bones of man.

v) Some fungi are concerned with destruction of substances like attacks textile materials, paper, leather goods, rubber even optical instruments.

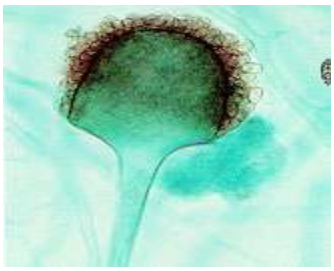
vi) Some fungi are not edible mushroom like different species Amanita.

Characteristic of Fungi

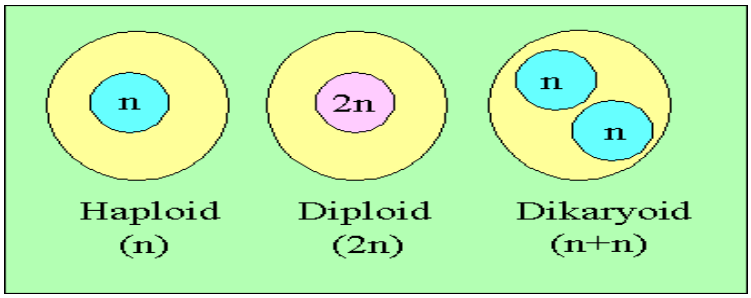
- **Nutritional modes**
  - Fungi are heterotrophs which are saprobes (consume dead material) or parasites
  - Fungi obtain nourishment by secreting digestive enzymes and then absorbing the broken-down substances
    - Fungi are basically an animal turned "inside out"
- **Body plans**
  - Fungi are multicellular organisms composed of filamentous **hyphae**



  - A mass of hyphae is called a **mycelium** (a mushroom is a mycelium)
  - Fungi also possess **sporangia** during their reproductive cycle

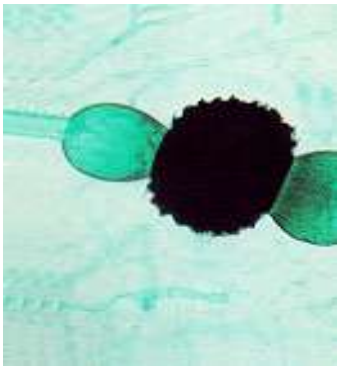


- Hyphae can be haploid (n), diploid (2n), or dikaryoid (n+n)



- **Reproductive cycle**
  - Asexual - sporangia produce haploid spores
  - Sexual - gametangia produce gametes
  - The asexual mode of reproduction is far more common
  - Most major taxonomic groups are differentiated by reproductive cycle

Fungal Taxonomy



Zygomycota

- Common representatives: black bread molds
- Hyphae are haploid
- Reproduction relies mainly on asexual spore production
- Sexual reproduction:
  - gametangia form
  - a zygospore is then formed where the gametes fuse
  - a sporangia forms, meiosis occurs, and haploid spores are released.

Ascomycota (sac fungi)

- Common representatives: yeasts, morels, and truffles
- Hyphae are haploid and dikaryoid
- Asexual reproduction relies on asexual spore production
- Sexual reproduction:
  - multicellular spore-producing structure (ascocarp) forms
  - nuclei in dikaryoid hyphae fuse
  - meiosis occurs and haploid spores are released

Basidiomycota (sac fungi)

- Common representatives: mushrooms and shelf fungi
- Hyphae are haploid and dikaryoid
- Asexual reproduction relies on asexual spore production
  - this is much rarer in basidiomycetes than in zygomycetes or ascomycetes
- Sexual reproduction:
  - multicellular spore-producing structures (basidiocarps) form
  - nuclei in dikaryoid hyphae fuse
  - meiosis occurs and haploid spores are released

Deuteromycota (fungi imperfecti)

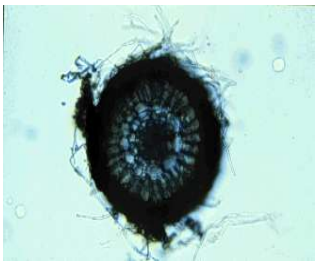
- The major divisions of fungi are based on sexual reproduction mode. What happens if the sexual stage of a fungi is not known?
- All of these fungi are lumped into a fourth group for simplicity
- Members include *Trichophyton* (Athlete's foot), *Penicillium* (Penicillin), and *Candida albicans* ("Yeast" infections).

Symbiosis between Fungi and Plants

Lichens

- Lichens are the product of a symbiosis between a fungi and an algae
  - The fungi envelops the algae and derive nutrition from the algae
  - The fungi provides water and shelter for the algae

Lichens can absorb minerals from rock and nitrogen from the air



Mycorrhizal fungi

- Mycorrhizae are fungi with a symbiotic relationship with land plants
- The fungi regulates ion flow into the plants and in turn is supplied with carbohydrates
- Some of the earliest terrestrial plant fossils show evidence of mycorrhizal associations

This implies that this symbiosis was important, if not crucial, for the colonization of the land by plants

(http://www.uic.edu/classes/bios/bios104/mike/fungi01.htm)

1. Zygomycetes: common in bread mould reproduses asexually unless conditions are poor the sexual reproduction occurs between mating strains
2. Ascomycetes: yeasts are unicellular cause mildew on plant leaves produse sexual spores called ascospores, and are mostly red brown blue green moulds that cause food spoilage
3. Basidiomycetes: known as club fungi, including mushroom some of which are edible other are poisenous or halloucinogens. Pproduce sexual spores called basidiospores
4. Deuteromycetes: known as fungi imperfecti either lost capacity for sexual production or has never been observed eg pencillium antibiotics, cheese aromas.

PROTOZOANS - GENERAL INTRODUCTION

Single-celled microorganisms belonging to the animal kingdom are classified as *Protozoa* (Greek *protos*—first; *zoon*—animal). Within its single cell, the protozoon contains all structures required for performing its various functions. Some free-living protozoa resemble plants in containing green plastids that enable them to perform photosynthesis. It is believed that these represent the earliest forms of animal life. Numerous varieties of protozoa have evolved to suit all manner of environmental conditions.

Free-living protozoa are found in all habitats—in deep ocean or in shallow fresh waters, in hot springs or in ice, under the soil or in snow on mountain tops. Parasitic protozoa have, however adapted to different host species, with more restricted physicochemical requirements.

Protozoa exhibit a wide range of size, shape and structure, yet all possess certain essential common features. The typical protozoan cell is bounded by a trilaminar unit membrane, supported by a sheet of contractile fibrils which enable the cell to change its shape and to move. The cytoplasm can often be differentiated into an outer rim of relatively homogeneous ectoplasm and a more granular inner endoplasm. The ectoplasm serves as the organ of locomotion and for engulfment of food materials by putting forth pseudopodial processes. It also functions in respiration, in discharging waste materials and also as a protective covering for the cell.

Within the endoplasm is the nucleus within a tough nuclear membrane. The nucleus is usually single, but may be double or multiple, some species having as many as a hundred nuclei in one cell. The nucleus contains one or more nucleoli or a central endosome or karyosome. The chromatin may be distributed along the inner surface of the nuclear membrane (peripheral chromatin) or as condensed masses around the karyosome. The endoplasm shows a number of structures—the endoplasmic reticulum, mitochondria and Golgi bodies. Contractile vacuoles may be present which serve to regulate the osmotic pressure. Several food vacuoles also may be seen. The active feeding and growing stage of the protozoa is called the *trophozoite* (G.trophos-nourishment). The cell may obtain nourishment from the environment by diffusion or by active transport across the plasma membrane. Larger food particles are taken in by phagocytosis through pseudopodia. Some species ingest food through special mouth-like structures or

cytostomes. Minute droplets of food may also enter by pinocytosis. Several species possess a resting or resistant cystic stage which enables prolonged survival under unfavourable conditions. The cystic stage may also involve reproduction by the nucleus dividing once or more to give rise to daughter trophozoites on excystation. The cyst is usually the infective stage for the vertebrate host.

Reproduction is usually asexual. The most common method is binary fission by mitotic division of the nucleus, followed by division of the cytoplasm. In amoebae, division occurs along any plane, but in flagellates division is along the longitudinal axis and in ciliates in the transverse plane. Some protozoa, as for instance the malaria parasites exhibit schizogony in which the nucleus undergoes several successive

divisions within the schizont to produce a large number of merozoites. Sexual stages are seen in ciliates and sporozoa. In ciliates the sexual process is conjugation in which two organisms join together and reciprocally exchange nuclear material. In sporozoa, male and female gametocytes are produced, which after fertilisation form the zygote giving rise to numerous sporozoites by sporogony.

CLASSIFICATION OF PROTOZOA

Protozoan parasites of medical importance have been classified into the following groups or Phyla: Sarcomastigophora, Apicomplexa, Microspora and Ciliophora.

A. Phylum Sarcomastigophora

Phylum Sarcomastigophora has been subdivided into two subphyla based on their modes of locomotion—Amoebae which have no permanent locomotory organs, but move about with the aid of temporary prolongations of the body called pseudopodia are grouped under subphylum *Sarcodina* (Sarcos, meaning flesh or body); and protozoa possessing whip-like flagella are grouped under subphylum *Mastigophora* (Mastix, meaning whip or flagellum).

Amoebae

These protean animalcules assume any shape and crawl along surfaces by means of foot-like projections called *pseudopodia* (literally meaning false feet).They are structurally very simple and are believed to have evolved from the flagellates by the loss of the flagella. Two groups of amoebae are of medical importance.

(a) *Amoebae of the alimentary canal:* The most important of these is *Entamoeba histolytica* which causes intestinal and extraintestinal amoebiasis. Amoebae are also present in the mouth.

(b) *Potentially pathogenic free-Iiving amoebae:* Several species of saprophytic amoebae are found in soil and water. Two of these, Naegleria and Acanthamoeba are of clinical interest because they can cause eye infections and fatal meningoencephalitis.

Flagellates

These protozoa have whip-like appendages called flagella as the organs of locomotion. The fibrillar structure of flagella is identical with that of spirochaetes and it has been suggested that they may have been derived from symbiotic spirochaetes which have become endoparasitic. In some species the flagellum runs parallel to the body surface, to which it is connected by a membrane called the undulating membrane.

Flagellates parasitic for man are divided into two groups:

(a) *Kinetoplastida:* These possess a kinetoplast from which arises a single flagellum. They are the *haemoflagellates* comprising the trypanosomes and leishmania which are transmitted by blood sucking insects and cause systemic or local infections.

(b) *Flagellates without kinetoplast:* These bear multiple flagella. Giardia, trichomonas and other *luminal flagellates* belong to this group. Because most of them live in the intestine, they are generally called *intestinal flagellates*.

B. Phylum Apicomplexa

Phylum Apicomplexa formerly known as Sporozoa, members of this group possess at some stage in their life cycle, a structure called the *apical complex* serving as the organ of attachment to host cells. They are tissue parasites. They have a complex life cycle with alternating sexual and asexual generations. To this group belong the malaria parasites (Suborder Haemosporina, Family Plasmodiidae); toxoplasma, sarcocystis, isospora and cryptosporidum (under the Suborder Eimeriina); babesia (under the Subclass Piroplasma); and the unclassified *Pneumocystis carinii*.

C. Phylum Microspora

Phylum microspora contains many minute intracellular protozoan parasites which frequently cause disease in immunodeficient subjects. They may rarely also cause illness in the immunocompetent.

D. Phylum Ciliophora

These protozoa are motile by means of cilia which cover their entire body surface. The only human parasite in this group is *Balantidium coli* which rarely causes dysentery. The zoological classification of protozoa is complex and subject to frequent revisions. The following is an abridged version of the classification proposed in 1980 by the Committee on Systematics and Evolution of the Society of Protozoologists, as applied to protozoa of medical importance.

Kingdom ANlMALIA

Subkingdom PROTOZOA

PHYLUM SARCOMASTIGOPHORA (having flagella or pseudopodia)

Subphylum MASTIGOPHORA (having one or more flagella)

Class ZOOMASTIGOPHORA

AMOEBAE

Amoebae are structurally simple protozoa which have no fixed shape. They are classified under the

Phylum -Sarcomastigophora

Subphylum -Sarcodina

Superclass -Rhizopoda

Order -Amoebida.

The cytoplasm is bounded by a unit membrane and can be differentiated into an outer ectoplasm and an inner endoplasm. Pseudopodia are formed by the ectoplasm thrusting out, being followed by the endoplasm flowing in, to produce blunt projections. Pseudopodial processes appear and disappear, producing quick changes in the shape of the cell. These are employed for locomotion and engulfment of food by phagocytosis. Amoebae may be free-Iiving or parasitic. A few of the free-living amoebae can, on occasion act as human pathogens, producing meningoencephalitis and other infections. Some of them can act as carriers of pathogenic bacteria. The parasitic amoebae inhabit the alimentary canal.

PARASITIC AMOEBAE

Parasitic amoebae belong to the following genera:

Genus	*Species*
1. *Entamoeba*	*E.histolytica, E.hartmanni, E.coli, E.polecki*
2. *Endolimax*	*E.nana*
3. *Iodamoeba*	*I.butschlii*
4. *Dientamoeba*	*D.fragilis* (now classified as Amoeboflagellate)

ENTAMOEBA HISTOLYTICA

History

Entamoeba histolytica was discovered in 1875 by Losch in the dysenteric feces of a patient in St Petersburg, Russia. He also observed it in colonic ulcers at autopsy and produced dysentery in a dog by inoculation through the rectum. In 1890, WilliamOsIer reported the case of a young man with dysentery who later died of liver abscess. Councilman and Lafleur in 1891 established the pathogenesis of intestinal and hepatic amoebiasis and introduced the terms 'amoebic dysentery' and 'amoebic liver abscess.'

Geographical Distribution

E. histolytica is world-wide in prevalence. It is much more common in the tropics than elsewhere, but it has been found wherever sanitation is poor, in all climatic zones, from Alaska (61° N) to the Straits of Magellan (52°S). It has been reported that about 10 per cent of the world's population and 50 per cent of the inhabitants

of some developing countries may be infected with the parasite. The infection is not uncommon even in affluent countries, about 1 per cent of Americans being reported to be infected. While the large majority of the infected are asymptomatic, invasive amoebiasis causes disabling illness in an estimated 50 million persons and death in 50,000 annually, mostly in the tropical belt of Asia. Africa and Latin America. It is the third leading parasitic cause of mortality, after malaria and schistosomiasis. *E. histolytica* is found in the human colon. Natural infection also occurs in monkeys, dogs and probably in pigs also but these animals do not appear to be relevant as sources of human infection. Infection is mostly asymptomatic. It commonly occurs in the lume of the colon as a commensal, but sometimes invades the intestinal tissues to become a pathogen.

Morphology

E. histolytica occurs in three forms—the trophozoite, precystic and cystic stages (Fig. 5.1).

Trophozoite

The trophozoite or the vegetative form is the growing or feeding stage of the parasite. It is irregular in shape and varies in size from about 12 to 60 μm. It is large and actively motile in freshly passed dysenteric stools, while in convalescents and carriers, it is much smaller. The parasite as it occurs free in the lumen as a commensal is generally smaller in size, about 15 to 20 μm and has been called the minuta form.

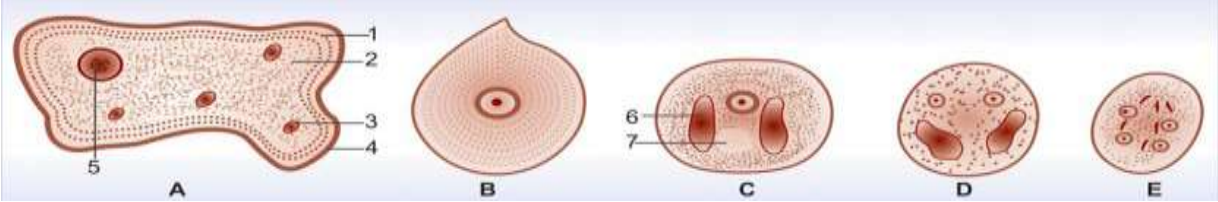


FIGURE 5.1: *Entamoeba histolytica.* (A) Trophozoite; (B) Precystic stage; (C) Uninucleate cyst; (D) Binucleate cyst; (E) Mature quadrinucleate cyst; 1—Ectoplasm; 2—Endoplasm; 3—Ingested erythrocytes; 4—Pseudopodium; 5—Nucleus; 6—Chromidial bar; 7—Glycogen mass

The protoplasm is differentiated into a thin outer layer of clear, transparent, refractive ectoplasm and an inner finely granular endoplasm having a ground glass appearance. Pseudopodia are formed by a sudden thrusting movement of the ectoplasm in one direction, followed by the streaming in of the whole endoplasm. The direction of movement may be changed suddenly, with another pseudopodium being formed at a different site, when the whole cytoplasm flows in the direction of the new pseudopodium. Typical amoeboid motility is a crawling or gliding movement and not a free-swimming one. The cell has to be attached to some surface or particle for it to move. In culture tubes, the trophozoites may be seen crawling up the side of the glass tube. Pseudopodium formation and motility are inhibited at low temperatures. The endoplasm contains the nucleus, food vacuoles and granules. The nucleus is not clearly seen in the living trophozoite, but can be distinctly demonstrated in preparations stained with iron-haematoxylin or Gomorri's trichrome stains. The nucleus is spherical, 4 to 6 μm in size and contains a small central karyosome surrounded by a clear halo. The karyosome is anchored to the inner surface of the nuclear membrane by fine radiating fibrils called the linin network giving a 'cartwheel appearance.' The delicate nuclear membrane is lined by a rim of chromatin distributed evenly as

small granules. The trophozoites from acute dysenteric stools often contain phagocytosed erythrocytes. This feature is diagnostic as phagocytosed red cells are not found in any other commensal intestinal amoebae. The trophozoite divides by binary fission once in about 8 hours. Trophozoites are delicate organisms and are killed by drying, heat and chemical disinfectants, They do not survive for any length of time in stools outside the body. Therefore, the infection is not transmitted by trophozoites. Even if live trophozoites from freshly passed stools are ingested, they are rapidly destroyed in the stomach and cannot initiate infection.

Precystic Stage

Some trophozoites undergo encystment in the intestinal lumen. Encystment does not occur in the tissues nor in feces outside the body. Before encystment the trophozoite extrudes its food vacuoles and becomes round or ovoid about 10 to 20 μm in size. This is the precystic stage of the parasite. It secretes a highly refractile cyst wall around it and becomes the cyst.

Cystic Stage

The cyst is spherical, about 10 to 20 μm in size. The early cyst contains a single nucleus and two other structures—a mass of glycogen and one to four chromatoid bodies or chromidial bars, which are cigar-shaped or oblong refractile rods with rounded ends. The chromatoid bodies are so called because they stain with haematoxylin like chromatin. As the cyst matures, the glycogen mass and chromidial bars disappear. The nucleus undergoes two successive mitotic divisions to form two and then four nuclei. The mature cyst is quadrinucleate. The nuclei and chromidial bodies can be made out in unstained films, but they appear more prominently in stained preparations. With iron-haematoxylin stain the nuclear chromatin and the chromatoid bodies appear deep blue-black, while the glycogen mass appears unstained. When stained with iodine the glycogen mass appears golden brown, the nuclear chromatin and karyosome bright yellow and the chromidial bars appear as clear spaces, being unstained.

Life Cycle

The infective form of the parasite is the mature cyst passed in the feces of convalescents and carriers. The cysts can remain viable under moist conditions for about ten days. The cysts

ingested in contaminated food or water pass through the stomach undamaged and enter the small intestine. When the surrounding medium becomes alkaline. The cyst wall is damaged by trypsin in the intestine, leading to excystation. The cytoplasm gets detached from the cyst wall and amoeboid movements appear causing a tear in the cyst wall through which the quadrinucleate amoeba emerges. This stage is called the *metacyst.* The nuclei in the metacyst immediately undergo division to form eight nuclei, each of which gets surrounded by its own cytoplasm to become eight small amoebulae or metacystic trophozoites. If excystation takes place in the small intestine, the metacystic trophozoites do not colonise there, but are carried to the caecum. The optimum habitat for the metacystic trophozoites is the caecal mucosa where they lodge in the glandular crypts and undergo binary fission. Some develop into precystic forms and cysts, which are passed in feces to repeat the cycle (Figs 5.2 and 5.3). The entire life cycle is thus completed in one host. Infection with *E. histolytica* does not necessarily lead to disease. Infact, in most cases it remains within the lumen of the large intestine, feeding on the colonic contents and mucus as a commensal without causing any ill effects. Such persons become carriers or asymptomatic cyst passers, as their stool contains cysts. They are responsible for the maintenance and spread of infection in the community. The infection may get spontaneously eliminated in many of them. Sometimes, the infection may be activated and clinical disease ensues. Such latency and reactivation are characteristicof amoebiasis.

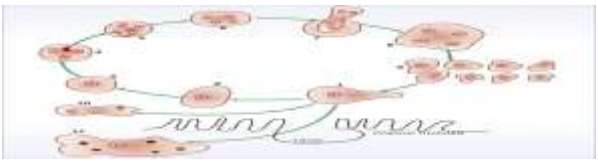


FIGURE 5.2: Life cycle of *E. histolytica.* (1) Trophozoite in gut lumen, (2) Precystic form, (3) Uninucleate cyst, (4) Binucleate cyst, (5) Quadrinucleate cyst, passed in faeces, (6) Mature cyst—infective when ingested, (7) Excystation in small intestine, (8) Metacystic form, (9) Eight daughter amoebulae, (10) Trophozoite shed in faeces—cannot encyst, (11) Tissue form of trophozoite in colonic ulcer—shows ingested erythrocytes

FIGURE 5.3: Life cycle of *E. histolytica* (Schematic)

.**FLAGELLATES**

Parasitic protozoa which possess whip-like flagella as their organs of locomotion are classified under the Phylum-Sarcomastigophora, Subphylum-Mastigophora, Class- Zoomastigophorea (from *mastix*-whip, *phoros*-bearing). Depending on their habitat, they can be considered under two headings:

1. *Lumen-dwelling flagellates:* Flagellates found in the alimentary and urogenital tracts.

2. *Haemoflagellates:* Flagellates found in blood and tissues.

INTESTINAL FLAGELLATES

Most luminal flagellates are nonpathogenic commensals. Two of them cause clinical disease, *Giardia lamblia* which can cause diarrhoea and *Trichomonas vaginalis* which can produce vaginitis and urethritis. Intestinal flagellates found in humans are listed below, with the sites affected by them shown in parenthesis.

1. *Giardia lamblia* (duodenum, jejunum)

2. (a)*Trichomonas vaginalis* (vagina, urethra); (b) *T. tenax* (mouth); (c) *T. hominis* (caecum)

3. *Chilomastix mesnili* (caecum)

4. *Enteromonas hominis* (colon)

5. *Retortamonas intestinalis* (colon)

6. *Dientamoeba fragilis*.

GIARDIA LAMBLIA

History and Distribution

This flagellate was observed by Leeuwenhoek (1681) in his own stools and was thus one of the earliest of protozoan parasites to have been recorded. It is named Giardia after Professor Giard of Paris and lamblia after Professor Lambl of Prague who gave a detailed description of the parasite. Worldwide in distribution, it is the most common intestinal protozoan pathogen. Infection may be asymptomatic or cause diarrhoea.

Morphology and Life Cycle

G. lamblia lives in the duodenum and upper jejunum and is the only protozoan parasite found in the lumen of the human small intestine. It occurs in the vegetative and cystic forms. The vegetative form or *trophozoite* is rounded anteriorly and pointed posteriorly, about 15 μm long, 9 μm wide and 4 μm thick. It has been described variously as pyriform, heart-shaped or racket-shaped. Dorsally it is convex and ventrally it has a concave *sucking disc* which occupies almost the entire anterior half of the body. It is bilaterally symmetrical and possesses 2 nuclei, one on either side of the midline, two *axostyles* running along the midline, 4 pairs of flagella and 2 sausage shaped *parabasal* or *median bodies* lying transversely posterior to the sucking disc (Fig. 5.7). The trophozoite is motile, with a slow oscillation about its long axis, which has been likened to the motion of a 'falling leaf.' It divides by longitudinal binary fission. It lives in the duodenum and upper part of the jejunum attached by means of the sucking disc to the epithelial cells of the villi and crypts feeding by pinocytosis. Encystation occurs in the colon. The trophozoite retracts its flagella into the axonemes which remain as curved bristles in the cyst. The cyst is ovoid about 12 μm by 8 μm in size and surrounded by a tough hyaline cyst wall. The young cyst contains two and the mature cyst four nuclei situated at one end. Cysts are passed in stools and remain viable in soil and water for several weeks. There may be up to 2,00,000 cysts present per gram of faeces. In diarrhoeic stools trophozoites also may be present, but they die outside and are not infectious. Infection is acquired by the ingestion of cysts in contaminated food and water. Infectivity is high, as few as 10 cysts being capable of initiating infection. Within half an hour of ingestion, the cyst hatches out into two trophozoites which multiply

successively by binary fission and colonise the duodenum. The trophozoites as they pass down the colon develop into cysts.

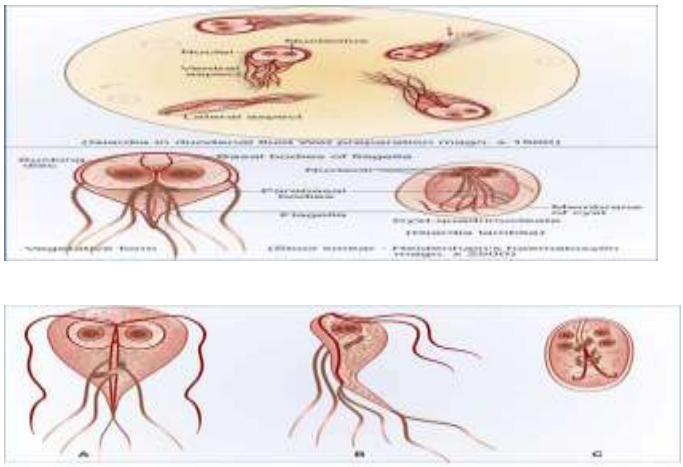


FIGURE 5.7: *Giardia lamblia.* (A) Trophozoite—ventral view (B) Trophozoite—lateral view (C) Cyst

TRICHOMONAS VAGINALIS

History

Trichomonas vaginalis was first observed by Donne (1836) in vaginal secretion.

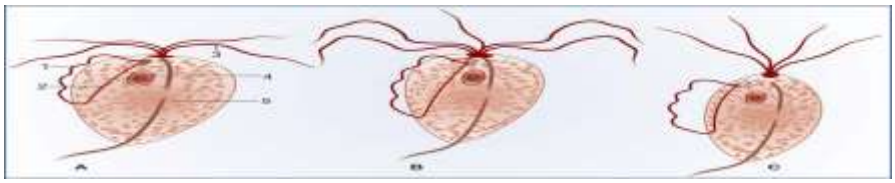


FIGURE 5.7: Trichomonas species. (A) T. vaginalis, (B) T. hominis, (C) T. tenax. 1—Undulating membrane, 2—Costa, 3—Flagella, 4—Nucleus, 5—Axostyle

Morphology and Life Cycle

T. vaginalis occurs only as the trophozoite, there being no cystic form in trichomonas. The trophozoite is ovoid or pear-shaped, about 10 to 30 μm long and 5 to 10 μm broad, with a short undulating membrane reaching up to the middle of the body. It has 4 anterior flagella and a fifth running along the outer margin of the undulating

membrane, which is supported at its base by a flexible rod, the costa. A prominent axostyle runs throughout the length of the body and projects posteriorly. The cytoplasm shows prominent granules which are most numerous alongside the axostyle and costa (Fig. 5.7).It lives in the vagina and cervix in the female, and may also be found in the Bartholin's glands, urethra and even the urinary bladder. In males, it occurs mainly in the anterior urethra, but it may also be found in the prostate and preputial sac. It is motile, with a jerky rapid movement. It divides by binary fission. As cysts are not formed, the trophozoite itself is the infective form.

TRICHOMONAS TENAX

Also known as T.buccalis , this is smaller (5 to 10 μm) than T.vaginalis. It is a harmless commensal which lives in the mouth. in the periodontal pockets, carious tooth cavities and less often in tonsillar crypts. It is transmitted by kissing, salivary droplets and fomites.

TRICHOMONAS HOMINIS

This measures 8 to 12 μm and carries 5 anterior flagella and an undulating membrane that extends the full length of the body. It is a very common harmless commensal of the caecum.

Malaria Parasites

Malaria is the most important parasitic disease of mankind. It accounts for over 300 million cases and 2 million deaths annually, the large majority of them in Subsaharan Africa. Once prevalent over much of the world, it is now confined to the tropical and subtropical areas of Asia, Africa, South and Central America. Even so, nearly half of the world's population may be exposed to the risk of malaria.

MALARIA

History

Malaria (or *ague*, as it was called earlier) has been known from antiquity . Seasonal intermittent fevers with chills and shivering, recorded in the religious and medical texts of ancient Indian, Chinese and Assyrian civilisations, are believed to have been malaria. Charaka and Susruta have described the disease and noted its association with mosquitoes. Hippocrates in Greece in the 5th

century BC gave a detailed account of the clinical picture and observed the prevalence of the disease in certain places and seasons. The relation between the disease and stagnant waters, swamps and marshy lands was recognised and measures to control the disease by effective drainage were practised in Rome and Greece by the 6th century AD. The name malaria (*mal*bad, *aria*-air) was given in the 18th century in Italy as it was believed to be caused by foul emanations from the marshy soil. Paludism, another name for malaria, also has a similar origin from *palus*, Latin for 'marsh'. The recent demonstration of a specific parasitic antigen in Egyptian mummies indicates that malaria was present thousands of years ago.

The specific causative agent of malaria was discovered in the red blood cells of a patient in 1880 by Alphonse Laveran, a French army surgeon in Algeria. In 1886, Golgi in Italy described the asexual development of the parasite in red blood cells (*erythrocytic schizogony*), which therefore came to be called the *Golgi cycle*. Romanowsky in Russia in 1891, developed a method of staining malaria parasites in blood films.Three different species of malaria parasites infecting man, *Plasmodium vivax, P. malariae* and *P.falciparum* were described in Italy between 1886 and 1890. The fourth species, *P.ovale* was identified only in 1922. The mode of transmission of the disease was established in 1897, when Ronald Ross in Secunderabad, India identified the developing stages of malaria parasites in mosquitoes. This led to various measures for the control and possible eradication of malaria by mosquito control. Both Ross (1902) and Laveran(1907) won the Nobel Prize for their discoveries in malaria.

Causative Agents

Four species of plasmodia cause malaria in man, *P. vivax, P. falciparum, P. malariae* and *P. ovale*. Two species, *P.vivax* and *P.falciparum* account for about 95 per cent of all malaria worldwide, the other two being of relatively minor importance.While these four species do not ordinarily infect animals, there is evidence that chimpanzees may act as a reservoir host for *P. malariae* in Africa, providing a possible source of human infection.

Malaria parasites belong to phylum-Apicomplexa, class-Sporozoea, Order- Eucoccidea, Suborder-Haemosporina. The genus *Plasmodium* is divided into 2 subgenera;—*P. vivax, P.*

malariae and *P. ovale* belong to the subgenus *Plasmodium* while *P. falciparum* is allocated to the subgenus Laverania because it differs in a number of respects from the other three species.

Several species of *Plasmodium* cause natural infection in birds and animals. Examples of monkey malaria parasites that have been used widely for experimental studies on malaria are *P.cyanomolgi, P.inui,* and *P.knowlesi.* While it is possible to produce experimental infection in man with some of these simian parasites, there is no evidence that this occurs to any significant extent in nature. *P.knowlesi,* a natural parasite of rhesus monkeys, is found to infect aborigines in the jungles of Malaysia. Examples of plasmodia infecting birds are *P.gallinaceum* and *P.elongatum*. *P. vivax, P. malariae* and *P.ovale* are closely related to other primate malaria parasites. *P. falciparum* on the other hand, is more related to bird malaria parasites, and appears to be a recent parasite of humans, in evolutionary terms. Perhaps for this reason, falciparum infection causes the severest form of malaria and is responsible for nearly all fatal cases.

Vectors

Human malaria is transmitted by the female Anopheles mosquito. The male mosquito feeds exclusively on fruit juices, but the female needs at least two blood meals before the first batch of eggs can be laid. Malaria parasites of animals (apes, monkeys, rodents) are transmitted by *Anopheles,* but bird malaria parasites are carried by *Culex, Aedes* and other genera of mosquitoes.

Life Cycle and Morphology

The life cycle of malaria parasites comprises two stages—an *asexual phase* occurring in humans and the *sexual phase* occurring in the mosquito. In the asexual phase the parasite multiplies by division or splitting, a process designated *schizogony* (from *schizo*-to split, and *gone*-generation). Because this asexual phase occurs in man it is also called the *vertebrate, intrinsic* or *endogenous phase*. In humans, schizogony occurs in two locations—in the red blood cell (*erythrocytic schizogony*) and in the liver cells (*exoerythrocytic schizogony* or the *tissue phase*). Because schizogony in the liver is an essential step before the parasites can invade erythrocytes, it is called *pre-erythrocytic schizogony*. The products of schizogony, whether erythrocytic or exoerythrocytic, are called *merozoites* (*meros*-a part, *zoon*-animal).

ii. The sexual phase takes place in the female Anopheles mosquito, even though the sexual forms of the parasite (*gametocytes*) originate in human red blood cells. Maturation and fertilisation take place in the mosquito, giving rise to a large number of sporozoites (from *sporos*-seed). Hence this phase of sexual multiplication is called *sporogony*. It is also called the *invertebrate, extrinsic*, or *exogenous phase*.

There is thus an *alternation of generations* in the life cycle of malaria parasites— asexual and sexual generations alternatively. There also occurs an *alternation of hosts*, as the asexual phase takes place in humans followed by the sexual phase in the mosquito. Therefore, the complete life cycle of the malaria parasite comprises an alternation of generations with an alternation of hosts. As the sexual phase occurs

in the mosquito, it is considered the *definitive host* of malaria parasites. Humans are the *intermediate host* as the human phase consists of asexual multiplication.

The Human Phase

Human infection comes through the bite of the infective female Anopheles mosquito. The sporozoites which are infective forms of the parasite are present in the salivary gland of the mosquito. They are injected into blood capillaries when the mosquito feeds on blood after piercing the skin. Usually 10 to 15 sporozoites are injected at a time, but occasionally many hundreds may be introduced. The sporozoites pass into the blood stream, where many are destroyed by the phagocytes, but some reach the liver and enter the parenchymal cells (hepatocytes).

Exo-erythrocytic (Tissue) Stage

Within an hour of being injected into the body by the mosquito, the sporozoites reach the liver and enter the hepatocytes to initiate the stage of pre-erythrocytic schizogony or *merogony*. The sporozoites which are elongated spindle-shaped bodies become rounded inside the liver cells. They enlarge in size and undergo repeated nuclear division to form several daughter nuclei, each of which is surrounded by

cytoplasm. This stage of the parasite is called the *pre-erythrocytic* or *exoerythrocytic schizont* or *meront*. The hepatocyte is distended by the enlarging schizont and the liver cell nucleus is pushed to the periphery. Unlike in erythrocytic schizogony, there is no pigment in liver schizonts. In 5.5 to 15 days the schizont becomes mature and bursts, releasing thousands of merozoites. They enter the blood stream and infect

the erythrocytes by a process of invagination. The interval between the entry of the sporozoites into the body and the first appearance of the parasites in blood is called the prepatent period. The duration of the pre-erythrocytic phase in the liver, the size of the mature schizont and the number of merozoites produced vary with the species of the parasite.

Pre-erythrocytic schizogony involves only a very small proportion of liver cells and causes no significant damage or clinical illness. Liver schizonts cannot be demonstrated in natural human infections, but have been observed in splenectomised chimpanzees or human volunteers experimentally infected with very large numbers of sporozoites (Table 5.1).

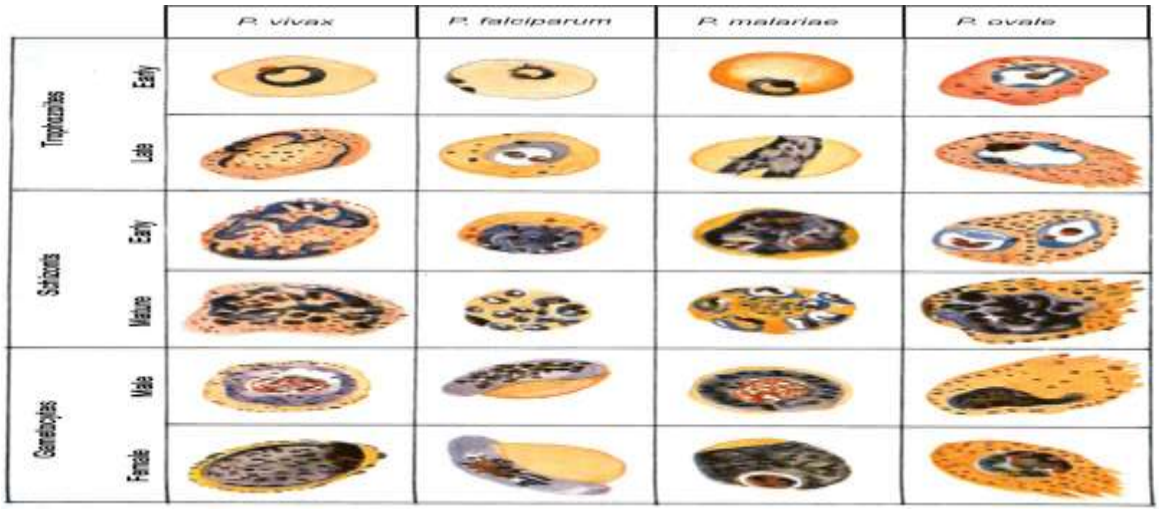


FIGURE 5.8: Malaria parasites—Erythrocytic stages of the four species (Giemsa stain. Magn × 2000)

Formerly, it was postulated that some merozoites released after the primary exoerythrocytic schizogony invaded other hepatocytes to initiate the secondary exoerythrocytic schizogony. Such exoerythrocytic schizogony was believed to be repeated for a few generations and was

considered to explain the occurrence of relapses in *P. vivax* and *P. ovale* infections. This view is no longer held. In *P.vivax* and *P. ovale*, two kinds of sporozoites are seen, some which multiply inside hepatocytes promptly to form schizonts and others which remain dormant. These latter forms are called *hypnozoites* (from *hypnos*-sleep). Hypnozoites remain inside the hepatocytes as uninucleated forms, 4 to 5 μm in diameter, for long periods. From time-to-time, some are activated to become schizonts and release merozoites, which go on to infect erythrocytes, producing clinical relapses. This is the present concept of relapses in vivax and ovale malaria.

Table 5.1: Features of pre-erythrocytic schizogony in human malaria parasites

	P.vivax	*P.falciparum*	*P.malariae*	*P.ovale*
Pre-erythrocytic stage (days)	8	5.5	15	9
Diameter of pre-erythrocytic schizont (μm)	45	60	55	60
No. of merozoites in pre-erythrocytic schizont	10,000	30,000	15,000	15,000

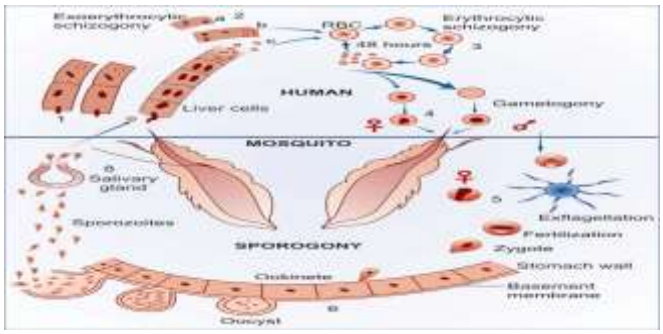


FIGURE 5.9: Life cycle of *Plasmodium vivax*. (1) Sporozoites present in thesalivary gland of female Anopheles mosquito are injected into skin capillaries when the mosquito bites humans. (2) They enter liver cells to initiate exoerythrocytic schizogony. Some sporozoites become dormant hypnozoites (2a,b) which are reactivated after varying intervals to produce relapses. Most sporozoites complete the pre-erythrocytic schizogony (2c). to form merozoites which infect red blood cells, to initiate the cycle of erythrocytic schizogony (3) which is repeated every 48 hours. Some merozoites initiate gametogony, forming male and female gametocytes (4) which are ingested by mosquito in its blood meal. Male gametocyte undergoes exflagellation.(5) one male gamete fertilises female gamete to form zygote. It develops into the motile ookinete, which

penetrates the stomach wall and becomes the oocyst inside which sporozoites develop. (6) Sporozoites released by rupture of mature oocyst (7) enter the haemocoele (8) and reach the salivary glandsof the mosquito.

Secondary exoerythrocytic schizogony is not believed to occur (Fig. 5.9). In *P.falciparum* and *P.malariae* no hypnozoites are formed and the parasites do not persist in the exoerythrocytic phase. However, a small number of erythrocytic parasites persist in the blood stream, and in course of time, multiply to reach significant numbers, resulting in clinical disease (*short-term relapse* or *recrudescence*). In falciparum malaria recrudescences are seen for one or two years, while in *P.malariae* infections, they may last for long periods, even up to 50 years.

Erythrocytic Stage

The merozoites released by pre-erythrocytic schizonts invade the red blood cells. The receptor for merozoites is glycophorin, which is a major glycoprotein on the red cell. The differences in the glycophorins of red cells of different species may account for the species specificity of malaria parasites. Merozoites are pear-shaped bodies about 1.5 μm in length, possessing an *apical complex* (*rhoptry*). They attach to erythrocytes by their apex, which has certain organelles that secrete a substance producing a pit on the erythrocyte membrane. The merozoite then enters the erythrocyte by endocytosis and the red cell membrane seals itself to form a vacuole (*parasitophorous vacuole*) enclosing the merozoite. The process of entry into the red cell takes about 30 seconds. Once inside the red cell the merozoite rounds up and loses its internal organelles. In the erythrocyte, the merozoite appears as a rounded body having a vacuole in the centre with the cytoplasm pushed to the periphery and the nucleus situated at one pole. When stained with Giemsa or other Romanowsky stains, the cytoplasm is stained blue and the nucleus red the central vacuole remaining unstained. This gives the parasite an annular or signet ring appearance. These young parasites are therefore called the *ring forms.*

KARPAGAM ACADEMY OF HIGHER EDUCATION FUNDAMENTALS OF MICROBIOLOGY AND CLASSIFICATION (17MBP101)	
Unit IIIQuestions	**Opt 1**
_____ or alcology is the study of algae.	Phycology
The term algae was originally used to simple _____	Marine plants
Dinoflagellates have chlorophylls_____	a & c
Diatom frustules are composed of crystallized _____	Calcium
Phycocyanin is a _____-	Red pigment
Agar, which is the solidifying agent in many bacterial culture media, is part	*Chlorophyta*
Red algae cell wall made up of _____.	Galactose
Starch is an energy storage material characteristic of	*Chlorophyta*
Which algal division never produces motile, flagellated cells among any of	*Chlorophyta*
Rhodophyta is a _____	*Red Algae*
Red Algae contain _____	Phycoerythrin
Blue pigment is known as _____	Phycocyanin
The classical classification of algae on recognizes seven divisions that were	Photosynthetic pi
The chloroplast have membrane bound sac called _____ that carryout t	Thylakoids
Starch synthesis in algae takes place in _____	Chloroblast
Chloplast contain chlorophyll a and b together with _____	Carotenoids
Contractile vacuole which present in euglenophyta , regulating the _____	Osmotic pressure
Chlamydomonas is_____	Red algae
Chlamydomonas consist of _____ nucleus	Multi
Algae reproduce asexually by producing_____	Zoospores
Example for non-mobile unicellular green algae such as_____	*Chollera*
Example of motile algae_____	Volvox
Euglenoids_____	Share with the ch
Chloroplast contain _____	Chlorophylls a &
The branch of microbiology that deals with the study of fungi is called ____	parasitology
The study and effect of fungal toxins and their effects is called _____	Mycoses
A character that promotes the pathogenic potential of fungus is called as __	toxin
A disease caused by a fungus is called _____	mycolysis
Fungi is _____	eukayotes
Fungi are	aerobic
Fungi are	chemolithotrophs
Fungi differ from the other eukaryotic microbes in having	flagella
Which of the following is not a member of the division Ascomycota?	*Aspergillus*
Mycorrhizae are mutualistic associations between fungi and	bacteria
Which of the following structures would not be associated with fungi?	Mitochondria
Fungi posses a cell membrane that contains _____	lipids
Ascospores are produced and enclosed in a sac like structure called _____	basidium
Basidiospores are borne in a specialised stalk called_____	basidium
Give an example for yeast like fungi____	*Cryptococcus*
Give an example for thermally dimorphic fungi_____	*Cryptococcus*

Give an example for filamentous fungi_____	*Cryptococcus*
Sporangium with sporangiospores is called ____	*Basidiomycete*
Ascus with ascospores is called _____	*Basidiomycete*
Basidium with basidiospores is called _____	*Basidiomycete*
Aspergillus flavus &*Aspergillus parasiticus* secretes _____	verotoxin
Which of the following does not represent a human disease caused by fungi	Ringworm
Specific media for the isolation of fungi is	brain heart infusi
Mechanism of pyrimidine	binds to sterol ca
Primary infection for coccidioidomycosis is _____	UTI
Fungi are important in the production of all of the following commercial pr	bread
Most fungi are soil _____	Parasites
Which is / are the medium/ media used to cultivate Entamoeba histolytica ?	Macconkey medi
Identify the commensal amoeba living in the mouth	Entamoeba gingi
Giardia interferes with the absorption of	Fat
Protozoans were first observed by _____	pasteur
In protozoa, in addition to cell membrane, a compound envelope of a modif	pedicle
Trichomonas belongs to _____ phyllum	protozoa
Trichomonas belongs to _____ class	sarcodina
Entamoeba belongs to _____ class	sarcodina
Plasmodium belongs to _____ class	sarcodina
Trichomonas comes under _____ order	kinetoplasta
Plasmodium vivax causes	quartan fever
Plasmodium malariae causes	quartan fever
Plasmodium falciparum causes	quartan fever
Giardia have _____ ribosome	20S
Plasmodium ovale causes _____	quartan fever
Microscopic examination of _____ will reveal the presence of trichom	blood
Who gave the name Entamoeba histolytica?	Lable
Protozoans are to be regarded as _____	unicellular
The term protozoa was first used by _____	Goldfuss
Cell membrane of protozoa is also called as _____	cellwall
Assembly of DNA virus occurs in _____ of the host.	Nucleus
Viruses require _____ for growth.	bacteria
RNA viruses get assembled in _____ of the host.	Capsid
The viral nucleocapsid is the combination of	genome and caps
Enveloped viruses are released from the host cell by the process of ____.	Lysogeny
Naked viruses are released from the host cell by _____.	Cell Lysis
Picorna Viruses are small _____ viruses.	DNA
Poliovirus comes under _____ genera of Picornaviruses.	Rhinoviruses
Total antigenic types of poliovirus include ___ types.	4
The nucleocapsid is covered by an outer membrane like structure called	envelope
Which of the following viruses has not been associated with human cancer?	*Hepatitis C virus*
The tail of phage T4 is _____ in length.	10
____ phage produces lysis of infected cells releasing large number of proge	Temperate

In ____ phage, phage DNA gets integrated into the bacterial chromosome.	Temperate
The integrated phage is known as _____ .	Coliphage
The bacteria with intetgrated phage is known as ______ .	Lysogen
In phage life cycle when the host is ruptured it is known as ______ cycle.	Lytic
____ phage enzymes weakens the cell wall during replication of phage.	Neuraminidase
The time interval between the infection of host cell and sudden increase in e	Eclipse period

Opt 2	**Opt 3**	**Opt 4**	**Opt 5**	**Opt 6**
Physiology	Mycology	Zoology		
Aquatic plants	Fresh water plants	Plants		
a & b	a, b &c	b&z		
Potassium	Silica	Cadmium		
Blue pigment	Brown pigment	Yellow pigment.		
Chrysophyta	*Pyrrophyta*	*Rhodophyta*		
Glucose	Galactans	Glucan		
Chrysophyta	*Phaeophyta*	*Rhodophyta*		
Chrysophyta	*Phaeophyta*	*Rhodophyta*		
Brown algae	*Blue algae*	*BGA*		
Elythesin	Elythrocytin	Cynin		
Pyocyami	Pyuredin	Cynin		
Cell wall	Cell constituents	Reproduction		
Cell wall	Pyrenoids	Flagella		
Vacuole	Contractile vacuole	Pyrenoids		
Chlorophyll	Chitin	Pyrenoids		
Temperature	Light	Boiling point		
Blue algae	Blue algae	Brown algae		
Two	Single	Three		
Ascospores	Basidiospores	Myxospores		
Diatoms	*Cyanobacteria*	*Actinomycetes*		
Tricoderma	*Chrysophyta*	*Rhodophyta*		
Share with the chrysoph	Share with the dialorin &	Share with the chlorophyta &cyar		
Chlorophyll a	Chlorophyll b	chorophyll z		
mycology	myology	fungyology		
Mycotoxin	Mycotoxicology	Mycology		
enzyme	byproducts	virulence factor		
virulence	mycosis	mycorrhizae		
prokaryotes	archae	Animalia		
obligate anaerobes	obligate anaerobes or fac	obligate aerobe or facultative ana		
chemoorganotrophs	lithotrophs	physicotrophs		
ergosterol	chloroplasts	an undulating membrane		
Claviceps	*Penicillium*	*Rhizopus*		
protozoa	unicellular green algae	vascular plants		
Cell walls	Chloroplasts	Spores		
Protein	Fat	Glycerol		
zygus	ascus	sporangium		
zygus	ascus	sporangium		
Candida albicans	*Aspergillus*	*Histopl;asma*		
Candida albicans	*Aspergillus*	*Histoplasma*		

Candida albicans	*Aspergillus*	*Histoplasma*		
Zygomycete	*Ascomycete*	*Sporangiomycete*		
Zygomycete	*Ascomycete*	*Sporangiomycete*		
Zygomycete	*Ascomycete*	*Sporangiomycete*		
endotoxin	exotoxin	aflatoxin		
Cryptococcosis	Malaria	Jock itch		
sabourauds dextrose aga	nutrient agar	muller hinton agar		
inhibit ergosterol	inhibit DNA,RNA synth	inhibit microtubule assay		
pulmanary infection	skin infection	RTI		
beer	cheese	rubber		
Obligate Parasites	Saprophytes	virulence factor		
Philips medium	Simple medium	Differential media		
Entamoeba histolytica	Entamoeba coli	Entamoeba nana		
Carbohydrate	Protein	None of the above		
robert hoek	fritch	leeuwenhoek		
pentent	pellicle	persistent		
animalia	fungi	plantae		
flagellata	sporozoa	acompixa		
flagellata	sporozoa	acompixa		
flagellata	sporozoa	acompixa		
rhizopoda	sarcodina	sporozoa		
tertian malaria	oval tertian malaria	malignant tertian malaria		
tertian malaria	oval tertian malaria	malignant tertian malaria		
tertian malaria	oval tertian malaria	malignant tertian malaria		
30S	50S	70S		
tertian malaria	oval tertian malaria	malignant tertian malaria		
fresh vaginal discharge	csf	urine		
Losch	Schavdin	Louis		
multicellular animal	cellular animal	acellular animal		
Losch	leeuwenhoek	Schavdin		
plasma membrane	vacuole	plasmalemma		
Cytoplasm	Capsid	Envelope		
plants	animals	living cells		
Nucleus	Cytoplasm	Envelope		
capsid and spikes	envelope and capsid	capsomere and genome		
Lysis	Budding	Endocytosis		
Budding	Endocytosis	Phaging		
RNA	Obligate	Plant		
Aphthoviruses	Cardioviruses	Enteroviruses		
2	3	5		
covering	membronocapsid	capsid		
Hepatitis B virus	*Varicella-Zoster virus*	*Herpes simplex virus type 2*		
1000	100	10000		
Lysogenic	Tryptic	Virulent		

Lysogenic	Cryptic	Virulent		
Prophage	Lytic phage	Prephage		
Colin	Plasmin	Dolphin		
Symbiosis	Lysogeny	Temperate		
Polymerase	Muramidase	cellulose		
Window period	Dormant period	Latent period		

Answer
Phycology
Aquatic plants
a & c
Potassium
Blue pigment

Rhodophyta

Galactose

Rhodophyta

Rhodophyta

Red Algae
Elythrocytin
Pyocyami
Photosynthetic pigments
Thylakoids
Pyrenoids
Chlorophyll
Osmotic pressure
Blue algae
Two
Zoospores
Chollera
Volvox

Share with the chlorophyta &charophyta

Chlorophyll b
mycology
Mycotoxicology
virulence factor
mycosis
eukayotes

obligate aerobe or facultative anaerobe

chemoorganotrophs
ergosterol
Rhizopus
vascular plants

Chloroplasts

lipids
ascus
basidium
Candida albicans
Histoplasma

Aspergillus
Zygomycete
Ascomycete
Basidiomycete
aflatoxin
Malaria
sabourauds dextrose agar
inhibit DNA,RNA synthesis
pulmanary infection

rubber

Saprophytes
Philips medium
Entamoeba gingivalis
Fat
leeuwenhoek
pellicle
protozoa
flagellata
sarcodina
sporozoa
kinetoplasta
tertian malaria
quartan fever
malignant tertian malaria
70S
oval tertian malaria
fresh vaginal discharge
Schavdin
acellular animal
Goldfuss
plasmalemma
Nucleus
living cells
Cytoplasm
genome and capsid
Budding
Cell Lysis
RNA
Enteroviruses
3
envelope

Varicella-Zoster virus

100
Virulent

Temperate
Prophage
Lysogen
Lytic
Muramidase
Latent period

DEPARTMENT OF MICROBIOLOGY
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I M.Sc MICROBIOLOGY - 2017 – 2019 Batch

FUNDAMENTALS OF MICROBIOLOGY AND CLASSIFICATION (17MBP101)

SEMESTER – I **4H – 4C**

POSSIBLE QUESTION

Unit III

TWO MARK

1. Define Algae.
2. What is meant by Phycology?
3. What is meant by algology?
4. Comment on planktonic.
5. Define neustonic.
6. What is phytoplankton?
7. What is zooplankton?
8. Write about photoautotrops?
9. What is fragmentation?
10. Define thallus.
11. Comment on aplanospore.
12. Briefly explain oogonia.
13. Write notes on antheridia?
14. What is zygote?
15. Define stigma.

16. Write notes on chlorella?
17. Define protothecosis.
18. Comment on rhodophyta.
19. Define Diatoms.
20. Define kelps
21. What is trophozoite?
22. Define phagocytosis.
23. Define pinocytosis.
24. List out the four phyla of medically important protozoan parasite.
25. Define pseudopodia.
26. Define Apical complex.
27. What are Amoebae? Give four examples for parasitic amoebae.
28. Define Metacyst.
29. What are types of flagellates? Write two examples.
30. Define Golgi Cycle.
31. What is meant by Schizogony?
32. What is meant by Sporogony?
33. What is meant by Merogony?
34. What is Meront?
35. What are the stages involved in the life cycle of Malarial Parasite?
36. What is Hypnozoite?

37. Define exflagellation.

38. Write about the ring form of malarial parasite.

39. What is Malarial Pigment?

40. What is called as Amoeboid form?

41. Explain the appearance of Malarial Pigments in different species.

42. What is Malignant Tertian?

43. What is Quartan Malaria?

44. What is Ovale Tertian?

45. Define Virus.

EIGHT MARKS

46. Discuss about the distribution of Algae.

47. Explain the classification of Algae.

48. Describe the ultra structure of algal cell and explain its morphology.

49. Explain the algal reproduction.

50. Discuss in detail about the brown algae.

51. Comment on red algae.

52. Give a detailed note on diatoms.

53. Write about the life cycle of chlamydomonas.

54. Write about classification of fungi?

55. Briefly explain about importance of fungi?

56. Explain –reproduction of fungi?

57. Give an account on life cycle of fungi?

58. Discuss about ecology of fungi?

59. Write beneficial and harmful activities of fungi?

60. What are the different types of amoebae which are medically important?

61. What are the different types of motility present in protozoa?

62. Illustrate trophozoiteof *Entamoeba histolytia.*

63. Write few lines about morphology of *Entamoeba histolytia.*

64. Illustrate *Giardia lamblia.*

65. Write few points about the morphology of *Giardia lamblia.*

66. Illustrate *Trichomonas vaginalis*.

67. Write about the morphology of *Trichomonas.*

68. Which protozoan is known as Malarial Parasite?

69. Write about the vectors which transmit the malarial parasite.

70. List out the name of the Malarial Parasite.

71. Write about the properties of Viruses.

72. How to Virus different from living cells?

73. What are four different types of capsid symmetry present in viruses?

74. Illustrate different structure of viruses.

75. Differentiate RNA and DNA viruses with example?

76. Write a detailed note on Protozoa.

77. Explain the classification of Protozoa?

78. Explain the morphology of *Entamoeba histolytica* with a neat diagram?

79. Explain the life cycle of *Entamoeba histolytica* with a neat diagram?

80. Illustrate and explain the morphology and life cycle of *Giardia lamblia.*

81. Write a short note on Trichomonas.

82. Explain the life cycle of Malarial Parasite with a neat diagram?

83. Distinguish between the characteristics of Malarial parasite.

84. Illustrate and explain the morphology of *Plasmodim vivax* and *Plasmodium malariae*?

85. Illustrate and explain the morphology of *Plasmodim ovale* and *Plasmodium falciparum*?

86. Write about the classification of RNA virus.

87. Write about the classification of DNA virus.

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SEMESTER – I **4H – 4C**

LECTURE PLAN

Unit IV

S.No	Duration	Topic	Reference
1	2	Sterilization and disinfection, culture methods	R2:pgs;469,W14, W15
2	2	Auxenic and synchronous, aerobic and anaerobic culture media	T1:139-146
3	2	Nutritional types and growth curve	R1:pgs 113,W15
4	2	Generation time and growth kinetics	R1: 175, W15
5	2	Factors influencing microbial growth.	W16
6	1	Preservation methods and quality control	T1:479-480,W16
7	1	Recapitulation and discussion of question	
	Total Hrs: 12		

W14: generalbacteriology.weebly.com

W15: www.biotopics.co.uk

W16: www.upendrats.blogspot.in

UNIT IV

Terminology

Sterilization

Sterilization is the killing or removal of all microorganisms, including bacterial spores which are highly resistant. Sterilization is an absolute term, i.e. the article must be sterile meaning the absence of all microorganisms.

Disinfection

Disinfection is the killing of many, but not all microorganisms. It is a process of reduction of number of contaminating organisms to a level that cannot cause infection, i.e. pathogens must be killed. Some organisms and bacterial spores may survive.

Disinfectants

Disinfectants are chemicals that are used for disinfection. Disinfectants should be used only on inanimate objects.

Antiseptics

It is a mild forms of disinfectants that are used externally on living tissues to kill microorganisms, e.g. on the surface of skin and mucous membranes.

Uses of Sterilization

1. Sterilization for Surgical Procedures: Gloves, aprons, surgical instruments, syringes etc. are to be sterilized.

2. Sterilization in Microbiological works like preparation of culture media, reagents and equipments where a sterile condition is to be maintained.

Thermal death time is a concept used to determine how long it takes to kill a specific microorganism at a specific temperature. It was originally developed for food canning and has found applications in cosmetics, producing salmonella-free feeds for animals (e.g. poultry) and pharmaceuticals.

4.2 Methods of Sterilization

(A).PhysicalAgents

1.Heat

2. Radiation

3. Filtration

B). Chemical Agents

In practice, certain methods are placed under sterilization which in fact do not fulfill the definition of sterilization such as boiling for 1/2 hr and pasteurization which will not kill spores.

STERILIZATION BY HEAT

Heat is most effective and a rapid method of sterilization and disinfection. Excessive heat acts by coagulation of cell proteins. Less heat interferes metabolic reactions. Sterilization occurs by heating above 100°C which ensure lolling of bacterial spores. Sterilization by hot air in hot air oven and sterilization by autoclaving are the two most common method used in the laboratory.

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Types of Heat:

A. Sterilization by moist heat

B. Sterilization by dry heat

A. Sterilization by Moist Heat

Moist heat acts by denaturation and coagulation of protein, breakage of DNA strands, and loss of functional integrity of cell membrane.

(I). Sterilization at 100°C

1. Boiling. Boiling at 100°C for 30 minutes is done in a water bath. Syringes, rubber goods and surgical instruments may be sterilized by this method. All bacteria and certain spores are killed. It leads to disinfection.

2. Steaming. Steam (100°C) is more effective than dry heat at the same temperature as: (a) Bacteria are more susceptible to moist heat, (b) Steam has more penetrating power, and (c) Steam has more sterilizing power as more heat is given up during condensation.

Steam Sterilizer. It works at 100°C under normal atmospheric pressure i.e. without extra pressure. It is ideally suitable for sterilizing media which may be damaged at a temperature higher than 100°C.

It is a metallic vessel having 2 perforated diaphragms (Shelves), one above boiling water, and the other about 4" above the floor. Water is boiled by electricity, gas or stove. Steam passes up. There is a small opening on the roof of the instrument for the escape of steam.

Sterilization is done by two methods:

(a) Single Exposure for 11/2 hours. It leads to disinfection.

(b) Tyndallization (Fractional Sterilization). Heat labile media like those containing sugar, milk, and gelatin can be sterilized by this method. Steaming at 100°C is done in steam sterilizer for 20 minutes followed by incubation at 37°C overnight. This procedure is repeated for another 2 successive days. That is 'steaming' is done for 3 successive days. Spores, if any, germinate to vegetative bacteria during incubation and are destroyed during steaming on second and third day. It leads to sterilization.

II. Sterilization above 100°C: Autoclaving

Autoclaving is one of the most common methods of sterilization. Principle: In this method sterilization is done by steam under pressure. Steaming at temperature higher than 100°C is used in autoclaving. The temperature of boiling depends on the surrounding atmospheric pressure. A higher temperature of steaming is obtained by employing a higher pressure. When the autoclave is closed and made air-tight, and water starts boiling, the inside pressures increases and now the water boils above 100°C. At 15 ib per sq. inch pressure, 121°C temperatures is obtained. This is kept for 15 minutes for sterilization to kill spores. It works like a pressure cooker.

'Sterilization holding time' is the time for which the entire load in the autoclave requires to be exposed.

Autoclave is a metallic cylindrical vessel. On the lid, there are : (1) A gauge for indicating the pressure, (2) A safety valve, which can be set to blow off at any desired pressure, and (3) A stopcock to release the pressure. It is provided with a perforated diaphragm. Water is placed below the diaphragm and heated from below by electricity, gas or stove. Working of Autoclave. (a) Place materials inside, (b) Close the lid. Leave stopcock open, (c) Set the safety

valve at the desired pressure, (d) Heat the autoclave. Air is forced out and eventually steam ensures out through the tap, (e) close the tap. The inside pressure now rises until it reaches the set level (i.e. 15 Win), when the safety valve opens and the excess steam escapes, (f) Keep it for 15 minutes (holding time), (g) Stop heating, (h) Cool the autoclave below 100°C, (i) Open the stopcock slowly to allow air to enter the autoclave.

Checking of Autoclave for Efficiency. Methods :

(i) Spores of Bacillus stearothermophilus are used. Spores withstand 121°C heat for up to 12 min. Strips containing this bacterium are included with the material being autoclaved. Strips are cultured between 50°C and 60°C for surviving spores. If the spores are killed the autoclave is functioning properly.

(ii)Automatic Monitoring System.

III. Sterilization below 100°C

1. Pasteurization. Pasteurization is heating of milk to such temperature and for such a period of time so as to kill pathogenic bacteria that may be present in milk without changing colour, flavour and nutritive value of the milk. Mycobacterium bovis, Salmonella species, Escherichia coli and Brucella species may be present in milk. It does not sterilize the milk as many living organisms including spores are not destroyed..

Methods of Pasteurization

(i) Flash Method. It is "high temperature- short time method". Heating is done at 72°C for 15 seconds.

(ii) Holding Method. Heating is done between 63°C and 66°C for 30 minutes.

2. Inspissation. Inspissation is done between 75°C to 80°C. Inspissation means stiffening of protein without coagulation as the temperature is below coagulation temperature. Media containing serum or egg is sterilized by heating for 3 successive days. It is done in 'Serum Inspissator'.

B. Sterilization by Dry Heat

Mechanisms. (1) Protein denaturation, (2) Oxidative damage, (3) Toxic effect of elevated electrolyte (in absence of water).Dry heat at 160°C (holding temperature for one hour is required to kill the most resistant spores). The articles remain dry. It is unsuitable for clothing which may be spoiled.

1.Red Heat. Wire loops used in microbiology laboratory are sterilized by heating to 'red' in bunsen burner or spirit lamp flame. Temperature is above 100°C. It leads to sterilization.

2.Flaming. The article is passed through flame without allowing it to become red hot, e.g. scalpel. Temperature is not high to cause sterilization.

3. Sterilization by Hot Air

Hot Air Oven (Sterilizer). It Is one of the most common method used for sterilization. Glass wares, swab sticks, all-glass syringes, powder and oily substances are sterilized in hot air oven. For sterilization, a temperature of 160°C is maintained (holding) for one hour. Spores are killed at this temperature. It leads to sterilization.

Hot Air Oven is an apparatus with double metallic walls and a door. There is an air space between these walls. The apparatus is heated by electricity or gas at the bottom. On heating, the air at the bottom becomes hot and passes between the two walls from below upwards, and then passes in the inner chamber through the holes on to top of the apparatus. A thermostat is fitted to maintain a constant temperature of 160°C.

Arnold Sterilizers

Arnold Sterilizer was made around 1940 by the Arnold This sterilizer is no longer in production.

Why was this sterilizer so unique? Because you could inject house steam into it, and maintain 100-102f temperature with pressure ZERO.The 100-102f (70-90c) temperature brings it down to liquad. Once the agar is brought into liquid, it destroys the cells of the agar. When it cools back again, the agar turns into solid again.

Today, you can have a state of the art sterilizer that will do that with digital programming of the time, and temperature. And by setting the parameters as per instructions, you may use it to sterilizer liquids or solid.

Filtration

Filtration is an excellent way to reduce the microbial population in solutions of heat-sensitive material, and sometimes it can be used to sterilize solutions. Rather than directly destroying contaminating microorganisms, the filter simply removes them. There are two types of filters. Depth filters consist of fibrous or granular materials that have been bonded into a thick

layer filled with twisting channels of small diameter. The solution containing microorganisms is sucked through this layer under vacuum, and microbial cells are removed by physical screening or entrapment and also by adsorption to the surface of the filter material. Depth filters are made of diatomaceous earth (Berkefield filters), unglazed porcelain (Chamberlain filters), asbestos, or other similar materials.

Membrane filters have replaced depth filters for many purposes. These circular filters are porous membranes, a little over 0.1 mm thick, made of cellulose acetate, cellulose nitrate, polycarbonate, polyvinylidene fluoride, or other synthetic materials. Although a wide variety of pore sizes are available, membranes with pores about 0.2 □m in diameter are used to remove most vegetative cells, but not viruses, from solutions ranging in volume from 1 ml to many liters.

The membranes are held in special holders and often preceded by depth filters made of glass fibers to remove larger particles that might clog the membrane filter. The solution is pulled or forced through the filter with a vacuum or with pressure from a syringe, peristaltic pump, or nitrogen gas bottle, and collected in previously sterilized containers. Membrane filters remove microorganisms by screening them out much as a sieve separates large sand particles from small ones.

These filters are used to sterilize pharmaceuticals, ophthalmic solutions, culture media, oils, antibiotics, and other heat-sensitive solutions. Air also can be sterilized by filtration. Two common examples are surgical masks and cotton plugs on culture vessels that let air in but keep microorganisms out. Laminar flow biological safety cabinets employing high-efficiency particulate air (HEPA) filters, which remove 99.97% of 0.3 □m particles, are one of the most important air filtration systems. Laminar flow biological safety cabinets force air through HEPA filters, and then project a vertical curtain of sterile air across the cabinet opening. This protects a worker from microorganisms being handled within the cabinet and prevents contamination of the room. A person uses these cabinets when working with dangerous agents such as Mycobacterium tuberculosis, tumor viruses, and recombinant DNA. They are also employed in research labs and industries, such as the pharmaceutical industry, when a sterile working surface is needed for conducting assays, preparing media, examining tissue cultures etc.

Filtration allows for the exclusion of organisms based upon size. There are many types of filtration techniques, but when sterilizing a system **membrane filtration** is used. Membrane filtration traps contaminants larger than the pore size on the surface of the membrane. If contaminants are smaller than the desired particle, decrease the membrane pore size and trap the product while passing the contaminants through the membrane. For greater system flexibility, filters can be added in parallel or series. When adding a filter of same pore size, in parallel, throughput increases. If instead a filter of differing pore size was added in series, separation of multiple microorganisms is possible.

Advantages:

Absolute sterilization - separates particles based on size

Used for heat sensitive media

Removal of multiple particle sizes

Allows for fairly high throughput

Disadvantages:

Each filter has a specific nominal pore size

Unable to separate microorganisms that have the same size

May require a high differential pressure

Radiation

The types of radiation and the ways in which radiation damages or destroys microorganisms have already been discussed. The practical uses of ultraviolet and ionizing radiation in sterilizing objects are briefly described next. Radiation and its effects on microorganisms. Ultraviolet (UV) radiation around 260 nm is quite lethal but does not penetrate glass, dirt films, water, and other substances very effectively. Because of this disadvantage, UV radiation is used as a sterilizing agent only in a few specific situations. UV lamps are sometimes placed on the ceilings of rooms or in biological safety cabinets to sterilize the air and any exposed surfaces. Because UV radiation burns the skin and damages eyes, people working in such areas must be certain the UV lamps are off when the areas are in use. Commercial UV units are available for water treatment.

Pathogens and other microorganisms are destroyed when a thin layer of water is passed under the lamps. Ionizing radiation is an excellent sterilizing agent and penetrates deep into objects. It will destroy bacterial endospores and vegetative cells, both procaryotic and eucaryotic; however, ionizing radiation is not always as effective against viruses. Gamma radiation from a cobalt 60 source is used in the cold sterilization of antibiotics, hormones, sutures, and plastic disposable supplies such as syringes. Gamma radiation has also been used to sterilize and "pasteurize" meat and other food. Irradiation can eliminate the threat of such pathogens as Escherichia coli O157:H7, Staphylococcus aureus, and Campylobacter jejuni. Both the Food and Drug Administration and the World Health Organization have approved food irradiation and declared it safe. A commercial irradiation plant operates near Tampa, Florida. However, this process has not yet been widely employed in the United States because of the cost and concerns about the effects of gamma radiation on food. The U.S. government currently approves the use of radiation to treat poultry, beef, pork, veal, lamb, fruits, vegetables, and spices. It will probably be more extensively employed in the future.

There are 2 general types of radiation used for sterilization, ionizing radiation and non-ionizing radiation. Ionizing radiation is the use of short wavelength, high-intensity radiation to destroy microorganisms. This radiation can come in the form of gamma or X-rays that react with DNA resulting in a damaged cell. Non-ionizing radiation uses longer wavelength and lower energy. As a result, non-ionizing radiation loses the ability to penetrate substances, and can only be used for sterilizing surfaces. The most common form of non-ionizing radiation is ultraviolet light, which is used in a variety of manners throughout industry.

One industrial application of non-ionizing radiation is the breakdown of ozone (O_3). By adding ozone to water, bacteria are unable to sustain life. Unfortunately, ozone also destroys process media. Therefore ozone must be broken down so water can be used for its designated purpose. Since ozone is very sensitive to ultraviolet light, pass the water stream under UV bulbs. This breaks the oxygen-oxygen bonds and results in safe process water. Here is a simple representation of the system.

Advantages:

- No degradation of media during sterilization, thus it can be used for thermally labile media
- Leaves no chemical residue
- Administration of precise dosage and uniform dosage distribution
- Immediate availability of the media after sterilization

Disadvantages:

This method is a more costly alternative to heat sterilization requires highly specialized equipment

The Use of Chemical Agents in Control

Although objects are sometimes disinfected with physical agents, chemicals are more often employed in disinfection and antisepsis. Many factors influence the effectiveness of chemical disinfectants and antiseptics as previously discussed. Factors such as the kinds of microorganisms potentially present, the concentration and nature of the disinfectant to be used, and the length of treatment should be considered. Dirty surfaces must be cleaned before a disinfectant or antiseptic is applied. The proper use of chemical agents is essential to laboratory and hospital safety. It should be noted that chemicals also are employed to prevent microbial growth in food. This is discussed in the chapter on food microbiology. Many different chemicals are available for use as disinfectants, and each has its own advantages and disadvantages. In selecting an agent, it is important to keep in mind the characteristics of a desirable disinfectant. Ideally the disinfectant must be effective against a wide variety of infectious agents (gram-positive and gram-negative bacteria, acid-fast bacteria, bacterial endospores, fungi, and viruses) at high dilutions and in the presence of organic matter. Although the chemical must be toxic for infectious agents, it should not be toxic to people or corrosive for common materials. In practice, this balance between effectiveness and low toxicity for animals is hard to achieve. Some chemicals are used despite their low effectiveness because they are relatively nontoxic. The disinfectant should be stable upon storage, odorless or with a pleasant odor, soluble in water and lipids for penetration into microorganisms, and have a low surface tension so that it can enter cracks in surfaces. If possible the disinfectant should be relatively inexpensive.

One potentially serious problem is the overuse of triclosan and other germicides. This antibacterial agent is now found in products such as deodorants, mouthwashes, soaps, cutting

boards, and baby toys. Triclosan seems to be everywhere. Unfortunately we are already seeing the emergence of triclosan-resistant bacteria. Pseudomonas aeruginosa actively pumps the antiseptic out the cell. Bacteria seem to be responding to antiseptic overuse in the same way as they reacted to antibiotic overuse. There is now some evidence that extensive use of triclosan also increases the frequency of antibiotic resistance in bacteria. Thus overuse of antiseptics can have unintended harmful consequences.

Phenolics

Phenol was the first widely used antiseptic and disinfectant. In 1867 Joseph Lister employed it to reduce the risk of infection during operations. Today phenol and phenolics (phenol derivatives) such as cresols, xylenols, and orthophenylphenol are used as disinfectants in laboratories and hospitals. The commercial disinfectant Lysol is made of a mixture of phenolics. Phenolics act by denaturing proteins and disrupting cell membranes. They have some real advantages as disinfectants: phenolics are tuberculocidal, effective in the presence of organic material, and remain active on surfaces long after application. However, they do have a disagreeable odor and can cause skin irritation.

Hexachlorophene has been one of the most popular antiseptics because it persists on the skin once applied and reduces skin bacteria for long periods. However, it can cause brain damage and is now used in hospital nurseries only in response to a staphylococcal outbreak.

Alcohols

Alcohols are among the most widely used disinfectants and antiseptics. They are bactericidal and fungicidal but not sporicidal; some lipid-containing viruses are also destroyed. The two most popular alcohol germicides are ethanol and isopropanol, usually used in about 70 to 80% concentration. They act by denaturing proteins and possibly by dissolving membrane lipids. A 10 to 15 minute soaking is sufficient to disinfect thermometers and small instruments.

Halogens

A halogen is any of the five elements (fluorine, chlorine, bromine, iodine, and astatine) in group VIIA of the periodic table. They exist as diatomic molecules in the free state and form saltlike compounds with sodium and most other metals. The halogens iodine and chlorine are important antimicrobial agents. Iodine is used as a skin antiseptic and kills by oxidizing cell constituents and iodinating cell proteins. At higher concentrations, it may even kill some spores.

Iodine often has been applied as tincture of iodine, 2% or more iodine in a water-ethanol solution of potassium iodide. Although it is an effective antiseptic, the skin may be damaged, a stain is left, and iodine allergies can result. More recently iodine has been complexed with an organic carrier to form an iodophor. Iodophors are water soluble, stable, and nonstaining, and release iodine slowly to minimize skin burns and irritation. They are used in hospitals for preoperative skin degerming and in hospitals and laboratories for disinfecting. Some popular brands are Wescodyne for skin and laboratory disinfection and Betadine for wounds.

Chlorine is the usual disinfectant for municipal water supplies and swimming pools and is also employed in the dairy and food industries. It may be applied as chlorine gas, sodium hypochlorite, or calcium hypochlorite, all of which yield hypochlorous acid (HclO) and then atomic oxygen. The result is oxidation of cellular materials and destruction of vegetative bacteria and fungi, although not spores.

Death of almost all microorganisms usually occurs within 30 minutes. Since organic material interferes with chlorine action by reacting with chlorine and its products, an excess of chlorine is added to ensure microbial destruction. One potential problem is that chlorine reacts with organic compounds to form carcinogenic trihalomethanes, which must be monitored in drinking water. Ozone sometimes has been used successfully as an alternative to chlorination in Europe and Canada.

Chlorine is also an excellent disinfectant for individual use because it is effective, inexpensive, and easy to employ. Small quantities of drinking water can be disinfected with halazone tablets. Halazone (parasulfone dichloramidobenzoic acid) slowly releases chloride when added to water and disinfects it in about a half hour. It is frequently used by campers lacking access to uncontaminated drinking water.

Chlorine solutions make very effective laboratory and house hold disinfectants. An excellent disinfectant-detergent combination can be prepared if a 1/100 dilution of household bleach (e.g., 1.3 fl oz of Clorox or Purex bleach in 1 gal or 10 ml/liter) is com- bined with sufficient nonionic detergent (about 1 oz/gal or 7.8 ml/liter) to give a 0.8% detergent concentration. This mixture will remove both dirt and bacteria.

Heavy Metals

For many years the ions of heavy metals such as mercury, silver, arsenic, zinc, and copper were used as germicides. More recently these have been superseded by other less toxic and more effectivegermicides (many heavy metals are more bacteriostatic than bac- tericidal). There are a few exceptions. A 1% solution of silver nitrate is often added to the eyes of infants to prevent ophthalmic gonorrhea (in many hospitals, erythromycin is used instead of silver nitrate because it is effective against Chlamydia as well as Neisseria). Silver sulfadiazine is used on burns. Copper sulfate is an effective algicide in lakes and swimming pools.

Heavy metals combine with proteins, often with their sulfhydryl groups, and inactivate them. They may also precipitate cell proteins. Quaternary Ammonium Compounds Detergents [Latin detergere, to wipe off or away] are organic molecules that serve as wetting agents and emulsifiers because they have both polar hydrophilic and nonpolar hydrophobic ends. Due to their amphipathic nature (see section 3.2), detergents solubilize otherwise insoluble residues and are very effective cleansing agents.

They are different than soaps, which are derived from fats. Although anionic detergents have some antimicrobial properties, only cationic detergents are effective disinfectants.

The most popular of these disinfectants are quaternary ammonium compounds characterized by positively charged quaternary nitrogen and a long hydrophobic aliphatic chain. They disrupt microbial membranes and may also denature proteins. Cationic detergents like benzalkonium chloride and cetylpyridinium chloride kill most bacteria but not M. tuberculosis or endospores. They do have the advantages of being stable, nontoxic, and bland but they are inactivated by hard water and soap. Cationic detergents are often used as disinfectants for food utensils and small instruments and as skin antiseptics. Several brands are on the market. Zephiran contains benzalkonium chloride and Ceepryn, cetylpyridinium chloride.

Aldehydes

Both of the commonly used aldehydes, formaldehyde and glutaraldehyde, are highly reactive molecules that combine with nucleic acids and proteins and inactivate them, probably by cross- linking and alkylating molecules. They are sporicidal and can be used as chemical sterilants. Formaldehyde is usually dissolved in water or alcohol before use. A 2% buffered solution of glutaraldehyde is an effective disinfectant. It is less irritating than formaldehyde and

is used to disinfect hospital and laboratory equipment. Glutaraldehyde usually disinfects objects within about 10 minutes but may require as long as 12 hours to destroy all spores.

Sterilizing Gases

Many heat-sensitive items such as disposable plastic petri dishes and syringes, heart-lung machine components, sutures, and catheters are now sterilized with ethylene oxide gas. Ethylene oxide (EtO) is both microbicidal and sporicidal and kills by combining with cell proteins. It is a particularly effective sterilizing agent because it rapidly penetrates packing materials, even plastic wraps. Sterilization is carried out in a special ethylene oxide sterlizer, very much resembling an autoclave in appearance, that controls the EtO concentration, temperature, and humidity. Because pure EtO is explosive, it is usually supplied in a 10 to 20% concentration mixed with either CO2 or dichlorodifluoromethane. The ethylene oxide concentration, humidity, and temperature influence the rate of sterilization.

A clean object can be sterilized if treated for 5 to 8 hours at 38°C or 3 to 4 hours at 54°C when the relative humidity is maintained at 40 to 50% and the EtO concentration at 700 mg/liter. Extensive aeration of the sterilized materials is necessary to remove residual EtO be- cause it is so toxic.

Betapropiolactone (BPL) is occasionally employed as a sterilizing gas. In the liquid form it has been used to sterilize vaccines and sera. BPL decomposes to an inactive form after several hours and is therefore not as difficult to eliminate as EtO. It also destroys microorganisms more readily than ethylene oxide but does not penetrate materials well and may be carcinogenic. For these reasons, BPL has not been used as extensively as EtO. Recently vapor-phase hydrogen peroxide has been used to decontaminate biological safety cabinets.

4.5 Phenol coefficient test

Testing of antimicrobial agents is a complex process regulated by two different federal agencies. The U.S. Environmental Protection Agency regulates disinfectants, whereas agents used on humans and animals are under the control of the Food and Drug Administration. Testing of antimicrobial agents often begins with an initial screening test to see if they are effective and at what concentrations. This may be followed by more realistic in-use testing. The best-known disinfectant screening test is the phenol co- efficient test in which the potency of a disinfectant is compared with that of phenol. A series of dilutions of phenol and the experimental disinfectant

are inoculated with the test bacteria Salmonella typhi and Staphylococcus aureus, then placed in a 20 or 37°C water bath. These inoculated disinfectant tubes are next subcultured to regular fresh medium at 5 minute intervals, and the subcultures are incubated for two or more days. The highest dilutions that kill the bacteria after a 10 minute exposure, but not after 5 minutes, are used to calculate the phenol coefficient. The reciprocal of the appropriate test disinfectant dilution is divided by that for phenol to obtain the coefficient. Suppose that the phenol dilution was 1/90 and maximum effective dilution for disinfectant X was 1/450. The phenol coefficient of X would be 5. The higher the phenol coefficient value, the more effective the disinfectant under these test conditions. A value greater than 1 means that the disinfectant is more effective than phenol.

The phenol coefficient test is a useful initial screening procedure, but the phenol coefficient can be misleading if taken as a direct indication of disinfectant potency during normal use. This is because the phenol coefficient is determined under carefully controlled conditions with pure bacterial strains, whereas disinfectants are normally used on complex populations in the presence of organic matter and with significant variations in environ- mental factors like pH, temperature, and presence of salts. To more realistically estimate disinfectant effectiveness, other tests are often used. The rates at which selected bacteria are destroyed with various chemical agents may be experimentally determined and compared. A use dilution test can also be carried out. Stainless steel cylinders are contaminated with specific bacterial species under carefully controlled conditions. The cylinders are dried briefly, immersed in the test disinfectants for 10 minutes, transferred to culture media, and incubated for two days. The disinfectant concentration that kills the organisms in the sample with a 95% level of confidence under these conditions is determined. Disinfectants also can be tested under conditions designed to simulate normal in-use situations. In-use testing techniques allow a more accurate determination of the proper disinfectant concentration for a particular situation.

4.6 Sterility testing

Sterility testing is used to ensure that pharmaceutical and biopharmaceutical therapeutics are sterile and safe for human use. Testing performed at Pacific BioLabs consists of three main categories:

Bioburden testing is performed on nonsterile products to determine the baseline microbiological level of products. Bioburden is performed as part of a sterilization validation, and may be done on a regular basis to measure whether the microbiological load on a product has changed over time.

Bacteriostasis/Fungistasis testing is performed to determine whether a product inhibits bacterial growth or fungal growth so as to ensure that false negatives do not occur during a sterility test.

Sterility testing determines whether a product is sterile and may often be used in the lot release process to verify that the sterilization process has been effective.

Sterility testing is required for monitoring all products that are manufactured according to GMP and purporting to be sterile. But not all sterility testing solutions are created equal. Quality, convenience and suitability can vary greatly – affecting the credibility of your findings.

Indeed, false negative results may lead to the release of contaminated products, with potentially severe consequences for both patients and manufacturers. On the other hand, cross-contamination and false positive results would imply lengthy and costly investigations, as well as possible product hold or rejection.

4.7 Serial Dilution

It is a common practice to determine microbial counts for both liquid and solid specimens---suspensions of *E. coli* in nutrient broth all the way to soil samples and hamburger meat. Most specimens have high enough numbers of microorganisms that the specimen has to be serially diluted to quantitate effectively.The following is a step-by-step procedure to working dilution problems, and includes some practice problems at the end.The purpose can be determination of bacterial, fungal, or viral counts. This protocol is specific for bacterial counts (colony-forming units, CFUs), but can be modified for fungi (CFUs) and viruses (plaque-forming units, PFUs for viral counts).

History

Robert Koch is credited with identifying a method for bacterial enumeration, used first for the study of water quality. His article, *About Detection Methods for Microorganisms in Water*. was published in 1883.The standard plate count is a reliable method for enumerating bacteria and fungi. A set of serial dilutions is made, a sample of each is placed into a liquefied

agar medium, and the medium poured into a petri dish. The agar solidifies, with the bacterial cells locked inside of the agar. Colonies grow within the agar, as well as on top of the agar and below the agar (between the agar and the lower dish). The procedure described above produces a set of pour plates from many dilutions, but spread plates (sample spread on top of solidified agar) can be used also. The agar plate allows accurate counting of the microorganisms, resulting from the equal distribution across the agar plate. This cannot be done with a fluid solution since 1) one cannot identify purity of the specimen, and 2) there is no way to enumerate the cells in a liquid.

Principles

THE STANDARD FORMULA

Colony count (CFUs) on an agar plate total dilution of tube (used to make plate for colony count) **X** volume plated.To work the problem, you need 3 values---a colony count from the pour or spread plates, a dilution factor for the dilution tube from which the countable agar plate comes, and the volume of the dilution that was plated on the agar plate.

PROTOCOL

STEP 1: Determine the appropriate plate for counting:

Look at all plates and finds the one with 30-300, Use the total dilution for the tube from where the plate count was obtained. If duplicate plates (with same amount plated) have been made from one dilution, average the counts together.

STEP 2: Determine the total dilution for the dilution tubes:

Dilution factor = amount of specimen transferred divided by the total volume after transfer [amount of specimen transferred + amount of diluent already in tube].

Determine the dilution factor for each tube in the dilution series.

Multiply the individual dilution factor for the tube and all previous tubes.

To calculate this dilution series:

Determine the dilution factor of each tube in the set.

Dilution factor for a tube = $\frac{\text{amount of sample}}{\text{Volume of specimen transferred + volume of diluents in tube}}$

But after the first tube, each tube is a dilution of the previous dilution tube. **So** total dilution factor = previous dilution factor of tube **X** dilution of next tube

FOR THE ABOVE DILUTION SERIES:

0.5 ml added to 4.5ml = 0.5/5.0 = 5/50 = 1/10 for 1st tube

1ml added to 9ml = 1/10 (2nd tube) **X** previous dilution of 1/10 (1st tube) = total dilution of 1/100 for 2nd tube.

STEP 3: Determine the amount plated (the amount of dilution used to make the particular pour plate or spread plate).There is nothing to calculate here: the value will be stated in the procedure, or it will be given in the problem.

STEP 4: Solve the problem

1. The countable plate is the one with **51** colonies.
2. The total dilution of the 2nd tube from which that pour plate was made = $1/10^2$
3. The amount used to make that pour plate = **0.1ml** (convert to 1/10 - it is easier to multiply fractions and decimals together).

51 colonies =51 X 10^3 = **5.1 X 10^4** (scientific notation) OR **51,000 CFUs/ml**

$1/10^2$ X 1/10

45 colonies = 45 X 10^4 = **4.5 X 10^5** (scientific notation) OR **450,000/ml**

$1/10^3$ X 1/10

Plate Count Method

- Estimate the number of microorganisms in soil using the plate count method.
- Describe the general activity of microorganisms in the soil.
- Distinguish between bacterial, actinomycete, and fungal colonies.

The soil is one of the main reservoirs of microbial life. Typical garden soil has millions of bacteria in each gram. The most numerous microbes in soil are bacteria. Although actinomycetes are bacteria, they are listed separately because conidiospores make their dry, powdery colonies easily recognizable. Soil bacteria include aerobes and anaerobes with a wide range of nutritional requirements, from photoautotrophs to chemoheterotrophs. As usable nutrients and suitable environmental conditions (such as light, aeration, temperature) become available, the microbial populations and their metabolic activity rapidly increase until the nutrients are depleted or physical conditions change, and they then return to lower levels.

Human pathogens, with the exception of endospore-formed bacteria, are uncommon in the soil. Soil microorganisms are responsible for recycling elements so they can be used over and over again. The numbers of bacteria and fungi in soil are usually estimated by the plate count

method. The actual number of organisms is probably much higher than the estimate, however, because a plate count only detects microbes that will grow under the conditions provided (such as nutrients and temperature).

In a plate count, the numbers of colony-forming units (c.f.u.) are determined. Each colony may arise from a group of cells rather than from one individual cell. The initial soil sample is diluted through serial dilutions in order to obtain a small number of colonies on each plate. A known volume of the diluted sample is plated on sterile nutrient agar. After incubation, the number of colonies is counted. Plates with between 25 and 250 colonies are suitable for counting. A plate with fewer than 25 colonies is inaccurate because a single contaminant could influence the results. A plate with greater than 250 colonies is extremely difficult to count. The microbial population in the original soil sample can then be calculated. For example, if 232 bacterial colonies were present on the plate containing 0.01 mL of the 1:1,000,000 dilution, the calculation would be

Viable plate counts

One of the most common methods of determining cell number is the viable plate count. A sample to be counted is diluted in a solution that will not harm the microbe, yet does not support its growth (so they do not grow during the analysis). In most cases a volume of liquid (or a portion of solid) from the sample is first diluted 10-fold into buffer and mixed thoroughly. In most cases, a 0.1-1.0 ml portion of this first dilution is then diluted a further 10-fold, giving a total dilution of 100-fold. This process is repeated until a concentration that is estimated to be about 1000 cells per ml is reached. In the spread-plate technique some of the highest dilutions (lowest bacterial density) are then taken and spread with a sterile glass rod onto a solid medium that will support the growth of the microbe. It is important that the liquid spread onto the plate soaks into the agar. This prevents left over liquid on the surface from causing colonies to run together and the need for dry plates restricts the volume to 0.1 ml or less.

A second method for counting viable bacteria is the pour plate technique, which consists of mixing a portion of the dilution with molten agar and pouring the mixture into a petri plate. In either case, sample dilution is high enough that individual cells are deposited on the agar and these give rise to colonies. By counting each colony, the total number of colony forming units

(CFUs) on the plate is determined. By multiplying this count by the total dilution of the solution, it is possible to find the total number of CFUs in the original sample

(A) A demonstration of a decimal series of dilutions. The 100 sample is a concentrated solution of methylene blue. A 0.2 ml portion of this was added to 1.8 ml (1:9 ratio) of 0.85% saline to create the 1:10 dilution. After mixing, 0.2 ml of the 10-1 dilution was added to a second tube containing 1.8 ml to create the 10-2 dilution. This was continued to generate the dilution series.

(B) A series of pour plates demonstrating the appearance of a viable plate count. The 3 plates show a 10-7, 10-8, and 10-9 dilution of a natural sample. Note how the number of colony forming units decreases 10 fold between the plates.

One major disadvantage of the viable plate count is the assumption that each colony arises from one cell. In species where cells grow together in clusters, a gross underestimation of the true population results. One example of this are species of Staphylococcus, which is known to form clumps of microorganisms in solution. Each clump is therefore counted as one colony. This problem is why the term CFUs per ml is used instead ofÃ¢ bacteria per mlÃ¢ for the results of such an analysis. It is a constant reminder that one colony does not equal one cell. Great care must also be taking during dilution and plating to avoid errors. Even one error in dilution can have large effects on the final numbers. The rate at which bacteria give rise to an observable colony can also vary. If too short an incubation time is used, some colonies may be missed. The temperature of incubation and medium conditions must also be optimized to achieve the largest colonies possible so that they are easily counted. Finally, this technique takes time. Depending on the organism, one day to several weeks might be necessary to determine the number of CFUs that were present when the experiment started. Such information may no longer be useful for many experiments.

Despite its shortcomings, the viable plate count is a popular method for determining cell number. The technique is sensitive and has the advantage of only counting living bacteria, which is often the important issue. Any concentration of microorganism can be easily counted, if the appropriate dilution is plated. It is even possible to concentrate a solution before counting, as is often done in water analysis, where bacterial populations are usually at low density. The equipment necessary for performing viable plate counts is readily available in any microbiology

lab and is cheap in comparison to other methods. Finally, by using a selective medium it is possible to determine the number of bacteria of a certain class, even in mixed populations. These advantages have made viable plate counts a favorite of food, medical, aquatic and research laboratories for the routine determination of cell number.

Culture Technique

Bacteria will grow on practically any source of organic food which provides **carbon** compounds to be respired for **energy**, and **nitrogen** compounds to be incorporated into **proteins** for **growth**. These substances are normally provided dissolved in water. However, in nature, bacteria can break down solid and insoluble substances by releasing **enzymes** into the substrate in which they are growing. These substances are thus broken down or digested to simpler substances and the process is called **extracellular digestion** because it takes place outside the bacterial cells.

The two normal **media** used in bacteriology are a clear soup-like liquid **nutrient broth**, usually in tubes, and **nutrient agar**, which is set into a jelly by the addition of a seaweed extract called agar, and when melted poured into glass or plastic **Petri dishes - also known as "plates"**.

Sometimes, substances are mixed into media, in order to suppress growth of other types of bacteria. There are many such selective media.

A standard **carbon source** is **glucose**, and **nitrogen** is often provided by **peptones** (partially digested **proteins**), or **inorganic salts**. Minerals and vitamins may also be provided, according to the growth requirements of the bacteria. Combinations of chemicals (buffers) may be used to keep the pH stable. Measured amounts of the concentrates are added to water, and dissolved to reconstitute the media.

These media must then be **sterilised** by heating in an autoclave (like a pressure cooker) at 121°C (pressure 1 bar or 15 lb/sq. in.) for 15 minutes, which kills all living organisms, including spores.

All apparatus used from this point onwards must be sterilised by heat (glassware - 160 °C for 2 hrs) or exposure to radiation.

Aseptic techniques must be used to reduce the likelihood of bacterial contamination. This usually involves **disinfection** of working areas, minimising possible access by bacteria from the air to exposed media, and use of **flames** to kill bacteria which might enter vessels as they are opened.

PREPARATION OF BROTH, SLANT & STAB CULTURES

Nutrient Broth Culture:

- Light your Bunsen burner.
- In one hand hold both the stock culture and the broth culture to be inoculated. Loosen the tube caps
- In your other hand hold the inoculating loop.
- Flame the inoculating loop to redness by holding it pointed down into the flame, starting near the handle and then moving the loop into the flame. This technique sterilizes the loop and, if wet with a culture, heats up the loop without spattering bacteria into the air and onto the surrounding area.
- Let the loop cool a minute. A hot loop will damage the bacteria cells.
- Using the fingers of the "loop hand" remove the cap from the stock culture tube and flame the tube mouth. DO NOT set the tube top down on the table.
- Insert the cooled sterilized loop into the culture tube being careful to not touch the sides of the tube. Touch the loop to the culture. You need not scrape a visible amount from the culture. Hold the tube as horizontal as possible to preclude particles from the air settling into the tube BUT do watch out for any condensate in the bottom of slant cultures. Don't let this fluid wash across the face of the culture.
- Remove the loop being careful again to not touch the tube sides.
- Flame the tube mouth and replace the cap.
- Remove the cap of the broth tube. Flame the top. Remember to hold the top in your fingers.
- Insert the loop into the broth and shake to remove the bacteria.
- Withdraw the loop, flame the tube mouth and replace the cap.
- Resterilize the inoculating loop and place it on the table. NEVER place a contaminated loop on the table.
- Return the stock culture to the rack or holder.
- Gently shake your broth culture. Label it with: today's date, microbe's name, your last name & section letter. The label should be placed so that it will not be in the way of later observations.

Inoculate TWO broths, one each from the two different stock cultures provided.

Incubate your broth cultures as directed. Most bacteria grow fully in broth cultures in 24-48hrs. The tops should be loosened when incubated to allow gas exchange. Observe & record observations at least once each day for the next three (3) days.

Nutrient Agar Slant Culture:

- Hold the tubes, flame the tops, and transfer the culture as outlined for the broth culture. Use the inoculating needle instead of the loop. Remember to sterilize it before and after each use.
- Inoculate your slant by moving the needle gently up the surface of the agar in a snake-like fashion. Be careful to not gouge the agar surface. If there is any liquid in the bottom of the slant tube avoid sticking the needle into this condensate.
- Label it with: today's date, microbe's name, your last name & section letter. The label should be placed so that it will not be in the way of later observations.

Inoculate TWO slants, one each from the two different stock cultures provided.

Incubate your slant cultures as directed. The tops should be loosened when incubated to allow gas exchange. Observe & record observations at least once each day for the next three (3) days.

Nutrient Agar Stab Culture:

- Handle the tubes and inoculating needle as outlined above.
- Inoculate your stab by thrusting the needle straight down into the agar center.
- Label your stabs with: today's date, microbe's name, your last name & section letter. The label should be placed so that it will not be in the way of later observations.

Inoculate TWO stabs, one each from the two different stock cultures provided.

Incubate your stab cultures as directed. The tops should be loosened when incubated to allow gas exchange. Observe & record observations at least once each day for the next three (3) days.

RECIPES

Nutrient Broth (100mL):

- Into a 250mL beaker place 0.3g beef extract, 0.5g peptone, 50mL distilled water
- Gently heat (stir) until dissolved (will be colored).
- Bring the volume to 100mL with distilled water.
- Adjust the pH to 7.0 using 0.1N NaOH or).1M HCl as needed.

Nutrient Agar:

Prepare exactly like nutrient broth BUT in step 1 add 1.5g of Agar.

Marine Agar (100mL):

1. Into a 250mL beaker place 0.5g peptone, 0.1g yeast extract, 0.01g FePO4, 1.5g Agar.
2. Bring the volume to 100mL with aged seawater.
3. Gently heat, (stir) until dissolved.
4. Adjust the pH to 7.5 – 7.8.

5.8 Types of Culture Media

Media are of different types on consistency and chemical composition.

A. On Consistency:

1. Solid Media. Advantages of solid media: (a) Bacteria may be identified by studying the colony character, (b) Mixed bacteria can be separated. Solid media is used for the isolation of bacteria as pure culture. 'Agar' is most commonly used to prepare solid media. Agar is polysaccharide extract obtained from seaweed. Agar is an ideal solidifying agent as it is : (a) Bacteriologically inert, i.e. no influence on bacterial growth, (b) It remains solid at 37°C, and (c) It is transparent.
2. Liquid Media. It is used for profuse growth, e.g. blood culture in liquid media. Mixed organisms cannot be separated.

B. On Chemical Composition :

1. Routine Laboratory Media
2.Synthetic Media. These are chemically defined media prepared from pure chemical substances. It is used in research work.

ROUTINE LABORATORY MEDIA

These are classified into six types: (1) Basal media, (2) Enriched media, (3) Selective media, (4) Indicator media, (5) Transport media, and (6) Storage media.

1.BASAL MEDIA. Basal media are those that may be used for growth (culture) of bacteria that do not need enrichment of the media. Examples: Nutrient broth, nutrient agar and peptone water. Staphylococcus and Enterobacteriaceae grow in these media.

2.ENRICHED MEDIA. The media are enriched usually by adding blood, serum or egg. Examples: Enriched media are blood agar and Lowenstein-Jensen media. Streptococci grow in blood agar media.

3.SELECTIVE MEDIA. These media favour the growth of a particular bacterium by inhibiting the growth of undesired bacteria and allowing growth of desirable bacteria. Examples: MacConkey agar, Lowenstein-Jensen media, tellurite media (Tellurite inhibits the growth of most of the throat organisms except diphtheria bacilli). Antibiotic may be added to a medium for inhibition.

4.INDICATOR (DIFFERENTIAL) MEDIA. An indicator is included in the medium. A particular organism causes change in the indicator, e.g. blood, neutral red, tellurite. Examples: Blood agar and MacConkey agar are indicator media.

5.TRANSPORT MEDIA. These media are used when specie-men cannot be cultured soon after collection. Examples: Cary-Blair medium, Amies medium, Stuart medium.\

6. STORAGE MEDIA. Media used for storing the bacteria for a long period of time. Examples: Egg saline medium, chalk cooked meat broth.

COMMON MEDIA IN ROUTINE USE

Nutrient Broth. 500 g meat, e.g. ox heart is minced and mixed with 1 litre water. 10 g peptone and 5 g sodium chloride are added, pH is adjusted to 7.3. Uses: (1) As a basal media for the preparation of other media, (2) To study soluble products of bacteria.

Nutrient Agar. It is solid at 37°C. 2.5% agar is added in nutrient broth. It is heated at 100°C to melt the agar and then cooled.

Peptone Water. Peptone 1% and sodium chloride 0.5%. It is used as base for sugar media and to test indole formation.

Blood Agar. Most commonly used medium. 5-10% defibrinated sheep or horse blood is added to melted agar at 45-50°C. Blood acts as an enrichment material and also as an indicator. Certain bacteria when grown in blood agar produce haemolysis around their colonies. Certain bacteria produce no haemolysis. Types of changes : (a) beta (p) haemolysis. The colony is surrounded by a clear zone of complete haemolysis, e.g. Streptococcus pyogenes is a beta haemolytic streptococci, (b) Alpha (a) haemolysis. The colony is surrounded by a zone of greenish discolouration due to formation of biliverdin, e.g. Viridans streptococci, (c) Gamma (y) haemolysis, or, No haemolysis. There is no change in the medium surrounding the colony,

Chocolate Agar or Heated Blood agar. Prepared by heating blood agar. It is used for culture of pneumococcus, gonococcus, meningococcus and Haemophilus. Heating the blood inactivates inhibitor of growths.

MacConkey Agar. Most commonly used for enterobacteriaceae. It contains agar, peptone, sodium chloride, bile salt, lactose and neutral red. It is a selective and indicator medium :

(1) Selective as bile salt does not inhibit the growth of enterobactericeae but inhibits growth of many other bacteria.

(2) Indicator medium as the colonies of bacteria that ferment lactose take a pink colour due to production of acid. Acid turns the indicator neutral red to pink. These bacteria are called 'lactose fermenter', e.g. Escherichia coll. Colourless colony indicates that lactose is not fermented, i.e. the bacterium is non-lactose fermenter, e.g. Salmonella. Shigella, Vibrio.

Mueller Hinton Agar. Disc diffusion sensitivity tests for antimicrobial drugs should be carried out on this media as per WHO recommendation to promote reproducibility and comparability of results.

Hiss's Serum Water Medium. This medium is used to study the fermentation reactions of bacteria which can not grow in peptone water sugar media, e.g. pneumococcus, Neisseria, Corynebacterium.

Lowenstein-Jensen Medium. It is used to culture tubercle bacilli. It contains egg, malachite green and glycerol. (1) Egg is an enrichment material which stimulates the growth of tubercle bacilli, (2) Malachite green inhibits growth of organisms other than mycobacteria, (3) Glycerol promotes the growth of Mycobacterium tuberculosis but not Mycobacterium bovis.

Dubos Medium. This liquid medium is used for tubercle bacilli. In this medium drug sensitivity of tubercle bacilli can be carried out. It contains 'tween 80', bovine serum albumin, casein hydrolysate, asparagin and salts. Tween 80 causes dispersed growth and bovine albumin causes rapid growth.

Loeffler Serum. Serum is used for enrichment. Diphtheria bacilli grow in this medium in 6 hours when the secondary bacteria do not grow. It is used for rapid diagnosis of diphtheria and to demonstrate volutin granules. It contains sheep, ox or horse serum.

Tellurite Blood Agar. It is used as a selective medium for isolation of Cotynebacterium diphtheriae. Tellurite inhibits the growth of most secondary bacteria without an inhibitory effect

on diphtheria bacilli. It is also an indicator medium as the diphtheria bacilli produce black colonies. Tellurite metabolized to tellbrism, which has black colour.

MB (Eosin-methylene blue) Agar. A selective and differential medium for enteric Gram-negative rods. Lactose-fermenting colonies are coloured and nonlactose-fermenting colonies are nonpigmented. Selects against gram positive bacteria.

XLD (Xylose Lysine Deoxychoiate). It is used to isolate Salmonella and Shigella species from stool specimens. This is a selective media.

SS (Salmonella-Shigella) Agar. It is a selective medium used to isolate Salmonella and Shigella species. SS Agar with additional bile salt is used if Yersinia enterocolitica is suspected.

DCA (Desoxycholate Citrate Agar). It is used for isolation of Salmonella and Shigella. The other enteric bacteria are mostly inhibited (a selective medium). It is also a differential (indicator) medium due to presence of lactose and neutral red.

Tetrathionate Broth. This medium is used for isolating Salmonella from stool. It acts as a selective medium. It inhibits normal intestinal bacteria and permits multiplication of Salmonella.

Selenite F Broth. Uses and functions are same as that of tetrathionate broth.

Thiosulphate-Citrate-Bile-Sucrose (TCBS) Agar. TCBS agar is a selective medium used to isolate Vibrio cholerae and other Vibrio species from stool.

Charcoal-yeast agar. Used for Legionella pneumophila. Increased concentration of iron and cysteine allows growth.

Tellurite-Gelatin Agar Medium (TGAM). It may be used as transport, selective and indicator medium.

Alkaline peptone water. See under Vibrio. (Chapter 51).

Campylobacter Medium. This selective medium is used to isolate Campylobacter jejuni and Campylobacter coli from stool.

Cary-Blair Medium. It is used as a transport medium for faeces that may contain Salmonella, Shigella, Vibrio or Campylobacter species.

Amies medium is used for gonococci and other pathogens.

Peptone Water Sugar Media. These indicator media are used to study 'Sugar fermentation'. 1 % solution of a sugar (lactose, glucose, mannitol etc) is added to peptone water containing Andrade's indicator in a test tube. A small inverted Durham tube is placed in the medium. The

media are colourless. After culture, change of a medium to red colour indicates acid production. Gas, if produced collects in Durham tube.

Motility Indole Urea (MIU) Medium. This is used to differentiate enterobacteria species by their motility, urease, and indole reactions.

TSI (Triple sugar iron) Agar-

KIA (Kligler Iron Agar). This is a differential slope medium used in the identification of enteric bacteria. The reactions are based on the fermentation of lactose and glucose and the production of hydrogen sulphide

Christensen's Urea Medium. This is used to identify urea splitting organisms, e.g. Proteus. A purple pink colour indicates urea splitting

Bordet-Gengou Medium. This medium is used for culture of Bordetella pertussis. Increased concentration of blood allows growth. It contains agar, potato, sodium chloride, glycerol, peptone and 50% horse blood. Penicillin may be added to it.

5.9 Auxenic culture

Since the microorganisms are too small to be seen with the aid of a microscope, it is not generally practical to work with a single microorganism. For this reason we study cultures that contain thousands, millions, or even billions of microorganisms. A culture that consists of a single kind of microorganisms (one living species), regardless of the number of individuals, in an environment free of other living organisms is called **auxenic culture**. Microbiologists customarily refer to such a culture as a **pure culture**, although in a strict technical sense a pure culture is one grown from, a single cell. If two or more kinds (species) of microorganisms grow together, as they commonly do in nature, this mixed population is referred to as mixed culture.

In biology, **axenic** describes the state of a culture in which only a single species, variety, or strain of organism is present and entirely free of all other contaminating organisms. The earliest axenic cultures were of bacteria or unicellular eukaryotes, but axenic cultures of many multicellular organisms are also possible.[1] Axenic culture is also an important tool for the study of symbiotic and parasitic organisms in a controlled manner.

Preparation

Axenic cultures of microorganisms are typically prepared by subculture of an existing mixed culture. This may involve use of a dilution series, in which a culture is successively

diluted to the point where subsamples of it contain only a few individual organisms, ideally only a single individual (in the case of an asexual species). These subcultures are allowed to grow until the identity of their constituent organisms can be ascertained. Selection of those cultures consisting solely of the desired organism produces the axenic culture. Subculture selection may also involve manually sampling the target organism from an uncontaminated growth front in an otherwise mixed culture, and using this as an inoculum source for the subculture.

Axenic cultures are usually checked routinely to ensure that they remain axenic. One standard approach with microorganisms is to spread a sample of the culture onto an agar plate, and to incubate this for a fixed period of time. The agar should be an enriched medium that will support the growth of common "contaminating" organisms. Such "contaminating" organisms will grow on the plate during this period, identifying cultures that are no longer axenic.

Experimental use

As axenic cultures are derived from very few organisms, or even a single individual, they are useful because the organisms present within them share a relatively narrow gene pool. In the case of an asexual species derived from a single individual, the resulting culture should consist of identical organisms (though processes such as mutation and horizontal gene transfer may introduce a degree of variability). Consequently, they will generally respond in a more uniform and reproducible fashion, simplifying the interpretation of experiments.

Problems

The axenic culture of some pathogens is complicated because they normally thrive within host tissues which exhibit properties that are difficult to replicate *in vitro*. This is especially true in the case of intracellular pathogens. However, careful replication of key features of the host environment can resolve these difficulties (e.g. host metabolites, dissolved oxygen), such as with the Q fever pathogen, *Coxiella burnetii*. [2]

Isolation of Pure Culture

Microorganisms are generally found in nature (air, soil and water) as mixed populations. Even the diseased parts of plants and animals contain a great number of microorganisms, which differ markedly from the microorganisms of other environments. To study the specific role played by a specific microorganism in its environment, one must isolate the same in pure culture. Pure culture involves not only isolation of individual microorganisms from a mixed population,

but also the maintenance of such individuals and their progenies in artificial media, where no other microorganisms find way to grow.

However, it is not easy to isolate the individual microorganisms from natural habitats and grow them under imposed laboratory conditions. For this, great deal of laboratory manipulation is required. If inoculums from any natural habitat is taken and allowed to grow in a culture medium, a large number of diverse colonies may develop that, due to crowdedness, may run together and, thereby, may lose individuality. Therefore, it is necessary to make the colonies well-isolated from each other so that each appears distinct, large and shows characteristic growth forms. Such colonies may be picked up easily and grown separately for detailed study. Several methods for obtaining pure cultures are in use. Some common methods are in everyday-use by a majority of microbiologists, while the others are methods used for special purposes.

5.10 Methods of isolation of pure culture

Pure culture of microorganisms that form discrete colonies on solid media, e.g., yeasts, most bacteria, many other microfungi, and unicellular microalgae, may be most commonly obtained by plating methods such as streak plate method, pour plate method and spread plate method. But, the microbes that have not yet been successfully cultivated on solid media and are cultivable only in liquid media are generally isolated by serial dilution method.

Streak Plate Method

This method is used most commonly to isolate pure cultures of bacteria. A small amount of mixed culture is placed on the tip of an inoculation loop/needle and is streaked across the surface of the agar medium. The successive streaks "thin out" the inoculums sufficiently and the microorganisms are separated from each other. It is usually advisable to streak out a second plate by the same loop/needle without reinoculation. These plates are incubated to allow the growth of colonies. The key principle of this method is that, by streaking, a dilution gradient is established across the face of the Petri plate as bacterial cells are deposited on the agar surface. Because of this dilution gradient, confluent growth does not take place on that part of the medium where few bacterial cells are deposited

Various methods of streaking

Presumably, each colony is the progeny of a single microbial cell thus representing a clone of pure culture. Such isolated colonies are picked up separately using sterile inoculating loop/ needle and restreaked onto fresh media to ensure purity.

Pour Plate Method

This method involves plating of diluted samples mixed with melted agar medium. The main principle is to dilute the inoculum in successive tubes containing liquefied agar medium so as to permit a thorough distribution of bacterial cells within the medium. Here, the mixed culture of bacteria is diluted directly in tubes containing melted agar medium maintained in the liquid state at a temperature of 42-45°C (agar solidifies below 42°C).The bacteria and the melted medium are mixed well. The contents of each tube are poured into separate Petri plates, allowed to solidify, and then incubated. When bacterial colonies develop, one finds that isolated colonies develop both within the agar medium (subsurface colonies) and on the medium (surface colonies). These isolated colonies are then picked up by inoculation loop and streaked onto another Petri plate to insure purity.

Pour plate method has certain disadvantages as follows: (i) the picking up of subsurface colonies needs digging them out of the agar medium thus interfering with other colonies, and (ii the microbes being isolated must be able to withstand temporary exposure to the 42-45° temperature of the liquid agar medium; therefore this technique proves unsuitable for the isolation of psychrophilic microorganisms.

However, the pour plate method, in addition to its use in isolating pure cultures, is also used for determining the number of viable bacterial cells present in a culture.

The isolated colonies are picked up and transferred onto fresh medium to ensure purity. In contrast to pour plate method, only surface colonies develop in this method and the microorganisms are not required to withstand the temperature of the melted agar medium.

Spread Plate Method

In this method the mixed culture of microorganisms is not diluted in the melted agar medium (unlike the pour plate method); it is rather diluted in a series of tubes containing sterile liquid, usually, water or physiological saline. A drop of so diluted liquid from each tube is placed on the centre of an agar plate and spread evenly over the surface by means of a sterilized bent-

glass-rod. The medium is now incubated. When the colonies develop on the agar medium plates, it is found that there are some plates in which well-isolated colonies grow. This happens as a result of separation of individual microorganisms by spreading over the drop of diluted liquid on the medium of the plate.

Serial Dilution Method

As stated earlier, this method is commonly used to obtain pure cultures of those microorganisms that have not yet been successfully cultivated on solid media and grow only in liquid media. A microorganism that predominates in a mixed culture can be isolated in pure form by a series of dilutions.

Spread plate method

The inoculum is subjected to serial dilution in a sterile liquid medium, and a large number of tubes of sterile liquid medium are inoculated with aliquots of each successive dilution. The aim of this dilution is to inoculate a series of tubes with a microbial suspension so dilute that there are some tubes showing growth ofonly one individual microbe. For convenience, suppose we have a culture containing 10 ml of liquid medium, containing 1,000 microorganisms i.e., 100 microorganisms/ml of the liquid medium.

Serial dilution method

If we take out 1 ml of this medium and mix it with 9 ml of fresh sterile liquid medium, we would then have 100 microorganisms in 10 ml or 10 microorganisms/ ml. If we add 1 ml of this suspension to another 9 ml. of fresh sterile liquid medium, each ml would now contain a single microorganism. If this tube shows any microbial growth, there is a very high probability that this growth has resulted from the introduction of a single microorganism in the medium and represents the pure culture of that microorganism.

The Growth Curve

Population growth is studied by analyzing the growth curve of a microbial culture. When microorganisms are cultivated in liquid medium, they usually are grown in a **batch culture** or closed system— that is, they are incubated in a closed culture vessel with a single batch of medium. Because no fresh medium is provided during incubation, nutrient concentrations decline and concentrations of wastes increase. The growth of microorganisms reproducing

by binary fission can be plotted as the logarithm of the number of viable cells versus the incubation time. The resulting curve has four distinct phases (**figure 6.1**).

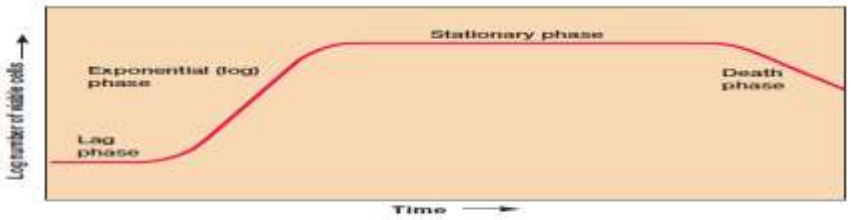


Figure 6.1 Microbial Growth Curve in a Closed System. The four phases of the growth curve are identified on the curve and discussed in the text.

Lag Phase

When microorganisms are introduced into fresh culture medium, usually no immediate increase in cell number occurs, and therefore this period is called the **lag phase.** Although cell division does not take place right away and there is no net increase in mass, the cell is synthesizing new components. A lag phase prior to the start of cell division can be necessary for a variety of reasons. The cells may be old and depleted of ATP, essential cofactors,

and ribosomes; these must be synthesized before growth can begin. The medium may be different from the one the microorganism was growing in previously. Here new enzymes would be needed to use different nutrients. Possibly the microorganisms have been injured and require time to recover. Whatever the causes, eventually the cells retool, replicate their DNA, begin to increase in mass, and finally divide. The lag phase varies considerably in length with the condition of the microorganisms and the nature of the medium. This phase may be quite long if the inoculum is from an old culture or one that has been refrigerated. Inoculation of a culture into a chemically different medium also results in a longer lag phase.

On the other hand, when a young, vigorously growing exponential

phase culture is transferred to fresh medium of the same composition,

the lag phase will be short or absent.

Exponential Phase

During the **exponential** or **log phase,** microorganisms are growing and dividing at the maximal rate possible given their genetic potential, the nature of the medium, and the conditions under which they are growing. Their rate of growth is constant during the exponential phase; that is, the microorganisms are dividing and doubling in number at regular intervals. Because each

individual divides at a slightly different moment, the growth curve rises smoothly rather than in discrete jumps (figure 6.1). The population is most uniform in terms of chemical and physiological properties during this phase; therefore exponential phase cultures are usually used in biochemical and physiological

studies. Exponential growth is **balanced growth.** That is, all cellular constituents are manufactured at constant rates relative to each other. If nutrient levels or other environmental conditions change, **unbalanced growth** results. This is growth during which the rates of synthesis of cell components vary relative to one another until a new balanced state is reached. This response is readily observed in a shift-up experiment in which bacteria are transferred from a nutritionally poor medium to a richer one. The cells first construct new ribosomes to enhance their capacity for protein synthesis. This is followed by increases in protein and DNA synthesis. Finally, the expected rise in reproductive rate takes place.

Unbalanced growth also results when a bacterial population is shifted down from a rich medium to a poor one. The organisms may previously have been able to obtain many cell components directly from the medium. When shifted to a nutritionally inadequate medium, they need time to make the enzymes required for the biosynthesis of unavailable nutrients. Consequently cell division and DNA replication continue after the shift-down, but net protein and RNA synthesis slow. The cells become smaller and reorganize themselves metabolically until they are able to grow again. Then balanced growth is resumed and the culture enters the exponential phase.

These shift-up and shift-down experiments demonstrate thatmicrobial growth is under precise, coordinated control and responds quickly to changes in environmental conditions. When microbial growth is limited by the low concentration of a required nutrient, the final net growth or yield of cells increases with the initial amount of the limiting nutrient present (**figure 6.2*a***). This is the basis of microbiological assays for vitamins and other growth factors. The rate of growth also increases with nutrient concentration (figure 6.2*b*), but in a hyperbolic manner much like that seen with many enzymes (*see figure 8.17*). The shape of the curve seems to reflect the rate of nutrient uptake by microbial transport proteins. At sufficiently high nutrient levels the transport systems are saturated, and the growth rate does not rise further with increasing nutrient concentration.

Stationary Phase

Eventually population growth ceases and the growth curve become horizontal (figure 6.1). This **stationary phase** usually is attained by bacteria at a population level of around 109 cells per ml. Other microorganisms normally do not reach such high population densities, protozoan and algal cultures often having maximum concentrations of about 106 cells per ml. Of course final population size depends on nutrient availability and other factors, as well as the type of microorganism being cultured. In the stationary phase the total number of viable microorganisms remains constant. This may result from a balance between cell division and cell death, or the population may simply cease to divide though remaining metabolically active. Microbial populations enter the stationary phase for several reasons. One obvious factor is nutrient limitation; if an essential nutrient is severely depleted, population growth will slow. Aerobic organisms often are limited by O2 availability. Oxygen is not very soluble and may be depleted so quickly that only the surface of a culture will have an O2 concentration adequate for growth. The cells beneath the surface will not be able to grow unless the culture is shaken or aerated in another way. Population growth also may cease due to the accumulation of toxic waste products. This factor seems to limit the growth of many anaerobic cultures (cultures growing in the absence of O2). For example, streptococci can produce so much lactic acid and other organic acids from sugar fermentation that their medium becomes acidic and growth is inhibited. Streptococcal cultures also can enter the stationary phase due to depletion of their sugar supply. Finally, there is some evidence that growth may cease when a critical population level is reached. Thus entrance into the stationary phase may result from several factors operating in concert. As we have seen, bacteria in a batch culture may enter stationary phase in response to starvation. This probably often occurs in nature as well because many environments have quite low nutrient levels.

Starvation can be a positive experience for bacteria. Many do not respond with obvious morphological changes such as endospore formation, but only decrease somewhat in overall size, often accompaniedby protoplast shrinkage and nucleoid condensation. The more important changes are in gene expression and physiology. Starving bacteria frequently produce a variety of **starvation proteins,** which make the cell much more resistant to damage in a variety

of ways. They increase peptidoglycan cross-linking and cell wall strength. The Dps (*D*NA-binding *p*rotein from *s*tarved cells) protein protects DNA. Chaperones prevent protein denaturation and renature damaged proteins. As a result of these and many other mechanisms, the starved cells become harder to kill and more resistant to starvation itself, damaging temperature changes, oxidative and osmotic damage, and toxic chemicals such as chlorine. These changes are so effective that some bacteria can survive starvation for years. Clearly, these considerations are of great practical importance in medical and industrial microbiology. There is even evidence that *Salmonella typhimurium* and some other bacterial pathogens become more virulent when starved.

Death Phase

Detrimental environmental changes like nutrient deprivation and the buildup of toxic wastes lead to the decline in the number of viable cells characteristic of the **death phase.** The death of a microbial population, like its growth during the exponential phase, is usually logarithmic (that is, a constant proportion of cells dies every hour). This pattern in viable cell count holds even when the total cell number remains constant because the cells simply fail to lyse after dying. Often the only way of deciding whether a bacterial cell is viable is by incubating it in fresh medium; if it does not grow and reproduce, it is assumed to be dead. That is, death is defined to be the irreversible loss of the ability to reproduce. Although most of a microbial population usually dies in a logarithmic fashion, the death rate may decrease after the population has been drastically reduced. This is due to the extended survival of particularly resistant cells. For this and other reasons, the death phase curve may be complex.

The Mathematics of Growth

Knowledge of microbial growth rates during the exponential phase is indispensable to microbiologists. Growth rate studies contribute to basic physiological and ecological research and the solution of applied problems in industry. Therefore the quantitative aspects of exponential phase growth will be discussed. During the exponential phase each microorganism is dividing at constant intervals. Thus the population will double in number during a specific length of time called the **generation time** or **doubling time.** This situation can be illustrated with a simple example.

Suppose that a culture tube is inoculated with one cell that divides every 20 minutes (**table 6.1**). The population will be 2 cells after 20 minutes, 4 cells after 40 minutes, and so forth. Because the population is doubling every generation, the increase in population is always 2*n* where *n* is the number of generations. The resulting population

increase is exponential or logarithmic (**figure 6.3**).

These observations can be expressed as equations for the generation time.

Let *N*0 = the initial population number

Nt = the population at time *t*

n = the number of generations in time *t*

Then inspection of the results in table 6.1 will show that

Nt=*N*0 X 2*n*.

Solving for *n*, the number of generations, where all logarithms

are to the base 10,

$$\log N_t = \log N_0 + n \cdot \log 2, \text{ and}$$

$$n = \frac{\log N_t - \log N_0}{\log 2} = \frac{\log N_t - \log N_0}{0.301}$$

The rate of growth during the exponential phase in a batch culture can be expressed in terms of the **mean growth rate constant *(k)*.** This is the number of generations per unit time, often expressed as the generations per hour.

$$k = \frac{n}{t} = \frac{\log N_t - \log N_0}{0.301t}$$

The time it takes a population to double in size—that is, the **mean generation time** or mean doubling time (*g*), can now be calculated. If the population doubles (*t* = *g*), then

Nt = 2 *N*0.

Substitute 2*N*0 into the mean growth rate equation and solve for *k.*

$$k = \frac{\log (2N_0) - \log N_0}{0.301g} = \frac{\log 2 + \log N_0 - \log N_0}{0.301g}$$

$$k = \frac{1}{g}$$

The mean generation time is the reciprocal of the mean growth rate constant.

$$g = \frac{1}{k}$$

The mean generation time (*g*) can be determined directly from a semilogarithmic plot of the growth data (**figure 6.4**) and the growth rate constant calculated from the *g* value. The generation time also may be calculated directly from the previous equations. For example, suppose that a bacterial population increases from 103 cells to 109 cells in 10 hours.

Generation times vary markedly with the species of microorganism and environmental conditions. They range from less than 10 minutes (0.17 hours) for a few bacteria to several days with some eucaryotic microorganisms (**table 6.2**). Generation times in nature are usually much longer than in culture.

The Influence of Environmental Factors on Growth

The growth of microorganisms also is greatly affected by the chemical and physical nature of their surroundings.

An understanding of environmental influences aids in the control of microbial growth and the study of the ecological distribution of microorganisms. The ability of some microorganisms to adapt to extreme and inhospitable environments is truly remarkable. Procaryotes are present anywhere life can exist. Many habitats in which prokaryotes thrive would kill most other organisms. Procaryotes such as *Bacillus infernus* even seem able to live over 1.5 miles below the Earth's surface, without oxygen and at temperatures above 60°C. Microorganisms that grow in such harsh conditions are often called **extremophiles.** Major emphasis will be given to solutes and water activity, pH, temperature, oxygen level, pressure, and radiation. **Table 6.3** summarizes the way in which microorganisms are categorized in terms of their response to these factors.

Table 6.3 Microbial Responses to Environmental Factors

Descriptive Term	Definition	Representative Microorganisms
Solute and Water Activity		
Osmotolerant	Able to grow over wide ranges of water activity or osmotic concentration	*Staphylococcus aureus, Saccharomyces rouxii*
Halophile	Requires high levels of sodium chloride, usually above about 0.2 M, to grow	*Halobacterium, Dunaliella, Ectothiorhodospira*
pH		
Acidophile	Growth optimum between pH 0 and 5.5	*Sulfolobus, Picrophilus, Ferroplasma, Acontium, Cyanidium caldarium*
Neutrophile	Growth optimum between pH 5.5 and 8.0	*Escherichia, Euglena, Paramecium*
Alkalophile	Growth optimum between pH 8.5 and 11.5	*Bacillus alcalophilus, Natronobacterium*
Temperature		
Psychrophile	Grows well at 0°C and has an optimum growth temperature of 15°C or lower	*Bacillus psychrophilus, Chlamydomonas nivalis*
Psychrotroph	Can grow at 0–7°C; has an optimum between 20 and 30°C and a maximum around 35°C	*Listeria monocytogenes, Pseudomonas fluorescens*
Mesophile	Has growth optimum around 20–45°C	*Escherichia coli, Neisseria gonorrhoeae, Trichomonas vaginalis*
Thermophile	Can grow at 55°C or higher; optimum often between 55 and 65°C	*Bacillus stearothermophilus, Thermus aquaticus, Cyanidium caldarium, Chaetomium thermophile*
Hyperthermophile	Has an optimum between 80 and about 113°C	*Sulfolobus, Pyrococcus, Pyrodictium*
Oxygen Concentration		
Obligate aerobe	Completely dependent on atmospheric O_2 for growth.	*Micrococcus luteus, Pseudomonas, Mycobacterium;* most algae, fungi, and protozoa
Facultative anaerobe	Does not require O_2 for growth, but grows better in its presence.	*Escherichia, Enterococcus, Saccharomyces cerevisiae*
Aerotolerant anaerobe	Grows equally well in presence or absence of O_2	*Streptococcus pyogenes*
Obligate anaerobe	Does not tolerate O_2 and dies in its presence.	*Clostridium, Bacteroides, Methanobacterium, Treponemas agilis*
Microaerophile	Requires O_2 levels below 2–10% for growth and is damaged by atmospheric O_2 (20%).	*Campylobacter, Spirillum volutans, Treponema pallidum*
Pressure		
Barophilic	Growth more rapid at high hydrostatic pressures.	*Photobacterium profundum, Shewanella benthica, Methanococcus jannaschii*

Solutes and Water Activity

Because a selectively permeable plasma membrane separates microorganisms from their environment, they can be affected by changes in the osmotic concentration of their surroundings. If a microorganism is placed in a hypotonic solution (one with a lower osmotic concentration), water will enter the cell and cause it to burst unless something is done to prevent the influx. The osmotic concentration of the cytoplasm can be reduced by use of inclusion bodies

. Procaryotes also can contain pressure-sensitive channels that open to allow solute escape when the osmolarity of the environment becomes much lower than that of the cytoplasm. Most bacteria, algae, and fungi have rigid cell walls that maintain the shape and integrity of the cell. When microorganisms with rigid cell walls are placed in a hypertonic environment, water leaves and the plasma membrane shrinks away from the wall, a process known as plasmolysis. This dehydrates the cell and may damage the plasma membrane; the cell usually becomes metabolically inactive and ceases to grow.

Many microorganisms keep the osmotic concentration of their protoplasm somewhat above that of the habitat by the use of compatible solutes, so that the plasma membrane is always pressed firmly against their cell wall. **Compatible solutes** are solutes that are compatible with metabolism and growth when at high intracellular concentrations. Most procaryotes increase their internal osmotic concentration in a hypertonic environment through the synthesis or uptake of choline, betaine, proline, glutamic acid, and other amino acids; elevated levels of potassium ions are also involved to some extent. Algae and fungi employ sucrose and polyols—for example, arabitol, glycerol, and mannitol—

for the same purpose. Polyols and amino acids are ideal solutes for this function because they normally do not disrupt enzyme structure and function. A few procaryotes like *Halobacterium salinarium* raise their osmotic concentration with potassium ions (sodium ions are also elevated but not as much as potassium). *Halobacterium*'s enzymes have been altered so that they actually require high salt concentrations for normal activity. Since protozoa do not have a cell wall, they must use contractile vacuoles to eliminate excess water when living in hypotonic environments.

The amount of water available to microorganisms can be reduced by interaction with solute molecules (the osmotic effect) or by adsorption to the surfaces of solids (the matric effect). Because the osmotic concentration of a habitat has such profound effects on microorganisms, it

is useful to be able to express quantitatively the degree of water availability. Microbiologists generally use **water activity (aw)** for this purpose (water availability also may be expressed as water potential, which is related to aw). The water activity of a solution is 1/100 the relative humidity of the solution (when expressed as a percent). It is also equivalent to the ratio of the solution's vapor pressure (*P*soln) to that of pure water (*P*water).

The water activity of a solution or solid can be determined by sealing it in a chamber and measuring the relative humidity after the system has come to equilibrium. Suppose after a sample is treated in this way, the air above it is 95% saturated—that is, the air contains 95% of the moisture it would have when equilibrated at the same temperature with a sample of pure water. The relative humidity would be 95% and the sample's water activity, 0.95.

Water activity is inversely related to osmotic pressure; if a solution has high osmotic pressure, its aw is low.

Microorganisms differ greatly in their ability to adapt to habitats with low water activity (**table 6.4**). A microorganism

must expend extra effort to grow in a habitat with a low aw value because it must maintain a high internal solute concentration to retain water. Some microorganisms can do this and are **osmotolerant;** they will grow over wide ranges of water activity or osmotic concentration. For example, *Staphylococcus aureus* can be cultured in media containing any sodium chloride concentration up to about 3 M. It is well adapted for growth on the skin. The

yeast *Saccharomyces rouxii* will grow in sugar solutions with aw values as low as 0.6. The alga *Dunaliella viridis* tolerates sodium chloride concentrations from 1.7 M to a saturated solution.

Although a few microorganisms are truly osmotolerant, most only grow well at water activities around 0.98 (the approximate aw for seawater) or higher. This is why drying food or adding large quantities of salt and sugar is so effective in preventing food spoilage. As table 6.4 shows, many fungi are osmotolerant and thus particularly important in the spoilage of salted or dried foods.

Halophiles have adapted so completely to hypertonic, saline conditions that they require high levels of sodium chloride to grow, concentrations between about 2.8 M and saturation (about 6.2 M) for extreme halophilic bacteria. The archaeon *Halobacterium* can be isolated from the Dead Sea (a salt lake between Israel and Jordan and

the lowest lake in the world), the Great Salt Lake in Utah, and other aquatic habitats with salt concentrations approaching saturation. *Halobacterium* and other extremely halophilic bacteria have significantly modified the structure of their proteins and membranes rather than simply increasing the intracellular concentrations of solutes, the

approach used by most osmotolerant microorganisms. These extreme halophiles accumulate enormous quantities of potassium in order to remain hypertonic to their environment; the internal potassium concentration may reach 4 to 7 M. The enzymes, ribosomes, and transport proteins of these bacteria require high levels of potassium for stability and activity. In addition, the plasma membrane and cell wall of *Halobacterium* are stabilized by high concentrations of

sodium ion. If the sodium concentration decreases too much, the wall and plasma membrane literally disintegrate. Extreme halophilic bacteria have successfully adapted to environmental conditions that would destroy most organisms. In the process they have become so specialized that they have lost ecological flexibility and can prosper

only in a few extreme habitats.

pH

pH is a measure of the hydrogen ion activity of a solution and is defined as the negative logarithm of the hydrogen ion concentration (expressed in terms of molarity). It is not surprising that pH dramatically affects microbial growth. Each species has a definite pH growth range and pH growth optimum. **Acidophiles** have their growth optimum between pH 0 and 5.5; **neutrophiles,** between pH 5.5 and 8.0; and **alkalophiles** prefer the pH range of 8.5 to 11.5. Extreme alkalophiles have growth optima at pH 10 or higher. In general, different microbial groups have characteristic pH preferences. Most bacteria and protozoa are neutrophiles. Most fungi prefer slightly acid surroundings, about pH 4 to 6; algae also seem to favor slight acidity. There are many exceptions to these generalizations. For example, the alga *Cyanidium caldarium* and the archaeon *Sulfolobus acidocaldarius* are common inhabitants of acidic hot springs; both grow well around pH 1 to 3 and at high temperatures. The Archaea *Ferroplasma acidarmanus* and *Picrophilus oshimae* can actually grow at pH 0, or very close to it.

Although microorganisms will often grow over wide ranges of pH and far from their optima, there are limits to their tolerance. Drastic variations in cytoplasmic pH can harm microorganisms by disrupting the plasma membrane or inhibiting the activity of enzymes and membrane

transport proteins. Procaryotes die if the internal pH drops much below 5.0 to 5.5. Changes in the external pH also might alter the ionization of nutrient molecules and thus reduce their availability to the organism. Several mechanisms for the maintenance of a neutral cytoplasmic pH have been proposed. The plasma membrane may be relatively impermeable to protons. Neutrophiles appear to exchange potassium for protons using an antiport transport system. Extreme alkalophiles like *Bacillus alcalophilus* maintain their internal pH closer to neutrality by exchanging internal sodium ions for external protons. Internal buffering also may contribute to pH homeostasis.

Microorganisms often must adapt to environmental pH changes to survive. In bacteria, potassium/proton and sodium/proton antiport systems probably correct small variations in pH. If the pH becomes too acidic, other mechanisms come into play. When the pH drops below about 5.5 to 6.0, *Salmonella typhimurium* and *E. coli* synthesize an array of new proteins as part of what has been called their acidic tolerance response. A proton-translocating ATPase contributes to this protective response, either by making more ATP or by pumping protons out of the cell. If the external pH decreases to 4.5 or lower, chaperones such as acid shock proteins and heat shock proteins are synthesized. Presumably these prevent the acid denaturation of proteins and aid in the refolding of denatured proteins.

Microorganisms frequently change the pH of their own habitat by producing acidic or basic metabolic waste products. Fermentative microorganisms form organic acids from carbohydrates, whereas chemolithotrophs like *Thiobacillus* oxidize reduced sulfur components to sulfuric acid. Other microorganisms make their environment more alkaline by generating ammonia through amino acid degradation.

Buffers often are included in media to prevent growth inhibition by large pH changes. Phosphate is a commonly used buffer and a good example of buffering by a weak acid (H2PO4 _) and its conjugate base (HPO4 2–). An increase in alkalinity is resisted because the weak acid will neutralize hydroxyl ions through protondonation to give water. Peptides and amino acids in complex media also have a strong buffering effect.

Temperature

Environmental temperature profoundly affects microorganisms, like all other organisms. Indeed, microorganisms are particularly susceptible because they are usually unicellular and their temperature varies with that of the external environment. For these reasons, microbial cell

temperature directly reflects that of the cell's surroundings. A most important factor influencing the effect of temperature on growth is the temperature sensitivity of enzymecatalyzed reactions. At low temperatures a temperature rise increasesthe growth rate because the velocity of an enzyme-catalyzed reaction, like that of any chemical reaction, will roughly double for every 10°C rise in temperature. Because the rate of each reaction increases, metabolism as a whole is more active at higher temperatures, and the microorganism grows faster. Beyond a certain point further increases actually slow growth, and sufficiently high temperatures are lethal. High temperatures damage microorganisms by denaturing enzymes, transport carriers, and other proteins. Microbial membranes are also disrupted by temperature extremes; the lipid bilayer simply melts and disintegrates. Thus, although functional enzymes operate more rapidly at higher temperatures, the microorganism may be damaged to such an extent that growth is inhibited because the damage cannot be repaired. At very low temperatures, membranes solidify and enzymes don't work rapidly. In summary, when organisms are above the optimum temperature, both function and cell structure are affected. If temperatures are very low, function is affected but not necessarily cell chemical composition and structure.

Because of these opposing temperature influences, microbial growth has a fairly characteristic temperature dependence with distinct **cardinal temperatures**—minimum, optimum, and maximum growth temperatures. Although the shape of the temperature dependence curve can vary, the temperature optimum is always closer to the maximum than to the minimum. The cardinal temperatures for a particular species are not rigidly fixed but often depend to some extent on other environmental factors such as pH and the available nutrients. For example, *Crithidia fasciculata,* a flagellated protozoan living in the gut of mosquitos, will grow in a simple medium at 22 to 27°C. However, it cannot be cultured at 33 to 34°C without the addition of extra metals, amino acids, vitamins, and lipids. The cardinal temperatures vary greatly between microorganisms (table 6.5). Optima normally range from 0°C to as high as 75°C, whereas microbial growth occurs at temperatures extending from _20°C to over 100°C. The major factor determining this growth range seems to be water. Even at the most extreme temperatures, microorganisms need liquid water to grow. The growth temperature range for a particular microorganism usually spans about 30 degrees. Some species (e.g., *Neisseria gonorrhoeae*) have a small range; others, like *Enterococcus faecalis*, will grow over a wide range

of temperatures. The major microbial groups differ from one another regarding their maximum growth temperature. The upper limit for protozoa is around 50°C. Some algae and fungi can grow at temperatures as high as 55 to 60°C. Procaryotes have been found growing at or close to 100°C, the boiling point of water at sea level (*see figure 20.8*). Recently strains growing at even higher temperatures have been discovered (**Box 6.1**). Clearly, prokaryotic organisms can grow at much higher temperatures than eucaryotes. It has been suggested that eucaryotes are not able to manufacture organellar membranes that are stable and functional attemperatures above 60°C. The photosynthetic apparatus also appears to be relatively unstable because photosynthetic organisms are not found growing at very high temperatures. The effect of temperature on growth rate. Microorganisms such as those in **table 6.5** can be placed in one of five classes based on their temperature ranges for growth.

1. **Psychrophiles** grow well at 0°C and have an optimum growth temperature of 15°C or lower; the maximum is around 20°C. They are readily isolated from Arctic and Antarctic habitats; because 90% of the ocean is 5°C or colder ,it constitutes an enormous habitat for psychrophiles. The psychrophilic alga *Chlamydomonas nivalis* can actually turn a snowfield or glacier pink with its bright red spores. Psychrophiles are widespread among bacterial taxa and found in such genera as *Pseudomonas, Vibrio, Alcaligenes, Bacillus, Arthrobacter, Moritella, Photobacterium,* and *hewanella.* The psychrophilic archaeon *Methanogenium* has recently been isolated from Ace Lake in Antarctica. Psychrophilic microorganisms have adapted to their environment in several ways. Their enzymes, transport systems, and protein synthetic mechanisms function well at low temperatures. The cell membranes of psychrophilic microorganisms have high levels of unsaturated fatty acids and remain semifluid when cold. Indeed, many psychrophiles begin to leak cellular constituents at temperatures higher than 20°C because of cell membrane disruption.
2. Many species can grow at 0 to 7°C even though they have optima between 20 and 30°C, and maxima at about 35°C. These are called **psychrotrophs** or **facultative psychrophiles.** Psychrotrophic bacteria and fungi are major factors in the spoilage of refrigerated foods

3. **Mesophiles** are microorganisms with growth optima around 20 to 45°C; they often have a temperature minimum of 15 to 20°C. Their maximum is about 45°C or lower. Most microorganisms probably fall within thiscategory. Almost all human pathogens are mesophiles, as might be expected since their environment is a fairly constant 37°C.
4. Some microorganisms are **thermophiles;** they can grow at temperatures of 55°C or higher. Their growth minimum is usually around 45°C and they often have optima between 55 and 65°C. The vast majority are procaryotes although a few algae and fungi are thermophilic (table 6.5). These organisms flourish in many habitats including composts, self-heating hay stacks, hot water lines, and hot springs. Thermophiles differ from mesophiles in having much more heat-stable enzymes and protein synthesis systems able to function at high temperatures. Their membrane lipids are also more saturated than those of mesophiles and have higher melting points; therefore thermophile membranes remain intact at higher temperatures.
5. As mentioned previously, a few thermophiles can grow at 90°C or above and some have maxima above 100°C. Procaryotes that have growth optima between 80°C and about 113°C are called **hyperthermophiles.** They usually do not grow well below 55°C. *Pyrococcus abyssi* and *Pyrodictium occultum* are examples of marine hyperthermophiles found in hot areas of the seafloor.

Oxygen Concentration

An organism able to grow in the presence of atmospheric O2 is an **aerobe,** whereas one that can grow in its absence is an **anaerobe.** Almost all multicellular organisms are completely dependent on atmospheric O2 for growth—that is, they are **obligate aerobes** (table 6.3). Oxygen serves as the terminal electron acceptor for the electron- transport chain in aerobic respiration. In addition, aerobic eucaryotes employ O2 in the synthesis of sterols and unsaturated fatty acids. **Facultative anaerobes** do not require O2 for growth but do grow better in its presence. In the presence of oxygen they will use aerobic respiration. **Aerotolerant anaerobes** such as *Enterococcus faecalis* simply ignore O2 and grow equally well whether it is present or not. In contrast, **strict** or **obligate anaerobes** (e.g., *Bacteroides, Fusobacterium, Clostridium pasteurianum, Methanococcus*) do not tolerate O2 at all and die in its presence. Aerotolerant and strict anaerobes cannot generate energy through respiration and must employ fermentation or

anaerobic respiration pathways for this purpose. Finally, there are aerobes such as *Campylobacter,* called **microaerophiles,** that are damaged by the normal atmospheric level of O2 (20%) and require O2 levels below the range of 2 to 10% for growth. The nature of bacterial O2 responses can be readily determined by growing the bacteria in culture tubes filled with a solid culture medium or a special medium like thioglycollate broth, which contains a reducing agent to lower O2 levels. A microbial group may show more than one type of relationship to O2. All five types are found among the procaryotes and protozoa. Fungi are normally aerobic, but a number of species— particularly among the yeasts—are facultative anaerobes.

Pressure

Most organisms spend their lives on land or on the surface of water, always subjected to a pressure of 1 atmosphere (atm), and are never affected significantly by pressure. Yet the deep sea (ocean of 1,000 m or more in depth) is 75% of the total ocean volume. The hydrostatic pressure can reach 600 to 1,100 atm in the deep sea, while the temperature is about 2 to 3°C. Despite these extremes, bacteria survive and adapt. Many are **barotolerant:** increased pressure does adversely affect them but not as much as it does nontolerant bacteria. Some bacteria in the gut of deep-sea invertebrates such as amphipods and holothurians are truly **barophilic**—they grow more rapidly at

high pressures. These gut bacteria may play an important role in nutrient recycling in the deep sea. One barophile has been recovered from the Mariana trench near the Philippines (depth about 10,500 m) that is actually unable to grow at pressures below about 400 to 500 atm when incubated at 2°C. Thus far, barophiles have been found among several bacterial genera (e.g., *Photobacterium, Shewanella, Colwellia*). Some members of the Archaea are thermobarophiles (e.g., *Pyrococcus* spp., *Methanococcus jannaschii*).

Radiation

Our world is bombarded with electromagnetic radiation of various types. This radiation often behaves as if it were composed of waves moving through space like waves traveling on the surface of water. The distance between two wave crests or troughs is the wavelength. As the wavelength of electromagnetic radiation decreases, the energy of the radiation increases—gamma rays and X rays are much more energetic than visible light or infrared waves. Electromagnetic radiation also acts like a stream of energy packets called photons, each photon

having a quantum of energy whose value will depend on the wavelength of the radiation. Sunlight is the major source of radiation on the Earth. It includes visible light, ultraviolet (UV) radiation, infrared rays, and radio waves. Visible light is a most conspicuous and important aspect of our environment: all life is dependent on the ability of photosynthetic organisms to trap the light energy of the sun. Almost 60% of the sun's radiation is in the infrared region rather than the visible portion of the spectrum. Infrared is the major source of the Earth's heat. At sea level, one finds very little ultravioletradiation below about 290 to 300 nm. UV radiation of wavelengths shorter than 287 nm is absorbed by O2 in the Earth's atmosphere; this process forms a layer of ozone between 25 and 30 miles above the Earth's surface. The ozone layer then absorbs somewhat longer UV rays and reforms O2. This elimination of UV radiation is crucial because it is quite damaging to living systems. The fairly even distribution of sunlight throughout the visible spectrum accounts for the fact that sunlight is generally "white."Many forms of electromagnetic radiation are very harmful to microorganisms. This is particularly true of **ionizing radiation,** radiation of very short wavelength or high energy, which can cause atoms to lose electrons or ionize. Two major forms of ionizing radiation are (1) X rays, which are artificially produced, and (2) gamma rays, which are emitted during radioisotope decay. Low levels of ionizing radiation will produce mutations and may indirectly result in death, whereas higher levels are directly lethal. Although microorganisms are more resistant to ionizing radiation than larger organisms, they will still be destroyed by a sufficiently large dose. Ionizing radiation can be used to sterilize items. Some procaryotes (e.g., *Deinococcus radiodurans*) and bacterial endospores can survive large d oses of ionizing radiation. A variety of changes in cells are due to ionizing radiation; it breaks hydrogen bonds, oxidizes double bonds, destroys ring structures, and polymerizes some molecules. Oxygen enhances these destructive effects, probably through the generation of hydroxyl radicals (OH·). Although many types of constituents can be affected, it is reasonable to suppose that destruction of DNA is the most important cause of death.

Ultraviolet (UV) radiation, mentioned earlier, kills all kinds of microorganisms due to its short wavelength (approximately from 10 to 400 nm) and high energy. The most lethal UV radiation has a wavelength of 260 nm, the wavelength most effectively absorbed by DNA. The primary mechanism of UV damage is the formation of thymine dimers in DNA. Two adjacent thymines

in a DNA strand are covalently joined to inhibit DNA replication and function. This damage is repaired in several ways. In **photoreactivation,** blue light is used by a photoreactivating enzyme to split thymine dimers. A short sequence containing the thymine dimer can also be excised and replaced. This process occurs in the absence of light and is called **dark reactivation.** Damage also can be repaired by the recA protein in recombination repair and SOS repair. When UV exposure is too heavy, the damage is so extensive that repair is impossible. Although very little UV radiation below 290 to 300 nm reaches the earth's surface, near-UV radiation between 325 and 400 nm can harm microorganisms. Exposure to near-UV radiation induces tryptophan breakdown to toxic photoproducts. It appears that these toxic tryptophan photoproducts plus the near-UV radiation itself produce breaks in DNA strands. The precise mechanism is not known, although it is different from that seen with 260 nm UV. Visible light is immensely beneficial because it is the source of energy for photosynthesis. Yet even visible light, when present in sufficient intensity, can damage or kill microbial cells. Usually pigments called photosensitizers and O2 are required. All microorganisms possess pigments like chlorophyll, bacteriochlorophyll, cytochromes, and flavins, which can absorb light energy, become excited or activated, and act as photosensitizers. The excited photosensitizer (P) transfers its energy to O2 generating **singlet oxygen** (1O2).

Special Methods of Isolation on of Pure Culture

1. **Single Cell Isolation methods**

An individual cell of the required kind is picked out by this method from the mixed culture and is permitted to grow. The following two methods are in use.

(i) **Capillary pipette method**

Several small drops of a suitably diluted culture medium are put on a sterile glass-coverslip by a sterile pipette drawn to a capillary. One then examines each drop under the microscope until one finds such a drop, which contains only one microorganism. This drop is removed with a sterile capillary pipette to fresh medium. The individual microorganism present in the drop starts multiplying to yield a pure culture.

(ii) **Micromanipulator method**

Micromanipulators have been built, which permit one to pick out a single cell from a mixed culture. This instrument is used in conjunction with a microscope to pick a single cell

(particularly bacterial cell) from a hanging drop preparation. The micro-manipulator has micrometer adjustments by means of which its micropipette can be moved right and left, forward, and backward, and up and down. A series of hanging drops of a diluted culture are placed on a special sterile coverslip by a micropipette.

Now a hanging drop is searched, which contains only a single microorganism cell. This cell is drawn into the micropipette by gentle suction and then transferred to a large drop of sterile medium on another sterile coverslip. When the number of cells increases in that drop as a result of multiplication, the drop is transferred to a culture tube having suitable medium. This yields a pure culture of the required microorganism.

The advantages of this method are that one can be reasonably sure that the cultures come from a single cell and one can obtain strains with in the species. The disadvantages are that the equipment is expensive, its manipulation is very tedious, and it requires a skilled operator. This is the reason why this method is reserved for use in highly specialized studies.

2. **Enrichment Culture Method**

Generally, it is used to isolate those microorganisms, which are present in relatively small numbers or that have slow growth rates compared to the other species present in the mixed culture. The enrichment culture strategy provides a specially designed cultural environment by incorporating a specific nutrient in the medium and by modifying the physical conditions of the incubation. The medium of known composition and specific condition of incubation favors the growth of desired microorganisms but, is unsuitable for the growth of other types of microorganisms.

Proof of Purity of Cultures

Assuming that one has isolated a pure culture, how does one establish that it is pure? A pure culture is one in which the cells are all of one kind, i.e., demonstrate "likeness". Hence, the proof of purity of cultures consists of demonstrating the "likeness" of microorganisms in the culture. It is based on certain criteria as follows:

1. The microorganisms look alike microscopically and stain in the same fashion.
2. When plated, all the colonies formed look alike.
3. Streaks, stabs, etc. are uniform.
4. Several isolated colonies perform identically, i.e., ferment the same sugars, and so on.

5.11 Maintenance and Preservation of Pure Cultures

Once a microorganism has been isolated and grown in pure culture, it becomes necessary to maintain the viability and purity of the microorganism by keeping the pure cultures free from contamination. Normally in laboratories, the pure cultures are transferred periodically onto or into a fresh medium (subculturing) to allow continuous growth and viability of microorganisms. The transfer is always subject to aseptic conditions to avoid contamination.

Since repeated sub culturing is time consuming, it becomes difficult to maintain a large number of pure cultures successfully for a long time. In addition, there is a risk of genetic changes as well as contamination. Therefore, it is now being replaced by some modern methods that do not need frequent subculturing. These methods include refrigeration, paraffin method, cryopreservation, and lyophilization (freeze drying).

Refrigeration

Pure cultures can be successfully stored at 0-4°C either in refrigerators or in cold-rooms. This method is applied for short duration (2-3 weeks for bacteria and 3-4 months for fungi) because the metabolic activities of the microorganisms are greatly slowed down but not stopped. Thus their growth continues slowly, nutrients are utilized and waste products released in medium. This results in, finally, the death of the microbes after sometime.

Paraffin Method

This is a simple and most economical method of maintaining pure cultures of bacteria and fungi. In this method, sterile liquid paraffin in poured over the slant (slope) of culture and stored upright at room temperature. The layer of paraffin ensures anaerobic conditions and prevents dehydration of the medium. This condition helps microorganisms or pure culture to remain in a dormant state and, therefore, the culture is preserved for several years.

Cryopreservation

Cryopreservation (i.e., freezing in liquid nitrogen at -196°C) helps survival of pure cultures for long storage times. In this method, the microorganisms of culture are rapidly frozen in liquid nitrogen at -196°C in the presence of stabilizing agents such as glycerol that prevent the formation of ice crystals and promote cell survival.

Lyophilization (Freeze-Drying)

In this method, the culture is rapidly frozen at a very low temperature (-70°C) and then dehydrated by vacuum. Under these conditions, the microbial cells are dehydrated and their metabolic activities are stopped; as a result, the microbes go into dormant state and retain viability for years. Lyophilized or freeze-dried pure cultures and then sealed and stored in the dark at 4°C in refrigerators. Freeze-drying method is the most frequently used technique by culture collection centers.

Culture collections Centre.

Microbial culture collections focus on the acquisition, authentication, production, preservation, cataloguing and distribution of viable cultures of standard reference microorganisms, cell lines and other materials for research in microbial systematic. Culture collection are also repositories of type strains.

Major national culture collections centre.

Collection Acronym	**Name**	**Location**
ATCC	American Type Culture Collection	Manassas, Virginia
NCTC	National Collection of Type Cultures	Health Protection Agency, London, United Kingdom
BCCM	Belgium Coordinated Collection of Microorganism	Ghent, Belgium
CIP	Collection d'Institut Pasteur	Paris, France
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen	Braunschweig, Germany
JCM	Japan Collection of Microorganisms	Tsukuba, Ibaraki, Japan
NCCB	Netherlands Culture Collection of Bacteria	Utrecht, Netherlands
NCIMB	National Collection of Industrial, Food and Marine Bacteria	Aberdeen, Scotland
STCC	Spanish type culture collection, Valencia University	Valencia, Spain

KARPAGAM ACADEMY OF HIGHER EDUCATION FUNDAMENTALS OF MICROBIOLOGY AND CLASSIFICATION (17MBP101)	
Unit IVQuestions	**Opt 1**
Fractional sterilisation was also called_____	Tyndallisation
Heat conduction is _____in dry than is moist air	rapid
The objects are protected from subsequent contamination in paper are expos	150ºC for 90 min
Steam must be used for the heat sterilisation of ____ solutions	aqueous
The scientist who introduced antiseptic principle	Louis Pasteur
sterilisation by discontinuous heating was developed by	Louis Pasteur
Killing effect of dry heat is due to ____	protein denaturat
Which of the following process is sporicidal	sanitisation
The widely used method of sterilisation of milk in diary industries is ____	Tyndallisation
Discontinuous heating is the basis of _____	Tyndallisation
Autoclaving is usually done at _____	100º C 15 lbs 15
Autoclave uses for sterilisation	steam
Sterilisation is done with filter pore size of _____	1.5 micron
Berkefeld filters are prepared by mixing diatomaceous earth with	asbestos
Asbestos is chemically composed of	magnesium silica
Phenol was first used as an antiseptic by _____	Louis Pasteur
The widely used fumigant is _____	ethylene
An example of a nonionizing type of radiation which is microbicidal is ___	gamma rays
Ultra violet light is strongly absorbed by _____ with in a cell.	DNA
Membrane filters are manufactured from _____	cellulose nitrate
Capillary pore membranes have pores produced by ____	irradation
The microbial media are solidified by using	Agar
_____ is the universal bacterial medium	Nutrient Agar
Give an example for a mechanical device for removing microorganism from	Hot air oven
Ultra violet light has a wavelength of between.	300-400nm
Which process force out electrons out of their shells in organic molecules	Ultra sonic vibrat
In ultra sonic vibration forms cavities is known as____	Cavitation
The process of destroying pathogens is called ____	Tyndallisation
Which phenolic compound hexachlorophene which used in toothpaste	Ipan
__ is the time in minutes needed to reduce the number of viable microorgan	steam
Name the isotope which release the x-rays, v-raysand cosmic rays	Uranium-60
Name the instrument which used to sterilize the surgical supplies	Hot air oven
What is the temperature which applied in moist heat sterilisation	160o
Disinfection accomplised by chemical agents called___	Sterilizer
sanitisers are commomnly applied to sterilise___	Skin
Egg albumin is coagulated by___	Heating
An agent that kills vegetative cells, but not resistant spore forms of germs	Fungicide
Give an example to the instrument that working under dry heat principle___	Hot air oven
Which kind of filter is used in Arnold steriliser	Porecelain filter
The process of energy transmitted through a space or material is called____	Acoustic

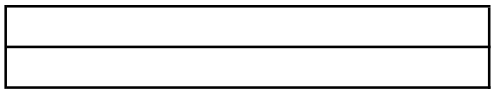
A laminar air flow hood has a ___ filter that removes microorganisms from	Porecelain filter
HEPA filters remove particulate material layer that about __ from the air	0.2µm
Chemicals can be used to clean inanimate objects and lower the number of r	Sanitation
Which one is the traditional method employed for cleaning municipal water	Sanitation
Which halogen compound is suitable for disinfection of dairy equipments	Calcium hypochl
Heavy metal toxicity in low concentration is called _____	Chlorination
What is the audible frequency of the sonic waves	5,900Hz
Example to man made radiation	gamma rays
In which country the X-ray was discovered	U.K
In France the natural radio activity was first idendified with ____	Cobalt
Surgical suture and disposable hypertonic syringes are contaminated with ar	Radiation
X-rays are effective in destroying bacterial___	Endotoxin
The organic acids are weaker in ___ ions than mineral acids	Cl+
The action of alkalies as disinfectants is dependent upon the release of __ io	Cl+
Name the alkali that is eapecially toxic to bacteria.	KOH
Which alkali have the lower germicidal activity	KOH
Commercial solution which capable of destroying anthrax spores in 1 hour.	H2O2
What is the color of the alcohol	White
Who introduced disinfectants to prevent infection of his surgical wounds.	Tyndall
Which chemical compound is called as "Gold standard of Disinfection"?	Dettol

Opt 2	**Opt 3**	**Opt 4**	**Opt 5**	**Opt 6**
Pasteurisation	Heating	Sterilisation		
less rapid	higher	nil		
120ºC for 90 min	170ºC for 90 min	160ºC for 90 min		
solid	semisolid	neutral		
John Tyndall	Joseph lister	Robert koch		
John Tyndall	Joseph lister	Robert koch		
elevated levels of electr	oxidative demand	Diffusion Process		
disinfection	antisepsis	sterilisation		
Pasteurisation	Heating	sterilisation		
Pasteurisation	Heating	sterilisation		
160ºC 15 lbs 60 min	115º C 15 lbs 15 min	121º C 15 lbs 15 min		
hot air	steam under pressure	cold		
0.85 micron	0.45 micron	1.0 micron		
aluminium	sand and clay	Copper		
mercuric sulphate	manganous chloride	mercapto ethanol		
John Tyndall	Joseph lister	Robert koch		
chlorine	formaldehyde	carbon-di-oxide		
UV rays	X- rays	Beta rays		
ribosomes	cell wall	cytoplasm		
poly carbonate and polye	cellulose diacetate	cellulose		
filtration	evaporation	respiration		
Peplone	Yeast extract	Tryptone		
Tryptone agar	Macconkey agar	Mannitol salt agar		
Autoclave	waterbath	Filter		
200-300nm	100-400nm	50-100nm		
Ionization radiation	Pasteurisation	Tyndallisation		
Ionization radiation	Pasteurisation	Tyndallisation		
Pasteurisation	Disinfection	sterilisation		
Mum	Dial	Phisohex		
hot air	L-value	D- value		
Cobalt-60	Tween-80	Nickle-10		
Cooker	Autoclave	Laminar air flow		
90o	60o	100o		
disinfectant	sanitiser `	chemical agent		
Animate objects	inanimate objects	Specimen		
cooling	Quenching	Disinfecting		
Germicide	Insecticide	Pesticide		
Cooker	Arnold sterilizer	Laminar air flow		
Asbestos filter	Membrane filter	HEPA filter		
Radiation	Electromagnetic	None of the above		

Asbestos filter	Membrane filter	HEPA filter		
0.1µm	0.3µm	0.03µm		
Pasteurisation	Disinfection	sterilisation		
Chlorination	Pasteurisation	Disinfection		
Chloride	Calcium	Iodide		
Sanitation	Oligodynamic action	Dynamic action		
6,900Hz	7,900Hz	8,900Hz		
UV rays	X- rays	Cosmic rays		
Germany	India	Africa		
Nickel	Aluminium	Uranium		
Filtration	disinfection	sanitation		
Exotoxin	Neurotoxin	Enterotoxin		
Na+	H+	OH-		
Na+	H+	OH-		
NaOH	HCHO	NH4OH		
NaOH	HCHO	NH4OH		
NH4OH	Hcl	HCHO		
Colorless	Pale yellow	Yellow		
Beijernick	Lister	Jenner		
Lysol	Glycerol	Phenol		

Answer
Tyndallisation
less rapid
1600C for 90 min
semisolid
Joseph lister
John Tyndall
protein denaturation
sterilisation
Pasteurisation
Tyndallisation
121o C 15 lbs 15 min
steam under pressure
1.0 micron
asbestos
manganous chloride
Joseph lister
formaldehyde
UV rays
DNA
cellulose nitrate
filtration
Agar
Tryptone agar
Filter
100-400nm
Ionization radiation
Cavitation
Disinfection
Ipan
D- value
Cobalt-60
Autoclave
100o
disinfectant
inanimate objects
Heating
Germicide
Arnold sterilizer
Porecelain filter
Radiation

HEPA filter
0.3µm
Sanitation
Chlorination
Calcium hypochloride
Oligodynamic action
8,900Hz
X- rays
Germany
Uranium
Radiation
Exotoxin
H+
OH-
KOH
NH4OH
H2O2
Colorless
Lister
Phenol



DEPARTMENT OF MICROBIOLOGY
KARPAGAM ACADEMY OF HIGHER EDUCATION
(Established Under Section 3 of UGC Act, 1956)
Eachanari Post, Coimbatore, Tamil Nadu, India – 641 021

I M.Sc MICROBIOLOGY - 2017 – 2019 Batch

FUNDAMENTALS OF MICROBIOLOGY AND CLASSIFICATION (17MBP101)

SEMESTER – I **4H – 4C**

POSSIBLE QUESTION

Unit IV

TWO MARK

1. Define sterilization.
2. Define disinfection.
3. Define antiseptic.
4. Define disinfectant.
5. Write about types of sterilization.
6. Comment on types of heat employed in sterilization.
7. What is pasteurization?
8. What is flaming?
9. What is filtration?
10. Expand HEPA.
11. Define radiation.
12. Comment on types of radiation.
13. Define ionizing radiation.
14. Define non- ionizing radiation.
15. Write notes on phenolics.
16. Define BPL.
17. What is CFU?
18. Expand MPN test.
19. What is clumping?
20. Write about EMB agar.
21. Define turbidity.
22. What is tyndallization.
23. What is flash process?

24. What is meant by hanging drop method?
25. Define heat fixation.
26. Write about simple staining method.
27. Define Capsule.
28. Define flagella.
29. Define spore.
30. Write about negative staining method.
31. Explain types of staining.
32. Write about extracellular digestion.
33. Define culture media.
34. Define selective media.
35. Define differential media.
36. Define transport media.
37. Define basal media.
38. Define indicator media.
39. Define pure culture.
40. Define auxenic culture.
41. What is cryopreservation?
42. Define lyophilization.
43. Expand ATCC & NCTC.
44. Expand MTCC & IMTECH.
45. Expand STCC & JCM.

EIGHT MARKS

1. Comment of types of filters.
2. Write about phenol coefficient test.
3. Give the advantage of plate count method.
4. Write about sterility testing.
5. Explain bacteriostasis.
6. Explain fungistasis.
7. Explain steam sterilizer.
8. Comment of membrane filtration.

9. Write the types of phenolic compounds.
10. What is meant by oxidative damage?
11. Comment on holding method.
12. Comment on Arnold sterilizer.
13. Write in detail about the process of sterilization and its applications.
14. Explain in detail about physical method of sterilization.
15. Write the working principle of Autoclave.
16. Write the working principle of Hot air oven.
17. Explain the process of pasteurization.
18. Explain filtration and various types of filters.
19. Write about radiation, its types and application.
20. Differentiate between ionizing and non ionizing radiation.
21. Explain in detail about chemical method of sterilization.
22. Explain in detail about gaseous sterilization.
23. Write the protocol for sterility testing.
24. Comment on serial dilution technique.
25. Explain in detail about plating technique.
26. Comment on plate count method.
27. Discuss about viable plate count.
28. Write about most probable number test in detail.
29. Explain wet mount.
30. Comment on freeze drying.
31. Explain extracellular digestion.
32. Explain in detail about hanging drop method.
33. Explain the preparation of nutrient agar slant.
34. Explain the preparation of nutrient broth.
35. Explain the preparation of nutrient agar deep.
36. Write about media and its types.
37. Comment on selective media.
38. Comment on properties of differential media.
39. Write about various transport media.
40. Give the importance of basal media.
41. Discuss about various types of streaking.

42. Write about the method of quadrant streaking.
43. Write about the method of simple streaking.
44. Explain the pour plate technique.
45. Explain the spread plate technique.
46. Discuss the preservation method of microorganism.
47. Comment on refrigeration and glycerol storage.
48. Explain the process of lyophilization.
49. Give a detailed note on culture collection centre with in India and international

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SEMESTER – I **4H – 4C**

LECTURE PLAN

Unit V

S.No	Duration	Topic	Reference
1	1	Modern Microbiology, Molecular taxonomy	R1:pgs; 426 -432
2	1	16S /18S rRNAs and its importance in identification of microorganisms.	R1:pgs; 426 -428
3	1	Phylogenetic tree	R1:pgs; 428
4	1	Molecular tools in assessing microbial diversity	R1:pgs; 432 -435
5	1	Probiotics and their applications	W17
6	1	Microbial fuel cells	W18, W19
7	1	Recapitulation and discussion of question	
8	1	Old question paper discussion (Last Five years)	
9	1	Old question paper discussion (Last Five years)	
	Total Hrs: 09		

W17: en.wikipedia.org

W18: books.google.co.in

W19: en.wikipedia.org

UNIT V

Modern Microbiology

16S ribosomal RNA (or 16S rRNA) is a component of the 30S small subunit of prokaryotic ribosomes. The genes coding for it are referred to as 16S rDNA and are used in reconstructing phylogenies, due to the slow rates of evolution of this region of the gene. Carl Woese and George E. Fox were two of the people who pioneered the use of 16S rRNA in phylogenies. Multiple sequences of 16S rRNA can exist within a single bacterium.

Functions

It has several functions:

- Like the large (23S) ribosomal RNA, it has a structural role, acting as a scaffold defining the positions of the ribosomal proteins.
- The 3' end contains the anti-Shine-Dalgarno sequence, which binds upstream to the AUG start codon on the mRNA. The 3'-end of 16S RNA binds to the proteins S1 and S21 known to be involved in initiation of protein synthesis; RNA-protein cross-linking by A.P. Czernilofsky et al. (FEBS Lett. Vol 58, pp 281–284, 1975).
- Interacts with 23S, aiding in the binding of the two ribosomal subunits (50S+30S)
- Stabilizes correct codon-anticodon pairing in the A site, via a hydrogen bond formation between the N1 atom of Adenine residues 1492 and 1493 and the 2'OH group of the mRNA backbone

Universal primers

The 16S rRNA gene is used for phylogenetic studies as it is highly conserved between different species of bacteria and archaea. Carl Woese pioneered this use of 16S rRNA. Some (hyper)thermophilic archaea (i.e. order Thermoproteales) contain 16S rRNA gene introns that are located in highly conserved regions and can impact the annealing of "universal" primers. Mitochondrial and chloroplastic rRNA is also amplified.

The most common primer pair was devised by Weisburg *et al.* and is currently referred to as 27F and 1492R; however, for some applications shorter amplicons may be necessary for example for 454 sequencing with Titanium chemistry (500-ish reads are ideal) the primer pair 27F-534R covering V1 to V3. Often 8F is used rather than 27F. The two primers are almost identical, but 27F has an M instead of a C. AGAGTTTGATC**M**TGGCTCAG compared with 8F.

Primer name	Sequence (5'-3')
8F	AGA GTT TGA TCC TGG CTC AG
U1492R	GGT TAC CTT GTT ACG ACT T
928F	TAA AAC TYA AAK GAA TTG ACG GG
336R	ACT GCT GCS YCC CGT AGG AGT CT
1100F	YAA CGA GCG CAA CCC
1100R	GGG TTG CGC TCG TTG
337F	GAC TCC TAC GGG AGG CWG CAG
907R	CCG TCA ATT CCT TTR AGT TT
785F	GGA TTA GAT ACC CTG GTA
805R	GAC TAC CAG GGT ATC TAA TC
533F	GTG CCA GCM GCC GCG GTA A
518R	GTA TTA CCG CGG CTG CTG G
27F	AGA GTT TGA TCM TGG CTC AG
1492R	CGG TTA CCT TGT TAC GAC TT

PCR applications

In addition to highly conserved primer binding sites, 16S rRNA gene sequences contain hypervariable regions that can provide species-specific signature sequences useful for identification of bacteria. As a result, 16S rRNA gene sequencing has become prevalent in medical microbiology as a rapid and cheap alternative to phenotypic methods of bacterial identification. Although it was originally used to identify bacteria, 16S sequencing was subsequently found to be capable of reclassifying bacteria into completely new species, or even genera. It has also been used to describe new species that have never been successfully cultured.

16S ribosomal databases

The 16S rRNA gene is used as the standard for classification and identification of microbes, because it is present in most microbes and shows proper changes. Type strains of 16S rRNA gene sequences for most bacteria and archaea are available on public databases such as NCBI.

However, the quality of the sequences found on these databases are often not validated. Therefore, secondary databases that collect only 16S rRNA sequences are widely used. The most frequently used databases are listed below:

1) **EzTaxon:** The EzTaxon-e database is an extension of the original EzTaxon database. It contains comprehensive 16S rRNA gene sequences of taxa with valid names as well as sequences of uncultured taxa. EzTaxon-e contains complete hierarchical taxonomic structure (from phylum rank to species rank) for the domain of bacteria and archaea.
2) **Ribosomal Database Project**: The Ribosomal Database Project (RDP) is a curated database that offers ribosome data along with related programs and services. The offerings include phylogenetically ordered alignments of ribosomal RNA (rRNA) sequences, derived phylogenetic trees, rRNA secondary structure diagrams and various software packages for handling, analyzing and displaying alignments and trees. The data are available via ftp and electronic mail. Certain analytic services are also provided by the electronic mail server.
3) **SILVA**: SILVA provides comprehensive, quality checked and regularly updated datasets of aligned small (16S/18S, SSU) and large subunit (23S/28S, LSU) ribosomal RNA (rRNA) sequences for all three domains of life as well as a suite of search, primer-design and alignment tools (Bacteria, Archaea and Eukarya).
4) **Greengenes**: Greengenes is a quality controlled, comprehensive 16S reference database and taxonomy based off a de novo phylogeny that provides standard operational taxonomic unit sets.

18S ribosomal RNA

18S ribosomal RNA (abbreviated **18S rRNA**) is a part of the ribosomal RNA. The S in 18S represents Svedberg units. 18S rRNA is a component of the small eukaryotic ribosomal subunit (40S). 18S rRNA is the structural RNA for the small component of eukaryotic cytoplasmic ribosomes, and thus one of the basic components of all eukaryotic cells.

It is the eukaryotic nuclear homologue of 16S ribosomal RNA in Prokaryotes and mitochondria.

The genes coding for 18S rRNA are referred to as **18S rDNA**. Sequence data from these genes is widely used in molecular analysis to reconstruct the evolutionary history of organisms, especially in vertebrates, as its slow evolutionary rate makes it suitable to reconstruct ancient divergences.

Uses in phylogeny

The small subunit (SSU) 18S rRNA gene is one of the most frequently used genes in phylogenetic studies and an important marker for random target polymerase chain reaction (PCR) in environmental biodiversity screening. In general, rRNA gene sequences are easy to access due to highly conserved flanking regions allowing for the use of universal primers. Their repetitive arrangement within the genome provides excessive amounts of template DNA for PCR, even in the smallest organisms. The 18S gene is part of the ribosomal functional core and is exposed to similar selective forces in all living beings. Thus, when the first large-scale phylogenetic studies based on 18S sequences were published - first and foremost phylogeny of the animal kingdom by Field *et al.*, (1988) - the gene was celebrated as the prime candidate for reconstructing the metazoan tree of life. And in fact, 18S sequences later provided evidence for the splitting of Ecdysozoa and Lophotrochozoa, thus contributing to the most recent revolutionary change in our understanding of metazoan relationships.

During recent years and with increased numbers of taxa included into molecular phylogenies, however, two problems became apparent. First, there are prevailing sequencing impediments in representatives of certain taxa, such as the mollusk classes Solenogastres and Tryblidia, selected bivalve taxa, and the enigmatic crustacean class Remipedia. Failure to obtain 18S sequences of single taxa is considered a common phenomenon but is rarely ever reported. Secondly, in contrast to initially high hopes, 18S cannot resolve nodes at all taxonomic levels and its efficacy varies considerably among clades. This has been discussed as an effect of rapid ancient radiation within short periods. Multigene analyses are currently thought to give more reliable results for tracing deep branching events in Metazoa but 18S still is extensively used in phylogenetic analyses.

Phylogenetic Trees

The tree is divided into three major branches representing the three primary groups: Bacteria, Archaea, and Eucarya. The archaea and bacteria first diverged, then the eucaryotes developed. These three primary groups are called domains and placed above the phylum and kingdom levels (the traditional kingdoms are distributed among these three domains). The domains differ markedly from one another. Eucaryotic organisms with primarily glycerol fatty acyl diester membrane lipids and eucaryotic rRNA belong to the Eucarya. The domain Bacteria contains

procaryotic cells with bacterial rRNA and membrane lipids that are primarily diacyl glycerol diesters. Procaryotes having isoprenoid glycerol diether or diglycerol tetraether lipids in their membranesand archaeal rRNA compose the third domain, Archaea.

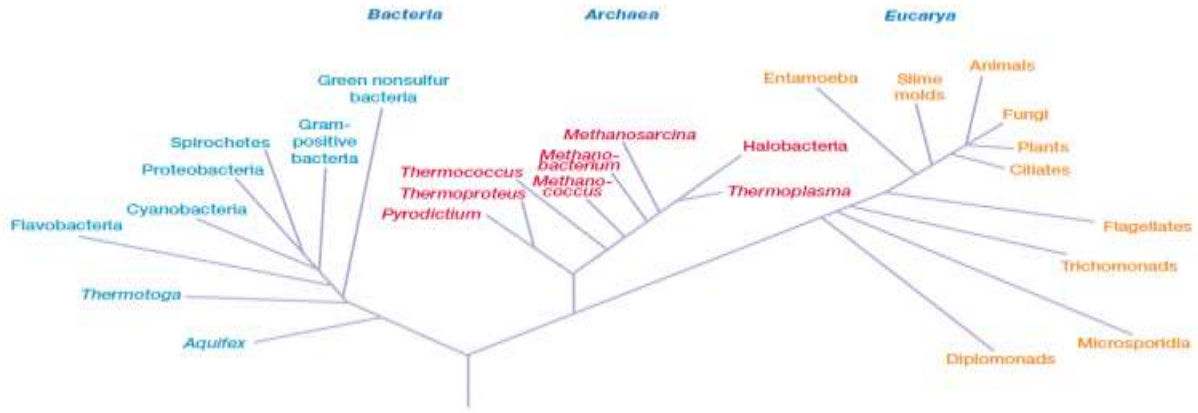


Figure: Universal Phylogenetic Tree.

The Major Divisions of Life

Since the beginning of biology, organisms have been classified as either plants or animals. However, discoveries in microbiology over the past century have shown that the two-kingdom system is oversimplified. Although not all biologists would agree, most microbiologists now believe that living forms can be divided into three distinctly different groups. We will first review this system in more detail, then turn to alternate views.

Domains

As mentioned earlier and illustrated in figure 1.1, Carl Woese and his collaborators have used rRNA studies to group all living organisms into three domains: Archaea, Bacteria, and Eucarya. Thus there are two quite different groups of procaryotes, the bacteria and the archaea. The bacteria comprise the vast majority of procaryotes. Among other properties, bacteria either have cell wall peptidoglycan containing muramic acid or are related to bacteria with such cell walls, and have membrane lipids with ester-linked, straight-chained fatty acids that resemble eukaryotic membrane lipids. The second group, the archaea differ from bacteria in many respects and resemble eucaryotes in some ways (table 19.8). Although the Archaea are described in more detail at a later point, it should be noted that they differ from bacteria in lacking muramic acid in their cell walls and in possessing:

(1) Membrane lipids with ether-linked branched aliphatic chains

(2) transfer RNAs without thymidine in the T or T+C arm

(3) Distinctive RNA polymerase enzymes

(4) Ribosomes of different composition and shape.

Thus although archaea resemble bacteria in their prokaryotic cell structure, they vary considerably on the molecular level. Both groups differ from eucaryotes in their cell ultrastructure and many other properties. However, inspection of table 19.8 shows that both bacteria and archaea do share some biochemical properties with eucaryotic cells. For example, bacteria and eucaryotes have ester-linked membrane lipids; archaea and eucaryotes are similar with respect to some components of the RNA and protein synthetic systems. Although the preceding view is the most widely accepted, other phylogenetic trees have been proposed. Six or more different trees relating the major domains have been proposed. **Figure 1.6** provides a simplified view of some of these. The first(figure 1.6a) indicates that the three groups are about equidistant from one another and fits with the early rRNA data. Figure 1.6b represents the currently most popular tree in which archaea and eucaryotes have a common ancestor; organisms like the bacteria may have existed before the other domains. The third tree, called the eocyte tree (figure 19.10c), is based on the proposal that sulfur-dependent, extremely thermophilic procaryotes called eocytes (dawn _ cell) are a separate group and more closely related to eucaryotes than are the archaea. Finally, some have proposed that eucaryotic cells are chimeric and arose from the fusion of a bacterium and archaeon (possibly a bacterium lacking a cell wall engulfed an eocytelike archaeon) (figure 1.6d). Clearly the situation is confused and more than one model has been proposed, though most microbiologists favor the three domain tree in figure 19.10b. When some protein sequences are used to construct phylogenetic trees, one does not even get a three domain pattern. Many factors may account for these problems. There could be unrecognized gene duplications that occurred before the domains formed, leading to confusing patterns. Unequal rates of evolution could distort the trees. Phylogenetically important information may have been lost in some molecular sequences. There may be significant sequence variation between the same molecules from different strains of the same species. Unless several strains are analyzed, false conclusions may be drawn. Thus inaccurate universal trees may result when only the sequences from a few molecules are employed (as is usually the case). One of the most important difficulties in constructing a satisfactory tree is widespread, frequent horizontal

or lateral gene transfer. Recent genome sequence studies have shown that there is extensive horizontal gene transfer within and between domains. Eucaryotes possess genes from both bacteria and archaea, and there has been frequent gene swapping between the two procaryotic domains. It appears that at least some bacteria even have acquired eucaryotic genes. Thus the pattern of microbial evolution is not as linear and treelike as previously thought. **Figure 1.7** depicts a morerealistic reticulated tree in which horizontal gene transfer playsa major role. This tree resembles a web or network with many lateral branches linking various trunks, each branch representing the transfer of one or a few genes. Instead of having a single main trunk or common ancestor at its base, this tree has several trunks or groups of primitive cells that contribute to the original gene pool. Although there is extensive gene transfer between the two procaryotic domains throughout their development, the eucaryotic domain seldom participates in horizontal gene transfer after the formation of fungi, plants, and animals. It is possible that eucaryotic cells originated in a complex process involving many gene transfers from both bacteria and archaea. This hypothesis still allows for the formation of mitochondria and chloroplasts by endosymbiosis with _-proteobacteria and cyanobacteria, respectively. Presumably the three domains remain separate because there are many more gene transfers within each than between domains. This brief discussion of the problems in developing a true universal phylogenetic tree is intended to show the difficulty in determining phylogenetic relationships. The best results will be obtained when all possible data, both molecular and phenotypic, are used in the analysis (for example, in polyphasic taxonomy). We will usually employ trees derived from 16S rRNA sequences because these data are most extensive and are used by most microbiologists. Keep in mind that such trees may well change as further data are collected and analyzed.

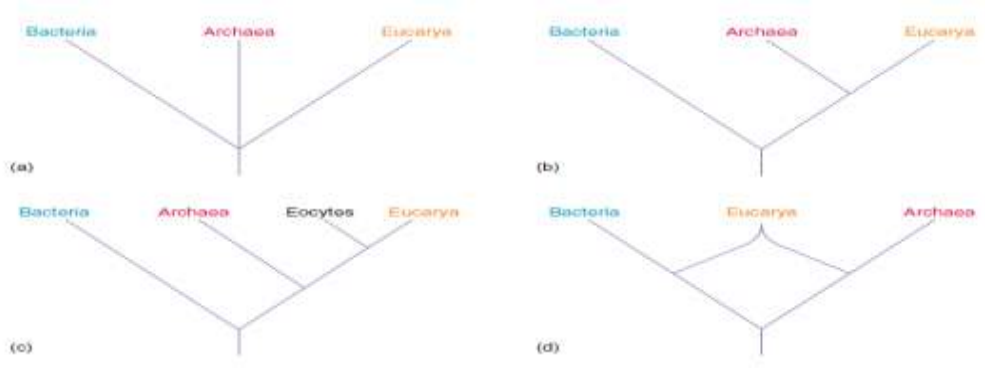


Figure 1.6 Variations in the Design of the “Tree of Life.”

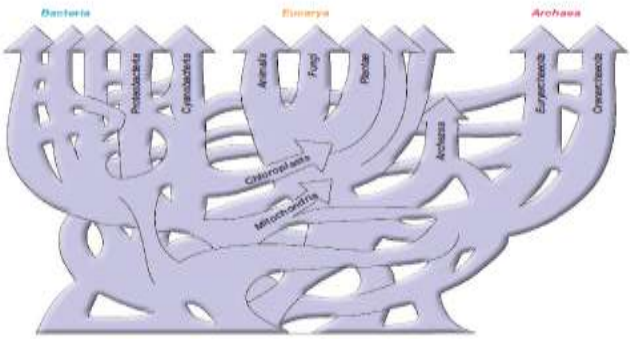


Figure 1.7 Universal Phylogenetic Tree with Frequent Horizontal or Lateral Gene Transfers.

Kingdoms

While most bacteriologists favor the three-domain system, many protozoologists, botanists, and zoologists still think in terms of five or more kingdoms. This section briefly summarizes the nature of some of these classification systems. The first classification system to have gained popularity in the last few decades is the five-kingdom system first suggested by Robert H. Whittaker in the 1960s. An overview of Whittaker's five-kingdom system is presented in **figure 1.8a.** Organisms are placed into five kingdoms based on at least three major criteria:

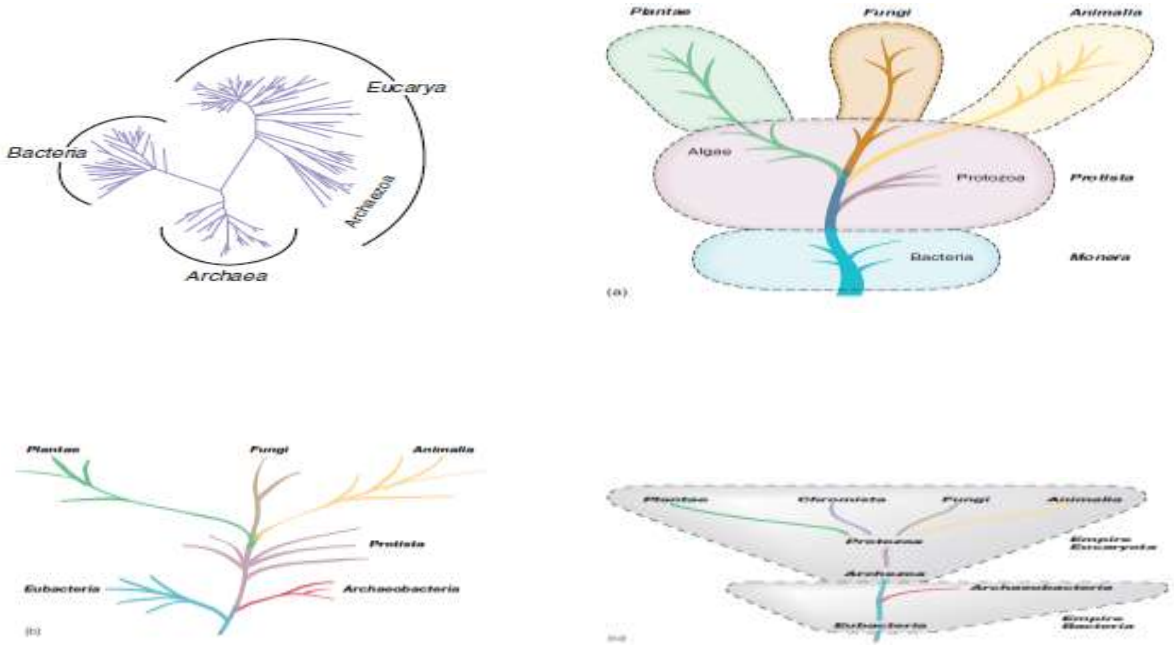
(1) Cell type—procaryotic or eucaryotic,

(2) Level of organization—solitary and colonial unicellular organization ormulticellular, and

(3) Nutritional type.

In this system the kingdom Animalia contains multicellular animals with wall-less eukaryotic cells and primarily ingestive nutrition, whereas the kingdom Plantae is composed of multicellular plants with walled eucaryotic cells and primarily photoautotrophic nutrition. Microbiologists study members of the other three kingdoms. The kingdom Monera or Procaryotae contains all procaryotic organisms. The kingdom Protista is the least homogeneous and hardest to define. **Protists** are eucaryotes with unicellular organization, either in the form of solitary cells or colonies of cells lacking true tissues. They may have ingestive, absorptive, or photoautotrophic nutrition, and they include most of the microorganisms known as algae, protozoa, and many of the simpler fungi. The kingdom Fungi contains eucaryotic and predominately multinucleate organisms, with nuclei dispersed in a walled and often septate mycelium; their nutrition is absorptive. The five-kingdom system is not accepted by many biologists. A major problem is its lack of distinction between archaea and bacteria. The kingdom Protista also may be too diverse

to be taxonomically useful. In addition, the boundaries between the kingdoms Protista, Plantae, and Fungi are ill-defined. For example, the brown algae are probably not closely related to the plants even though the five-kingdom system places them in the Plantae. Because of such problems with the five-kingdom system, various alternatives have been suggested. The six-kingdom system is the simplest option; it divides the kingdom Monera or Procaryotae into two kingdoms, the Eubacteria and Archaeobacteria (figure1.8b). Many attempts have been made to divide the protists into several better-defined kingdoms. The eight-kingdom system of Cavalier-Smith is a good example (figure 1.8c). Cavalier-Smith believes that differences in cellular structure and genetic organization are exceptionally important in determining phylogeny; thus he has used ultrastructural characteristics as well as rRNA sequences and other molecular data in developing his classification. He divides all organisms into two empires and eight kingdoms.



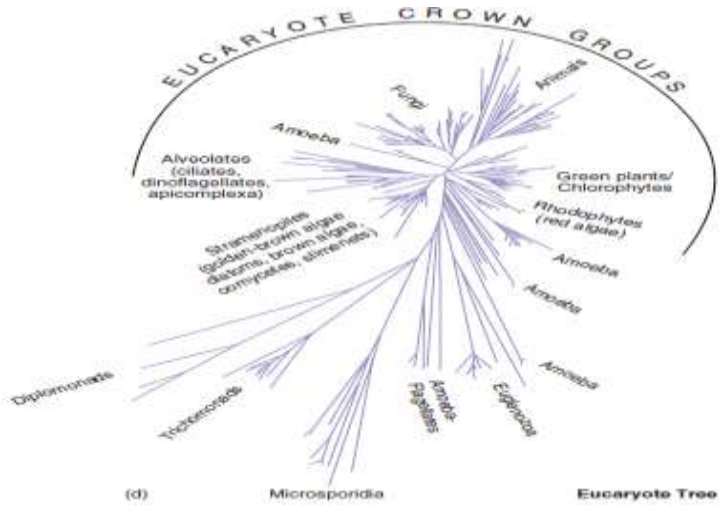


Figure 1.8 Systems of Eucaryotic and Procaryotic Phylogeny. Simplified schematic diagrams of the (**a**) five-kingdom system (Whittaker), (**b**) six-kingdom system, (**c**) eight-kingdom system (Cavalier-Smith), and (**d**) the universal and eucaryotic trees according to Sogin.

The empire Bacteria contains two kingdoms, the Eubacteria and the Archaeobacteria. The second empire, the Eucaryota, contains six kingdoms of eucaryotic organisms. There are two new kingdoms of eucaryotes. The Archezoa are primitive eucaryotic unicellular organisms such as Giardia that have 70S ribosomes and lack Golgi apparatuses, mitochondria, chloroplasts, and peroxisomes. The kingdom Chromista contains mainly photosynthetic organisms that have their chloroplasts within the lumen of the rough endoplasmic reticulum rather than in the cytoplasmic matrix (as is the case in the kingdom Plantae). Diatoms, brown algae, cryptomonads, and oomycetes are all placed in the Chromista. The boundaries of the remaining four kingdoms—Plantae, Fungi, Animalia, and Protozoa—have been adjusted to better define each kingdom and distinguish it from the others. Sogin and his coworkers do not cluster the eucaryotes into a few major divisions, but rather consider them to be a single domain or empire composed of a collection of independently evolved lineages (figure 1.8d). In this scheme the protists do not comprise a separate kingdom, but simply represent a level of organization with many separate lineages and tremendous diversity.

Assessing Microbial Phylogeny

Procaryotic taxonomy is changing rapidly. This is caused by ever increasing knowledge of the biology of procaryotes and remarkable advances in computers and the use of molecular

characteristics to determine phylogenetic relationships between prokaryotic groups. This section briefly describes some of the ways in which phylogenetic relationships are determined.

Molecular Chronometers

The sequences of nucleic acids and proteins change with time and are considered to be **molecular chronometers.** This concept, first suggested by Zuckerkandl and Pauling (1965), is important in the use of molecular sequences in determining phylogenetic relationships and is based on the assumption that there is an evolutionary clock. It is thought that the sequences of many rRNAs and proteins gradually change over time without destroying or severely altering their functions. One assumes that such changes are selectively neutral, occur fairly randomly, and increase linearly with time. When the sequences of similar molecules are quite different in two groups of organisms, the groups diverged from one another a long time ago. Phylogenetic analysis using molecular chronometers is somewhat complex because the rate of sequence change can vary; some periods are characterized by especially rapid change. Furthermore, different molecules and various parts of the same molecule can change at different rates. Highly conserved molecules such as rRNAs are used to follow large-scale evolutionary changes, whereas rapidly changing molecules are employed in following speciation. Not everyone believes that molecular chronometers, and particularly protein clocks, are very accurate. Further studies will be required to establish their accuracy and usefulness.

Phylogenetic Trees

Phylogenetic relationships are illustrated in the form of branched diagrams or trees. A **phylogenetic tree** is a graph made of branches that connect nodes (**figure 1.5**). The nodes represent taxonomic units such as species or genes; the external nodes, those at the end of the branches, represent living organisms. The tree may have a time scale, or the length of the branches may represent the number of molecular changes that have taken place between the two nodes. Finally, a tree may be nrooted or rooted. An unrooted tree (figure 1.5a) simply represents phylogenetic relationships but does not provide an evolutionary path. Figure 1.5a shows that A is more closely related to C than it is to either B or D, but does not specify the common ancestor for the four species or the direction of change. In contrast, the rooted tree (figure 1.5b) does give a node that serves as the common ancestor and shows the development of the four species from this root. It is much more difficult to develop a rooted tree. For example, there are 15 possible

rooted trees that connect four species, but only three possible unrooted trees. Phylogenetic trees are developed by comparing molecular sequences. To compare two molecules their sequences must first be aligned so that similar parts match up. The object is to align and compare homologous sequences, ones that are similar because they had a common origin in the past. This is not an easy task, and computers plus fairly complex mathematics must be employed to minimize the number of gaps and mismatches in the sequences being compared.

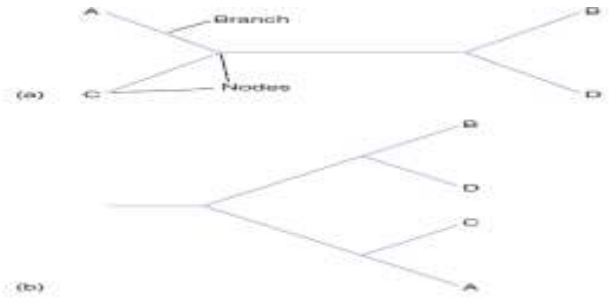


Figure 1.5 Examples of Phylogenetic Trees. (**a**) Unrooted tree joining four taxonomic units. (**b**) Rooted tree.

Once the molecules have been aligned, the number of positions that vary in the sequences can be determined. These data are used to calculate a measure of the difference between the sequences. Often the difference is expressed as the **evolutionary distance.** This is simply a quantitative indication of the number of positions that differ between two aligned macromolecules. Statistical adjustments can be made for back mutations and multiple substitutions that may have occurred. Organisms are then clustered together based on similarity in the sequences. The most similar organisms are clustered together, then compared with the remaining organisms to form a larger cluster associated together at a lower level of similarity or evolutionary distance. The process continues until all organisms are included in the tree. Phylogenetic relationships also can be estimated by techniques such as parsimony analysis. In this approach, relationships are determined by estimating the minimum number of sequence changes required to give the final sequences being compared. It is presumed that evolutionary change occurs along the shortest pathway with the fewest changes or steps from an ancestor to the organism in question. The tree or pattern of relationships is favored that is simplest and requires the fewest assumptions.

rRNA, DNA, and Proteins as Indicators of Phylogeny

Although a variety of molecular techniques are used in estimating the phylogenetic relatedness of procaryotes, the comparison of 16S rRNAs isolated from thousands of strains is of particular

importance. Recall that either complete rRNA or rRNA fragments can be sequenced and compared (p. 432). The association coefficients or Sab values from rRNA studies are assumed to be a true measure of relatedness; the higher the Sab values obtained from comparing two organisms, the more closely the organisms are related to each other. If the sequences of the 16S rRNAs of two organisms are identical, the Sab value is 1.0. Sab values also are a measure of evolutionary time. A group of procaryotes that branched off from other prokaryotes long ago will exhibit a large range of Sab values because it has had more time to diversify than a group that developed more recently. That is, the narrower the range of Sab values in a group of procaryotes, the more modern it is. After Sab values have been determined, a computer calculates the relatedness of the organisms and summarizes their relationships in a tree or dendrogram. Ribosomal RNA sequence studies have uncovered a feature of great practical importance. The 16S rRNA of most major phylogenetic groups has one or more characteristic nucleotide sequences called oligonucleotide signatures. **Oligonucleotide signature sequences** are specific oligonucleotide sequences that occur in most or all members of a particular phylogenetic group. They are rarely or never present in other groups, even closely related ones. Thus signature sequences can be used to place microorganisms in the proper group. Signature sequences have been identified for bacteria, archaea, eucaryotes, and many major prokaryotic groups. Although rRNA comparisons are useful above the species level, DNA similarity studies sometimes are more effective in categorizing individual species and genera. These comparisons can be carried out using G+C content or hybridization studies, as already discussed. Techniques such as direct sequence analysis and analysis of DNA restriction fragment patterns also can be used. There are advantages to DNA comparisons. As with rRNA, the DNA composition of a cell does not change with growth conditions. DNA comparisons also are based on the complete genomes rather than a fraction, and make it easier to precisely define a species based on the 70% relatedness criterion. Full sequences of genomes now are being published and will make it easier to study the impact on phylogenetic schemes of such processes as lateral gene transfer as will be discussed later. Many protein sequences are currently used to develop phylogenetic trees. This approach does have some advantages over rRNA comparisons. A sequence of 20 amino acids has more information per site than a sequence of four nucleotides. Protein sequences are less affected by organism-specific differences in G+ C content than are DNA and RNA sequences.

Finally, protein sequence alignment is easier because it is not dependent on secondary structure as is an rRNA sequence. Proteins evolve at different rates, as might be expected. Indispensable proteins with constant functions do not change as rapidly (e.g., histones and heat-shock proteins), whereas proteins such as immunoglobulins evolve quite rapidly. Thus not all proteins are suitable for studying large-scale changes that occur over long periods. As mentioned earlier, there is a question about the adequacy of protein based clocks. It is clear that sequences of all three macromolecules can provide valuable phylogenetic information. However, different sequences sometimes produce different trees, and it may be difficult to decide which result is most accurate. Presumably more molecular data plus further study of phenotypic properties will help resolve uncertainties.

Polyphasic Taxonomy

Because phylogenetic results vary with the data used in analysis, many taxonomists believe that all possible valid data should be employed in determining phylogeny. In the approach called **polyphasic taxonomy,** taxonomic schemes are developed using a wide range of phenotypic and genotypic information ranging from molecular properties to ecological characteristics. The techniques that are appropriate for grouping organisms depend on the level of taxonomic resolution needed. For example, serological techniques can be used to identify strains, but not genera or species. Protein electrophoretic patterns are useful in determining species, but not genera or families. DNA hybridization and the analysis of % G+C can be used in studying species and genera. Characteristics such as chemical composition, DNA probe results, rRNA sequences, and DNA sequences can be used to define species, genera, and families. Where possible, as many properties as possible are used to get more stable and reliable results. Successful polyphasic approaches often will help one select techniques for rapid identification of the microorganism. Because rRNA sequences have been used so extensively, we will focus mainly on the phylogenetic trees derived from rRNA studies.

Metagenomics

Defining Statement

Metagenomics is the study of the collective genomes ofthe members of a microbial community. It involves cloning and analyzing the genomes without culturing the organisms in the

community, thereby offering the opportunity to describe the planet's diverse microbialinhabitants, many of which cannot yet be cultured.

Introduction

Prokaryotes are the most physiologically diverse andmetabolically versatile organisms on our planet. Bacteria vary in the ways that they forage for food, transducer energy, contend with competitors, and associate with allies. But the variations that we know are only the tip of the microbial iceberg. The vast majority of microorganisms have not been cultivated in the laboratory, and almost all of our knowledge of microbial life is based on organisms raised in pure culture. The variety of the rest of the uncultured microbial world is staggering and will expand our view of what is possible in biology.

The challenge that has frustrated microbiologists for decades is how to access the microorganisms that cannot be cultured in the laboratory.Many clever cultivation methods have been devised to expand the range of organisms that can be cultured, but knowledge of the uncultured world is slim, so it is difficult to use a process based on rational design to coax many of these organisms into culture. Metagenomics provides an additional set of tools to study uncultured species. This new field offers an approach to studying microbial communities as entire units, without cultivating individual members. Metagenomics entails extraction of DNA from a community so that all of the genomes of organisms in the community are pooled. These genomes are usually fragmented and cloned into an organism that can be cultured to create 'metagenomic libraries', and these libraries are then subjected to analysis based on DNA sequence or on functions conferred on the surrogate host by the metagenomic DNA. Although this field of microbiology is quite young, discoveries have already been made that challenge existing paradigms and made substantial contributions to biologists' quest to piece together the puzzle of life.

Building Metagenomic Libraries

DNA has been isolated from microbial communities inhabiting diverse environments. Early metagenomic projects focused on soil and sea water because of the richness of microbial species (e.g., 5000–40 000 species/g soil) as well as the abundance of biocatalysts and natural products

known to be in these environments from culture-based studies. While soil has been most sampled for metagenomic libraries, aquatic sediments, biofilms, and industrial effluents have also been successfully tapped, often because of their unique physicochemistries, for various biological activities. Metagenomic libraries constructed from DNA extracted from animal-associated microbial communities have also been the source of a number of novel biocatalysts (i.e., hydrolases, laccases, and xylanases), antibiotic resistance genes, and inter/intraspecies communication molecules.

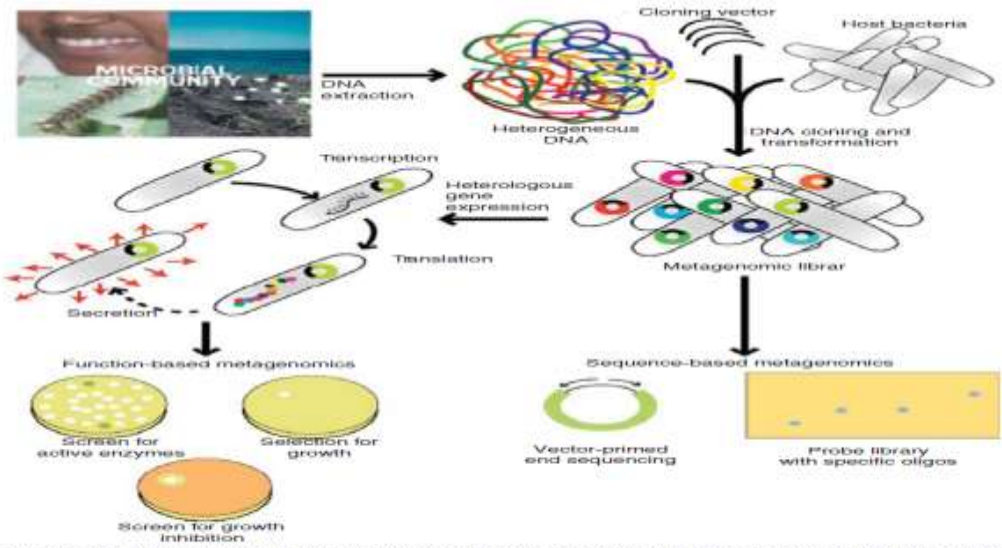


Figure 1 Metagenomics. Metagenomics is the study of the collective genomes of the members of a community. DNA is extracted directly from the community, cloned into a surrogate host, and then studied by sequencing or screening for expression of activities of interest. Many microbial communities have been tapped for metagenomic analyses. Following construction of a metagenomic library, two approaches can be taken to access the genomic information. Functional metagenomics requires that the host bacterium can express the recombinant DNA in either screens for active enzymes or antibiotic production or selections for growth under growth-suppressive conditions (e.g., nutrient deficiency or presence of an antibiotic). In sequence-based metagenomics, cloned DNA is randomly sequenced using vector-based primers or a specific gene is sought using complimentary oligonucleotides (oligos) to hybridize to arrayed metagenomic clones.

Prebiotics

Prebiotics is a general term to refer to chemicals that induce the growth or activity of microorganisms (e.g., bacteria and fungi) that contribute to the well-being of their host. The most common example is in the gastrointestinal tract, where prebiotics can alter the composition of organisms in the gut microbiome. However, in principle it is a more general term that can refer to other areas of the body as well. For example, certain hand moisturizers have been proposed to act as prebiotics to improve the activity or composition of the skin microbiota.

In diet, prebiotics are typically non-digestible fiber compounds that pass undigested through the upper part of the gastrointestinal tract and stimulate the growth or activity of advantageous

bacteria that colonize the large bowel by acting as substrate for them. They were first identified and named by Marcel Roberfroid in 1995.[2] As a functional food component, prebiotics, like probiotics, are conceptually intermediate between foods and drugs. Depending on the jurisdiction, they typically receive an intermediate level of regulatory scrutiny, in particular of the health claims made concerning them.

Definition

"A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health."

Researchers now also focus on the distinction between short-chain, long-chain, and full-spectrum prebiotics. "Short-chain" prebiotics, e.g. oligofructose, contain 2–8 links per saccharide molecule and are typically fermented more quickly in the ascending colon of the colon providing nourishment to the bacteria in that area. Longer-chain prebiotics, e.g. inulin, contain 9-64 links per saccharide molecule, and tend to be fermented more slowly, nourishing bacteria predominantly in the descending colon. Full-spectrum prebiotics provide the full range of molecular link-lengths from 2-64 links per molecule, and nourish bacteria throughout the colon, e.g. Oligofructose-Enriched Inulin (OEI). The majority of research done on prebiotics is based on full-spectrum prebiotics, typically using OEI as the research substance.

Prebiotics are a source of food for probiotics to grow, multiply and survive in the gut. Prebiotics are fibres which cannot be absorbed or broken down by the body and therefore serve as a great food source for probiotics, in particular the Bifidobacteria genus, to increase in numbers. Prebiotics by nature do not stimulate the growth of bad bacteria or other pathogens; the official definition of prebiotics is: "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, which can improve host health."

Prebiotics occur naturally in our diet and prebiotic fibres can be found in Jerusalem artichokes, garlic, chicory, and onions amongst other things. One may have to eat large quantities of these foods to have a ‘bifidogenic’ effect – that is to increase the levels of friendly bacteria in our intestines. For this reason many people find it easier to take a prebiotic supplement, or a combination probiotic and prebiotic supplement (called a synbiotic) to ensure they are feeding

their levels of friendly bacteria. Research shows that there are different types of prebiotics, in a similar manner as there are different types of probiotics. With prebiotics, the key differentiating factor is the length of the chemical chain – short chain; medium chain or long chain determines where in the gastrointestinal tract the prebiotic has its effect, and how the benefits may be felt by the host. Common prebiotics include: inulin, Fructooligosaccharides (FOS), galactooligosaccharides (GOS), lactulose and lafinose. Within the OptiBac Probiotics range, the prebiotic FOS can be found in 'For every day', 'One week flat' (formerly called 'For a flat stomach'), 'For babies & children' and 'Bifidobacteria & fibre' (formerly 'For maintaining regularity'). Fructooligosaccharides prebiotics are low molecular weight carbohydrates which only promote growth of probiotics. FOS is probably the most researched of the prebiotic fibres. For more information on prebiotics, healthcare professionals can read the in-depth article and research analysis by Dr Georges Mouton, 'The Uses of Prebiotics'.

NB: In some cases, prebiotics can cause minor disturbance / flatulence in the first few days of taking the but after 3-4 days of continued use (once the intestines have adapted to the prebiotics) this discomfort tends to disappear. Many practitioners make their clients aware of this when recommending prebiotics, especially in higher doses.

How Prebiotics Work

Prebiotics are really the new kid on the block. The term was coined in 1995. A prebiotic is a special type of soluble fiber that is used mostly by the beneficial good bacteria as a fuel. These good bacteria, in turn, produce certain substances that acidify the colon (a very good thing) and serve as a nutrition source for the colon's own cells. Isn't this remarkable? The colon provides a warm, oxygen-free environment for these beneficial bacteria to grow. These bacteria, in turn, manufacture the nutrition source for the colon itself. This is a true symbiotic relationship where both the bacteria and colon depend on each other and promote each others' health. Of course, the body benefits even more as some, rather remarkable health benefits occur when this system is operating maximally.

So what are these prebiotics? The ones with the most science behind them are inulin and oligofructose. Inulin, itself, is remarkable in that it has been around in the plant world for a very long time. It has been found in over 36,000 different plants, so it somehow has been a vital food source for plant-eating animals and humans for a very long time. Interestingly, as our food

industries and agriculture have developed, the foods in which we get inulin have become limited. We now find inulin in: wheat, onions, bananas, garlic, leeks, chicory root, Jerusalem artichokes, wild yam, agava, and jicama.

In the U.S., most people get very little of this valuable fiber, perhaps only 2-3 grams a day on average with 70% of this coming from wheat and 20% from onions. Europeans eat three to five times this amount of inulin containing foods.

A prebiotic:

1. is not digested by the small intestine
2. is used as fuel, or fermented, by some colon bacteria
3. produces health benefits by objective measurements

According to some leading authorities, only inulin and oligofructose have fulfilled these three criteria. There are only a few others, but none with as much medical research.

So, the proven prebiotics fibers are a relatively new discovery. More than this, they have been found by careful research to provide significant health benefits, not only to the colon but to the body as a whole.

Function

The prebiotic definition does not emphasize a specific bacterial group. Generally, however, it is assumed that a prebiotic should increase the number or activity of bifidobacteria and lactic acid bacteria. The importance of the bifidobacteria and the lactic acid bacteria (LABs) is that these groups of bacteria may have several beneficial effects on the host, especially in terms of improving digestion (including enhancing mineral absorption) and the effectiveness and intrinsic strength of the immune system. A product that stimulates bifidobacteria is considered a bifidogenic factor. Some prebiotics may thus also act as a bifidogenic factor and vice versa, but the two concepts are not identical.

Sources

Top 10 Foods Containing Prebiotics	
Food	**Prebiotic Fiber Content by Weight**
Gum Arabic	85%
Raw, Dry Chicory Root	64.6%

Raw, Dry Jerusalem Artichoke	31.5%
Raw, Dry Dandelion Greens	24.3%
Raw, Dry Garlic	17.5%
Raw, Dry Leek	11.7%
Raw, Dry Onion	8.6%
Raw Asparagus	5%
Raw Wheat bran	5%
Whole Wheat flour, Cooked	4.8%
Raw Banana	1%
Source:[18]	

While there is no broad consensus on an ideal daily serving of prebiotics, recommendations typically range from 4 to 8 grams (0.14–0.28 oz) for general digestive health support, to 15 grams (0.53 oz) or more for those with active digestive disorders. Given an average 6 grams (0.21 oz) serving, below are the amounts of prebiotic foods required to achieve a daily serving of prebiotic fiber:

Food	**Amount of food to achieve 6g serving of prebiotics**
Raw Chicory Root	9.3g (0.33oz)
Raw Jerusalem Artichoke	19g (0.67oz)
Raw Dandelion Greens	24.7g (0.8oz)
Raw Garlic	34.3g (1.21oz)
Raw Leek	51.3g (1.81oz)
Raw Onion	69.8g (2.46oz)
Cooked Onion	120g (4.2oz)
Raw Asparagus	120g (4.2oz)
Raw Wheat Bran	120g (4.2oz)
Whole Wheat Flour, Cooked	125g (4.4oz)

Raw Banana	600g (1.3lb)

Research

Preliminary research has demonstrated potential effects on calcium and other mineral absorption, immune system effectiveness, bowel acidity, reduction of colorectal cancer risk, inflammatory bowel disease (Crohn's disease or ulcerative colitis) hypertension and defecation frequency. Prebiotics may be effective in decreasing the number of infectious episodes needing antibiotics and the total number of infections in children aged 0–24 months.

While research demonstrates that prebiotics lead to increased production of short-chain fatty acids (SCFA), more research is required to establish a direct causal connection. Prebiotics may be beneficial to inflammatory bowel disease or Crohn's disease through production of SCFA as nourishment for colonic walls, and mitigation of ulcerative colitis symptoms.

The immediate addition of substantial quantities of prebiotics to the diet may result in an increase in fermentation, leading to increased gas production, bloating or bowel movement. Production of SCFA and fermentation quality are reduced during long-term diets of low fiber intake. Until bacterial flora are gradually established to rehabilitate or restore intestinal bacteria, nutrient absorption may be impaired and colonic transit time temporarily increased with an immediate addition of higher prebiotic intake.

Genetic modification

Genetically modified plants have been created in research labs with upregulated inulin production.

Probiotics

Probiotics are live bacteria and yeasts that are good for your health, especially your digestive system. We usually think of bacteria as something that causes diseases. But your body is full of bacteria, both good and bad. Probiotics are often called "good" or "helpful" bacteria because they help keep your gut healthy.

Probiotics are naturally found in your body. You can also find them in some foods and supplements. It's only been since about the mid-1990s that people have wanted to know more about probiotics and their health benefits. Doctors often suggest them to help with digestive problems. And because of their newfound fame, you can find them in everything from yogurt to chocolate.

How Do They Work?

- When you lose "good" bacteria in your body (like after you take antibiotics, for example), probiotics can help replace them.
- They can lower the amount of "bad" bacteria in your system that can cause infections or other problems.
- They can help balance your "good" and "bad" bacteria to keep your body working like it should.

Types of Probiotics

Many types of bacteria are classified as probiotics. They all have different benefits, but most come from two groups.

Lactobacillus. This may be the most common probiotic. It's the one you'll find in yogurt and other fermented foods. Different strains can help with diarrhea and may help with people who can't digest lactose, the sugar in milk.

Bifidobacterium. Can also find it in some dairy products. It may help ease the symptoms of irritable bowel syndrome (IBS).

What Do They Do?

Probiotics help move food through your gut. Researchers are still trying to figure out which are best for certain health problems. Some common conditions they treat are:

- Irritable bowel syndrome
- Inflammatory bowel disease (IBD)
- Infectious diarrhea (caused by viruses, bacteria, or parasites)
- Antibiotic-related diarrhea

There is also some research to show they ease the symptoms of non-stomach-related problems. For example, some people say they have helped with:

- Skin conditions, like eczema
- Urinary and vaginal health
- Preventing allergies and colds
- Oral health

How to Use Them Safely

The FDA regulates probiotics like foods, not like medications. Unlike drug companies, makers of

probiotic supplements don't have to show their products are safe or that they work. Ask your doctor for more information about the correct product and dose for you.

In general, probiotic foods and supplements are thought to be safe. Mild side effects might include upset stomach, diarrhea, gas, and bloating for the first couple of days after you start them. They may also trigger allergic reactions. Stop taking them and talk to your doctor if you have problems.

Probiotics are microorganisms that are believed to provide health benefits when consumed. The term **probiotic** is currently used to name ingested microorganisms associated with beneficial effects to humans and animals. Introduction of the concept is generally attributed to Nobel Prize recipient Élie Metchnikoff, who in 1907 suggested that "the dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes". A significant expansion of the potential market for probiotics has led to higher requirements for scientific substantiation of putative beneficial effects conferred by the microorganisms. Studies on the medical benefits of probiotics have yet to reveal a cause-effect relationship, and their medical effectiveness has yet to be conclusively proven for most of the studies conducted thus far.

Commonly claimed benefits of probiotics include the decrease of potentially pathogenic gastrointestinal microorganisms, the reduction of gastrointestinal discomfort, the strengthening of the immune system, the improvement of the skin's function, the improvement of bowel regularity, the strengthening of the resistance to cedar pollen allergens, the decrease in body pathogens, the reduction of flatulence and bloating, the protection of DNA, the protection of proteins and lipids from oxidative damage, and the maintaining of individual intestinal microbiota in subjects receiving antibiotic treatment.

Scientific evidence to date has been insufficient to substantiate any antidisease claims or health benefits from consuming probiotics

Etymology

Some literature gives it a full Greek etymology, but the term appears to be a composite of the Latin preposition *pro* ("for") and the Greek adjective βιωτικός (*biotic*), the latter deriving from the noun βίος (*bios*, "life").

Definition

The World Health Organization's 2001 definition of probiotics is "live micro-organisms which,

when administered in adequate amounts, confer a health benefit on the host". Following this definition, a working group convened by the FAO/WHO in May 2002 issued the "Guidelines for the Evaluation of Probiotics in Food". This first global effort was further developed in 2010, two expert groups of academic scientists and industry representatives made recommendations for the evaluation and validation of probiotic health claim. The same principles emerged from those groups as the ones expressed in the Guidelines of FAO/WHO in 2002. This definition, although widely adopted, is not acceptable to the European Food Safety Authority because it embeds a health claim which is not measurable.

A consensus definition of the term "probiotics", based on the available information and scientific evidence, was adopted after a joint Food and Agricultural Organization of the United Nations and World Health Organization expert consultation. In October 2001, this expert consultation defined probiotics as: "live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host". The FAO/WHO consultation was also a first effort towards the assessment of probiotics efficacy and resulted in May 2002 in a document named "Guidelines for the Evaluation of Probiotics in Food". This effort is accompanied by local governmental and supra-governmental regulatory bodies requirements to better characterize health claims substantiations.

Probiotics have to be alive when administered. One of the concerns throughout the scientific literature resides in the viability and reproducibility on a large scale of the observed results, as well as the viability and stability during use and storage, and finally the ability to survive in stomach acids and then in the intestinal ecosystem. Probiotics must have undergone controlled evaluation to document health benefits in the target host. Only products containing live organisms shown in reproducible human studies to confer a health benefit can actually claim to be a probiotic. The correct definition of health benefit, backed with solid scientific evidence, is a strong element for the proper identification and assessment of the effect of a probiotic. This aspect represents a major challenge for scientific and industrial investigations because several difficulties arise, such as variability in the site for probiotic use (oral, vaginal, intestinal) and mode of application.

The probiotic candidate must be a taxonomically defined microbe or combination of microbes (genus, species, and strain level). It is commonly admitted that most effects of probiotic are strain-specific and cannot be extended to other probiotics of the same genus or species. This calls for a precise identification of the strain, i.e. genotypic and phenotypic characterization of the tested

microorganism.

Probiotics must be safe for their intended use. The 2002 FAO/WHO guidelines recommend that, though bacteria may be generally recognized as safe (GRAS), the safety of the potential probiotic should be assessed by the minimum required tests:

- Determination of antibiotic resistance patterns
- Assessment of certain metabolic activities (e.g., D-lactate production, bile salt deconjugation)
- Assessment of side effects during human studies
- Epidemiological surveillance of adverse incidents in consumers (after market)
- If the strain under evaluation belongs to a species that is a known mammalian toxin producer, it must be tested for toxin production. One possible scheme for testing toxin production has been recommended by the EU Scientific Committee on Animal Nutrition.
- If the strain under evaluation belongs to a species with known hemolytic potential, determination of hemolytic activity is required

In Europe, EFSA has adopted a premarket system for safety assessment of microbial species used in food and feed productions, to set priorities for the need of risk assessment. The assessment is made for a selected group of microorganisms, which if favorable, leads to the "Qualified Presumption of Safety" status.

Finally, probiotics must be supplied in adequate numbers, which may be defined as the number able to trigger the targeted effect on the host. It depends on strain specificity, process, and matrix, as well as the targeted effect. Most of reported benefits demonstrated with the traditional probiotics have been observed after ingestion of a concentration around 10^7 to 10^8 probiotic cells per gram, with a serving size around 100 to 200 mg per day.

History

Probiotics have received renewed attention recently from product manufacturers, research studies, and consumers. The history of probiotics can be traced to the first use of cheese and fermented products that were well known to the Greeks and Romans who recommended their consumption. The fermentation of dairy foods represents one of the oldest techniques for food preservation.

The original modern hypothesis of the positive role played by certain bacteria was first introduced by Russian scientist and Nobel laureate Élie Metchnikoff, who in 1907 suggested that it would be possible to modify the gut flora and to replace harmful microbes with useful microbes.[3]

Metchnikoff, at that time a professor at the Pasteur Institute in Paris, proposed the hypothesis that the aging process results from the activity of putrefactive (proteolytic) microbes producing toxic substances in the large bowel. Proteolytic bacteria such as clostridia, which are part of the normal gut flora, produce toxic substances including phenols, indols, and ammonia from the digestion of proteins. According to Metchnikoff, these compounds were responsible for what he called "intestinal autointoxication", which would cause the physical changes associated with old age.

It was at that time known that milk fermented with lactic-acid bacteria inhibits the growth of proteolytic bacteria because of the low pH produced by the fermentation of lactose. Metchnikoff had also observed that certain rural populations in Europe, for example in Bulgaria and the Russian steppes, who lived largely on milk fermented by lactic-acid bacteria were exceptionally long lived. Based on these observations, Metchnikoff proposed that consumption of fermented milk would "seed" the intestine with harmless lactic-acid bacteria and decrease the intestinal pH, and that this would suppress the growth of proteolytic bacteria. Metchnikoff himself introduced in his diet sour milk fermented with the bacteria he called "Bulgarian Bacillus" and found his health benefited. Friends in Paris soon followed his example and physicians began prescribing the sour-milk diet for their patients.

Bifidobacteria were first isolated from a breast-fed infant by Henry Tissier, who also worked at the Pasteur Institute. The isolated bacterium named *Bacillus bifidus communis* was later renamed to the genus *Bifidobacterium*. Tissier found that bifidobacteria are dominant in the gut flora of breast-fed babies and he observed clinical benefits from treating diarrhea in infants with bifidobacteria. The claimed effect was bifidobacterial displacement of proteolytic bacteria causing the disease.

During an outbreak of shigellosis in 1917, German professor Alfred Nissle isolated a strain of *Escherichia coli* from the feces of a soldier who was not affected by the disease. Methods of treating infectious diseases were needed at that time when antibiotics were not yet available, and Nissle used the *E. coli* Nissle 1917 strain in acute gastrointestinal infectious salmonellosis and shigellosis.

In 1920, Rettger and Cheplin reported that Metchnikoff's "Bulgarian Bacillus", later called *Lactobacillus delbrueckii* subsp. *bulgaricus*, could not live in the human intestine. They conducted experiments involving rats and humans volunteers, by feeding them with *Lactobacilus acidophilus*. They observed changes in composition of fecal microbiota, which they described as “transformation

of the intestinal flora". Rettger further explored the possibilities of *L. acidophilus* and reasoned that bacteria originating from the gut were more likely to produce the desired effect in this environment. In 1935, certain strains of *L. acidophilus* were found to be very active when implanted in the human digestive tract. Trials were carried out using this organism, and encouraging results were obtained, especially in the relief of chronic constipation.

The term "probiotics" was first introduced in 1953 by Werner Kollath to describe organic and inorganic food supplements applied to restore health to patients suffering from malnutrition. Contrasting antibiotics, probiotics were defined as microbially derived factors that stimulate the growth of other microorganisms. A definition of probiotics that has been widely used: "*A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance*". Fuller's definition emphasizes the requirement of viability for probiotics and introduces the aspect of a beneficial effect on the host.

The term "probiotic" originally referred to microorganisms that have effects on other microorganisms. The conception of probiotics involved the notion that substances secreted by one microorganism stimulated the growth of another microorganism. The term was used again to describe tissue extracts which stimulated microbial growth. The term probiotics was taken up by Parker, who defined the concept as, "organisms and substances that have a beneficial effect on the host animal by contributing to its intestinal microbial balance". Later, the definition was greatly improved by Fuller, whose explanation was very close to the definition used today. Fuller 89 described probiotics as a "live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance". He stressed two important facts of probiotics: the viable nature of probiotics and the capacity to help with intestinal balance.

In the following decades, intestinal lactic acid bacterial species with alleged health beneficial properties have been introduced as probiotics, including *Lactobacillus rhamnosus*, *Lactobacillus casei*, and *Lactobacillus johnsonii*.

Research

Probiotics are under considerable research, as the concept holds promise for human health and well-being, and corresponding commercial opportunities. Protection of consumers requires health claims to be confirmed with sufficient scientific evidence. Overall scientific demonstration of probiotic effects requires defining a healthy microbiota and interactions between microbiota and host, and the

difficulty to characterize probiotic effectiveness in health and disease. Recent developments of high-throughput sequencing technology and the consequent progresses of metagenomics represent a new approach for the future of probiotics research.

Studies are examining whether probiotics affect mechanisms of intestinal inflammation, diarrhea, or urogenital infections. Through 2012, however, in all cases proposed as health claims to the European Food Safety Authority, the scientific evidence remains insufficient to prove a cause and effect relationship between consumption of probiotic products and any health benefit.

Research into the potential health effects of supplemental probiotics has included the molecular biology and genomics of *Lactobacillus* in immune function, cancer, and antibiotic-associated diarrhea, travellers' diarrhea, pediatric diarrhea, inflammatory bowel disease, and irritable bowel syndrome. Testing of a probiotic applies to a specific strain under study. The scientific community cautions against extrapolating an effect from a tested strain to an untested strain.

Although research does suggests that the relationship between gut flora and humans is a mutualistic relationship, very little evidence supports claims that probiotic dietary supplements have any health benefits. Improved health through gut flora modulation appears to be directly related to long-term dietary changes.

In a 2009, one expert reasoned that preliminary clinical results exist for some applications, such as treating diarrhea, but wider health benefits claimed by probiotic proponents lack plausibility since the body's "ecosystem" is sufficiently complex that adding a few bacteria is unlikely to have the claimed effect. Accordingly, he reasoned, "the alleged health benefits of probiotics are often an example of spin". Since then, there has been an increase in the body of scientific evidence supporting the use of specific probiotics to improve health (See table on Probiotic strains section). Although the body's complex microbial community is incompletely understood at present, there is strong scientific consensus on the benefits of using of probiotics to address certain medical states or conditions.

Claims that some lactobacilli may contribute to weight gain in some humans remain controversial.

Allergies

Probiotics are ineffective in preventing allergies in children, with the possible exception of eczema.

Diarrhea

Some probiotics are suggested as a possible treatment for various forms of gastroenteritis, and a

Cochrane Collaboration meta-analysis on the use of probiotics to treat acute infectious diarrhea based on a comprehensive review of medical literature through 2010 (35 relevant studies, >4500 participants) reported that use of any of the various tested probiotic formulations appeared to reduce the duration of diarrhea by a mean of 25 hours (vs. control groups, 95% confidence interval, 16–34 hours), also noting, however, that "the differences between the studies may be related to other unmeasured and unexplored environmental and host factors" and that further research was needed to confirm reported benefits.

Antibiotic-associated diarrhea

Some of the best evidence in support of probiotic health benefits is in the treatment of antibiotic-associated diarrhea (AAD). Antibiotics are a common treatment for children, and 20% of antibiotic-treated children develop diarrhea. AAD results from an imbalance in the colonic microbiota caused by antibiotic therapy. Microbiota alteration changes carbohydrate metabolism, with decreased short-chain fatty acid absorption and osmotic diarrhea as a result. The preventive role of some probiotics has been correctly assessed in randomized, controlled clinical trials. A review assessing the work of 16 different studies representing the evaluation of more than 3,400 patients concluded that the evidence gathered suggested a protective effect of some probiotics in this condition. In adults, some probiotics showed a beneficial role in reducing the occurrence of AAD. Another consequence of antibiotic therapy leading to diarrhea is the overgrowth of potentially pathogenic organisms such as *Clostridium difficile*.

Probiotic treatment might reduce the incidence and severity of AAD as indicated in several meta-analyses. For example, treatment with probiotic formulations including *L. rhamnosus* may reduce the risk of AAD, improve stool consistency during antibiotic therapy, and enhance the immune response after vaccination. However, further documentation of these findings through randomized, double-blind, placebo-controlled trials is required to confirm specific effects and obtain regulatory approval, which currently does not exist.

The potential efficacy of probiotic AAD prevention is dependent on the probiotic strain(s) used and on the dosage. A Cochrane Collaboration systematic review, in which 16 randomized clinical trials (n=3432 participants) were analyzed, concluded that treatments with less than 5000 million CFUs/day did not show a significant decrease of AAD. However, patients treated with ≥5000 million CFUs/day (including *L. rhamnosus* and *Saccharomyces boulardii*) had 60% lower relative

risk (95%CI: 44–71%) of experiencing AAD than untreated patients.

Lactose intolerance

Ingestion of certain active strains may help lactose-intolerant individuals tolerate more lactose than they would otherwise have tolerated.

Cholesterol

Preliminary human and animal studies have demonstrated the efficacy of some strains of lactic acid bacteria (LAB) for reducing serum cholesterol levels, presumably by breaking down bile in the gut, thus inhibiting its reabsorption (where it enters the blood as cholesterol).

A meta-analysis that included five double-blind trials examining the short-term (2–8 weeks) effects of a yogurt with probiotic strains on serum cholesterol levels found a minor change of 8.5 mg/dL (0.22 mmol/L) (4% decrease) in total cholesterol concentration, and a decrease of 7.7 mg/dL (0.2 mmol/L) (5% decrease) in serum LDL concentration.

A slightly longer study evaluating the effect of a yogurt with probiotic strains on 29 subjects over six months found no statistically significant differences in total serum cholesterol or LDL values. However, the study did note a significant increase in serum HDL from, 50 to 62 mg/dL (1.28 to 1.6 mmol/L) following treatment. This corresponds to a possible improvement of LDL/HDL ratio.

Studies specifically on hyperlipidemic subjects are still needed.

Blood pressure

The consumption of probiotics may effect a modest benefit in helping to control high blood pressure.

Immune function and infections

Some strains of LAB may affect pathogens by means of competitive inhibition (i.e., by competing for growth) and some evidence suggests they may improve immune function by increasing the number of IgA-producing plasma cells and increasing or improving phagocytosis, as well as increasing the proportion of T lymphocytes and natural killer cells. Clinical trials have demonstrated that probiotics may decrease the incidence of respiratory-tract infections and dental caries in children. LAB products might aid in the treatment of acute diarrhea, and possibly affect rotavirus infections in children and travelers' diarrhea in adults, but no products are approved for such indications.

Helicobacter pylori

Some strains of LAB may affect *Helicobacter pylori* infections (which may cause peptic ulcers) in adults when used in combination with standard medical treatments, but no standard in medical practice or regulatory approval exists for such treatment.

Inflammation

Some strains of LAB may modulate inflammatory and hypersensitivity responses, an observation thought to be at least in part due to the regulation of cytokine function. Clinical studies suggest they can prevent reoccurrences of inflammatory bowel disease in adults, as well as improve milk allergies. How probiotics may influence the immune system remains unclear, but a potential mechanism under research concerns the response of T lymphocytes to proinflammatory stimuli.

Irritable bowel syndrome and colitis

Probiotics may help people with irritable bowel syndrome, although uncertainty remains around which type of probiotic works best, and around the size of the effect.

No good evidence indicates taking probiotics helps maintain remission from ulcerative colitis.

Necrotizing enterocolitis

Several clinical studies provide evidence for the potential of probiotics to lower the risk of necrotizing enterocolitis (NEC) and mortality in premature infants. One meta-analysis indicated that probiotics reduce all-cause mortality and risk of having NEC by more than 50% compared with controls.

Vitamin production

Probiotic treatment has been studied as a means of addressing maladies associated with vitamin deficiency, e.g., of vitamin K, folic acid, and vitamin B12.

Eczema

Probiotics are commonly given to breast-feeding mothers and their young children to prevent eczema, but some doubt exists over the strength of evidence supporting this practice.

Bacterial Vaginosis

Probiotic treatment of bacterial vaginosis is the application or ingestion of bacterial species found in the healthy vagina to cure the infection of bacteria causing bacterial vaginosis. This treatment is based on the observation that 70% of healthy females have a group of bacteria in the genus *Lactobacillus* that dominate the population of organisms in the vagina. Currently, the success of

such treatment has been mixed since the use of probiotics to restore healthy populations of *Lactobacillus* has not been standardized. Often, standard antibiotic treatment is used at the same time that probiotics are being tested. In addition, some groups of women respond to treatment based upon ethnicity, age, number of sexual partners, pregnancy, and the pathogens causing bacterial vaginosis. In 2013, researchers found that administration of hydrogen peroxide producing strains, such as *L. acidophilus* and *L. rhamnosus*, were able to normalize vaginal pH and rebalance vaginal flora, preventing and alleviating bacterial vaginosis.

Side effects

In some situations, such as where the person consuming probiotics is critically ill, probiotics could be harmful. In a therapeutic clinical trial conducted by the Dutch Pancreatitis Study Group, the consumption of a mixture of six probiotic bacteria increased the death rate of patients with predicted severe acute pancreatitis.

In a clinical trial aimed at showing the effectiveness of probiotics in reducing childhood allergies, researchers gave 178 children either a probiotic or a placebo for the first six months of their lives. Those given the probiotic were more likely to develop a sensitivity to allergens.

Some hospitals have reported treating *Lactobacillus* septicaemia, which is a potentially fatal disease caused by the consumption of probiotics by people with lowered immune systems or who are already very ill.

Probiotics taken orally can be destroyed by the acidic conditions of the stomach. A number of microencapsulation techniques are being developed to address this problem.

One 2009 paper cited a 2007 study in chickens as a support for causally linked probiotic products such as yogurts with obesity trends. However, this is contested as the link to obesity, and other health-related issues with yogurt may link to its dairy and calorie attributes.

Some experts are skeptical on the efficacy of many strains and believe not all subjects will benefit from the use of probiotics.

Strains

Live probiotic cultures are available in fermented dairy products and probiotic fortified foods. However, tablets, capsules, powders, and sachets containing the bacteria in freeze-dried form are also available.

Only preliminary evidence exists for most probiotic health claims. Even for the most studied strains,

few have been sufficiently developed in basic and clinical research to warrant approval for health claim status to a regulatory agency such as the Food and Drug Administration or European Food Safety Authority, and to date, no claims have been approved by those two agencies.

For the variety of strains with imputed potential health benefits, see the research section above. Some additional forms of lactic acid bacteria include *Lactobacillus bulgaricus, Streptococcus thermophilus,* and "*Lactobacillus bifidus*".

Some fermented products are reported to contain similar lactic acid bacteria, including pickled vegetables, fermented bean paste such as tempeh, miso, and doenjang, kefir, buttermilk or karnemelk, kimchi, pao cai, sauerkraut, soy sauce, and *zha cai*.

Commercial probiotics

Labeling ambiguities

The National Yogurt Association gives a Live & Active Cultures seal to yogurt products which contain 10^8 CFU/mL cultures per gram (at the time of manufacture). In 2002, the US FDA and World Health Organization recommended that "the minimum viable numbers of each probiotic strain at the end of the shelf-life" be reported on labeling, but most companies that give a number report the viable cell count at the date of manufacture, a number probably much higher than existing at the moment of consumption. Because of variability in storage conditions and time before eating, it is difficult to tell exactly how much active culture remains at the time of consumption. Due to these ambiguities, in 2012, the European Commission placed a ban on putting the word "probiotic" on the packaging of products because such labeling misleads consumers to believe a health benefit is provided by the product when no scientific proof exists to demonstrate that health effect.

History and modern products

The first commercially sold dairy-based probiotic was Yakult, a fermented milk with added *Lactobacillus casei*. Since then, many more probiotic foods have come on the market, mostly in the form of dairy products. Recently, nondairy and unfermented probiotics have been produced, including breakfast cereal and snack bars, whereas other probiotic products include kefir, yogurt, kombucha, kimchi, sauerkraut, and other fermented foods and beverages.

Global consumption

Sales of probiotic products have a rising trend from 2010 to 2014, increasing globally by 35% from US$23.1 billion to $31.3 billion. Some regions have increased their use by even more than the

average, including Eastern Europe (67%), Asia Pacific (67%), and Latin America (47%), comprising nearly half of probiotics sold globally in 2014. By geographic region, the leading consumers of probiotics in 2014 were Western Europe ($8.3 billion), Asia Pacific ($7 billion), Japan ($5.4 billion), Latin America ($4.8 billion), North America ($3.5 billion), and Eastern Europe ($2.3 billion).

EFSA scientific review of probiotics

The European Food Safety Authority has so far rejected 260 claims on probiotics in Europe due to insufficient research and thus inconclusive proof. The review did not refute the potential for effectiveness, but rather that a cause-effect relationship had not been sufficiently established in studies to date. The claims **rejected** include:

- *Lactobacillus paracasei* LMG P 22043 decreases potentially pathogenic gastrointestinal microorganisms or reduce gastrointestinal discomfort.
- *Lactobacillus johnsonii* BFE 6128 - immunity and skin claims (all too general for consideration)
- *Lactobacillus fermentum* ME-3 decreases potentially pathogenic gastrointestinal microorganisms.
- *Lactobacillus plantarum* BFE 1685 - immunity claim (deemed too general)
- *Bifidobacterium longum* BB536 improves bowel regularity, resists cedar pollen allergens, and decreases pathogens.
- *Lactobacillus plantarum* 299v reduces flatulence and bloating and protects DNA, proteins, and lipids from oxidative damage.
- *Lactobacillus rhamnosus* LB21 NCIMB 40564 helps maintain individual intestinal microbiota in subjects receiving antibiotic treatment.

Multiple probiotics

Preliminary research is evaluating the potential physiological effects of multiple probiotic strains, as opposed to a single strain. As the human gut may contain several hundred microbial species, one theory indicates that this diverse environment may benefit from consuming multiple probiotic strains, an effect that remains scientifically unconfirmed.

PREBIOTIC VS PROBIOTIC	
PREBIOTICS	**PROBIOTICS**
PREBIOTICS are a special form of dietary fiber	PROBIOTICS are live bacteria in yogurt, dairy products and pills. There are hundreds of probiotic species available. Which of the hundreds of available probiotics is best for the average healthy person is still unknown.
PREBIOTIC powders are not affected by heat, cold, acid or time.	PROBIOTIC bacteria must be kept alive. They may be killed by heat, stomach acid or simply die with time.
PREBIOTICS provide a wide range of health benefits to the otherwise healthy person. Most of these have been medically proven.	PROBIOTICS are still not clearly known to provide health benefits to the otherwise healthy. Some are suspected but still not proven.
PREBIOTICS nourish the good bacteria that everyone already has in their gut.	PROBIOTICS must compete with the over 1000 bacteria species already in the gut.
PREBIOTICS may be helpful for several chronic digestive disorders or inflammatory bowel disease.	Certain PROBIOTIC species have been shown to be helpful for childhood diarrhea, irritable bowel disease and for recurrence of certain bowel infections such as C. difficile.

Microbial fuel cell:

A microbial fuel cell (MFC) or biological fuel cell is a bio-electrochemical system that drives a current by using bacteriaand mimicking bacterial interactions found in nature. MFCs can be grouped into two general categories, those that use a mediator and those that are mediator-less. The first MFCs, demonstrated in the early 20th century, used a mediator: a chemical that transfers electrons from the bacteria in the cell to the anode. Mediator-less MFCs are a more recent development dating to the 1970s; in this type of MFC the bacteria typically have electrochemically active redox proteins such as cytoachromes on their outer membrane that can

transfer electrons directly to the anode. Since the turn of the 21st century MFCs have started to find a commercial use in the treatment of wastewater.

History:

The idea of using microbial cells in an attempt to produce electricity was first conceived in the early twentieth century. M. Potter was the first to perform work on the subject in 1911. A professor of botany at the University of Durham, Potter managed to generate electricity from E. coli, but the work was not to receive any major coverage. In 1931, however, Barnet Cohen drew more attention to the area when he created a number of microbial half fuel cells that, when connected in series, were capable of producing over 35 volts, though only with a current of 2 milliamps.

More work on the subject came with a study by DelDuca et al. who used hydrogen produced by the fermentation of glucose by Clostridium butyricum as the reactant at the anode of a hydrogen and air fuel cell. Though the cell functioned, it was found to be unreliable owing to the unstable nature of hydrogen production by the micro-organisms. Although this issue was later resolved in work by Suzuki et al. in 1976 the current design concept of an MFC came into existence a year later with work once again by Suzuki.

Definition

A microbial fuel cell is a device that converts chemical energy to electrical energy by the catalytic reaction ofmicroorganisms.

A typical microbial fuel cell consists of anode and cathode compartments separated by a cation (positively charged ion) specific membrane. In the anode compartment, fuel is oxidized by microorganisms, generating CO_2, electrons and protons. Electrons are transferred to the cathode compartment through an external electric circuit, while protons are transferred to the cathode compartment through the membrane. Electrons and protons are consumed in the cathode compartment, combining with oxygen to form water.

More broadly, there are two types of microbial fuel cell: mediator and mediator-less microbial fuel cells.

Mediator microbial fuel cell

Most of the microbial cells are electrochemically inactive. The electron transfer from microbial cells to the electrode is facilitated by mediators such as thionine, methyl viologen, methyl blue, humic acid, and neutral red. Most of the mediators available are expensive and toxic.

Mediator-free microbial fuel cell

A plant microbial fuel cell (PMFC)

Mediator-free microbial fuel cells do not require a mediator but use electrochemically active bacteria to transfer electrons to the electrode (electrons are carried directly from the bacterial respiratory enzyme to the electrode). Among the electrochemically active bacteria are, Shewanella putrefaciens, Aeromonas hydrophila, and others. Some bacteria, which have pili on their external membrane, are able to transfer their electron production via these pili. Mediator-less MFCs are a more recent area of research and, due to this, factors that affect optimum efficiency, such as the strain of bacteria used in the system, type of ion-exchange membrane, and system conditions (temperature, pH, etc.) are not particularly well understood.

Mediator-less microbial fuel cells can, besides running on wastewater, also derive energy directly from certain plants. This configuration is known as a plant microbial fuel cell. Possible plants include reed sweetgrass, cordgrass, rice, tomatoes, lupines, and algae. Given that the power is thus derived from living plants (in situ-energy production), this variant can provide additional ecological advantages.

Microbial electrolysis cell

Main article: Microbial electrolysis cell

A variation of the mediator-less MFC is the microbial electrolysis cells (MEC). Whilst MFC's produce electric current by the bacterial decomposition of organic compounds in water, MECs partially reverse the process to generate hydrogen or methane by applying a voltage to bacteria to supplement the voltage generated by the microbial decomposition of organics sufficiently lead to the electrolysis of water or the production of methane. A complete reversal of the MFC principle is found in microbial electrosynthesis, in which carbon dioxide is reduced by bacteria using an external electric current to form multi-carbon organic compounds.

Soil-based microbial fuel cell

A soil-based MFC

Soil-based microbial fuel cells adhere to the same basic MFC principles as described above, whereby soil acts as the nutrient-rich anodic media, the inoculum, and the proton exchange membrane (PEM). The anode is placed at a certain depth within the soil, while the cathoderests on top the soil and is exposed to the oxygen in the air above it.

Soils are naturally teeming with a diverse consortium of microbes, including the electrogenic microbes needed for MFCs, and are full of complex sugars and other nutrients that have accumulated over millions of years of plant and animal material decay. Moreover, the aerobic(oxygen consuming) microbes present in the soil act as an oxygen filter, much like the expensive PEM materials used in laboratory MFC systems, which cause the redox potential of the soil to decrease with greater depth. Soil-based MFCs are becoming popular educational tools for science classrooms.

Sediment microbial fuel cells (SMFCs) application for wastewater treatment is a relatively new field. SMFCs with simple structures can generate electrical energy while decontaminatingwastewater. Most SMFCs used for wastewater treatment contain plants to mimic constructed wetlands. Both synthetic and real wastewaters have been used as substrates in SMFCs that achieved satisfactory performance in organic removal. SMFC tests have reached more than 150 L.

In 2015 researchers announced an SMFC application that extracts energy and charges a battery. Salts found in the waste dissociates into positively and negatively charged ions in water, and move and adhere to the respective negative and positive electrodes, charging the battery and making it possible to remove the salt effecting microbial capacitive desalination. The microbes produce more energy than is required for the desalination process.

Phototrophic biofilm microbial fuel cell

Phototrophic biofilm MFCs (PBMFCs) are the ones that make use of anode with a phototrophic biofilm containing photosynthetic microorganism like chlorophyta, cyanophyta etc., since they

could carry out photosynthesis and thus they act as both producers of organic metabolites and also as electron donors.

A study conducted by Strik et al. reveals that PBMFCs yield one of the highest power densities and, therefore, show promise in practical applications. Researchers face difficulties in increasing their power density and long-term performance so as to obtain a cost-effective MFC.

The sub-category of phototrophic microbial fuel cells that use purely oxygenic photosynthetic material at the anode are sometimes called biological photovoltaic systems.

Nanoporous membrane microbial fuel cells

The United States Naval Research Laboratory (NRL) developed the nanoporous membrane microbial fuel cells which operate the same as most MFCs, but use a non-PEM to generate passive diffusion within the cell. The membrane used instead is a nonporous polymer filter (nylon, cellulose, or polycarbonate) which generates comparable power densities asNafion (a well known PEM) while remaining more durable than Nafion. Porous membranes allow passive diffusion thereby reducing the necessary power supplied to the MFC in order to keep the PEM active and increasing the total output of energy from the cell.

MFCs that do not use a membrane can deploy anaerobic bacteria in aerobic environments however, membrane-less MFCs will experience cathode contamination by the indigenous bacteria and the power-supplying microbe. The novel passive diffusion of nanoporous membranes can achieve the benefits of a membrane-less MFC without worry of cathode contamination.

Nanoporous membranes are also eleven times cheaper than Nafion (Nafion-117, $0.22/cm2 vs. polycarbonate, <$0.02/cm2).

Electrical generation process:

When micro-organisms consume a substance such as sugar in aerobic conditions, they produce carbon dioxide and water. However, when oxygen is not present, they produce carbon dioxide, protons, and electrons, as described below:

$C_{12}H_{22}O_{11} + 13H_2O \rightarrow 12CO_2 + 48H^+ + 48e^-$ (Eqt. 1)

Microbial fuel cells use inorganic mediators to tap into the electron transport chain of cells and channel electrons produced. The mediator crosses the outer cell lipid membranes and bacterial outer membrane; then, it begins to liberate electrons from the electron transport chain that normally would be taken up by oxygen or other intermediates.

The now-reduced mediator exits the cell laden with electrons that it transfers to an electrode where it deposits them; this electrode becomes the electro-generic anode (negatively charged electrode). The release of the electrons means that the mediator returns to its original oxidised state ready to repeat the process. It is important to note that this can happen only under anaerobic conditions; if oxygen is present, it will collect all the electrons, as it has a greater electronegativity than mediators.

In a microbial fuel cell operation, the anode is the terminal electron acceptor recognized by bacteria in the anodic chamber. Therefore, the microbial activity is strongly dependent on the redox potential of the anode. In fact, it was recently published that a Michaelis-Menten curve was obtained between the anodic potential and the power output of an acetate driven microbial fuel cell. A critical anodic potential seems to exist at which a maximum power output of a microbial fuel cell is achieved.

A number of mediators have been suggested for use in microbial fuel cells. These include natural red, methylene blue, thionine, or resorufin.

This is the principle behind generating a flow of electrons from most micro-organisms (the organisms capable of producing an electric current are termed exoelectrogens). In order to turn this into a usable supply of electricity, this process has to be accommodated in a fuel cell. In order to generate a useful current it is necessary to create a complete circuit, and not just transfer electrons to a single point.

The mediator and micro-organism, in this case yeast, are mixed together in a solution to which is added a suitable substrate such as glucose. This mixture is placed in a sealed chamber to stop

oxygen entering, thus forcing the micro-organism to useanaerobic respiration. An electrode is placed in the solution that will act as the anode as described previously.

In the second chamber of the MFC is another solution and electrode. This electrode, called the cathode is positively charged and is the equivalent of the oxygen sink at the end of the electron transport chain, only now it is external to the biological cell. The solution is an oxidizing agent that picks up the electrons at the cathode. As with the electron chain in the yeast cell, this could be a number of molecules such as oxygen. However, this is not particularly practical as it would require large volumes of circulating gas. A more convenient option is to use a solution of a solid oxidizing agent.

Connecting the two electrodes is a wire (or other electrically conductive path, which may include some electrically powered device such as a light bulb) and completing the circuit and connecting the two chambers is a salt bridge or ion-exchange membrane. This last feature allows the protons produced, as described in Eqt. 1 to pass from the anode chamber to the cathode chamber.

The reduced mediator carries electrons from the cell to the electrode. Here the mediator is oxidized as it deposits the electrons. These then flow across the wire to the second electrode, which acts as an electron sink. From here they pass to an oxidising material.

Applications:

Power generation

Microbial fuel cells have a number of potential uses. The most readily apparent is harvesting electricity produced for use as a power source. The use of MFCs is attractive for applications that require only low power but where replacing batteries may be time-consuming and expensive such as wireless sensor networks. Virtually any organic material could be used to feed the fuel cell, including coupling cells to wastewater treatment plants.

Education

Soil-based microbial fuel cells are popular educational tools, as they employ a range of scientific disciplines (microbiology, geochemistry, electrical engineering, etc.), and can be made using commonly available materials, such as soils and items from the refrigerator. There are also kits

available for classrooms and hobbyists, and research-grade kits for scientific laboratories and corporations.

Biosensor

Since the current generated from a microbial fuel cell is directly proportional to the energy content of wastewater used as the fuel, an MFC can be used to measure the solute concentration of wastewater (i.e., as a biosensor system).

The strength of wastewater is commonly evaluated as biochemical oxygen demand (BOD) values. BOD values are determined incubating samples for 5 days with proper source of microbes; usually activate sludge collected from sewage works. When BOD values are used as a real-time control parameter, 5 days' incubation is too long.

Water treatment

Microbial Fuel Cells are being used in the water treatment process to harvest energy utilizing anaerobic digestion (a method used in the microbial fuel cell to collect bioenergy from wastewater). The process is well developed and can handle a high volume of wastewater and reduce pathogens. However, the process requires high temperatures (upwards of 30 degrees Celsius) and requires an extra step in order to convert biogas to electricity. Spiral spacers may also be used to increase electricity generation by creating a helical flow in the microbial fuel cells. The challenge is that it is difficult to scale up the MFCs for practical wastewater treatment because of the power output challenges of a larger surface area MFC.

KARPAGAM ACADEMY OF HIGHER EDUCATION FUNDAMENTALS OF MICROBIOLOGY AND CLASSIFICATION (17MBP101)	
Unit V Questions	Opt 1
G+C content is determined from _____ of DNA	cooling temperat
G+C content can be determined by_____	HPLC
Give an example for comparison of protiens	aspartic acid
choose a correct indirect method followed for comparison of protiens	SDS PAGE
choose a correct indirect method followed for comparison of protiens	biochemical test
what type of centrifugation is subjected for DNA to locate its density ?	caesium chloride
In DNA homology experiment when heteroduplex forms that indicates____	two strains are fa
DNA structure can be directly compared by _____	G+C content dete
Full form of Sab value is ___	association coeffi
The similarity between genomes can be compared more directly by_____	Morphological C
In Nucleic acid hybridization, the single stand DNA fragments made radioactive with _____	32P
Which Technique is used to study only closly related micro-organisms	RNA-RNA hybr
Which Technique is used to detect distant relationship micro-organisms	RNA-RNA hybri
In hybridization technique even more accurate measurment of homology is obtained by _____	Finding the pH
Which method is used to amplify 16Sr DNA from bacterial genome	PCR
What is the name of the method that is used for sequencing the amplfied rDNA	Chargoff's metho
The G+C content is often determined from the _____ of DNA	Optimum PH
At which point the rising curve gives the melting temperature, a direct measure of the G+C content	End point
On five kingdom classification, the organisms are based on	Pigmentation
The very first comprehensive system of bacterial classification was propose	Pasteur
In microbial taxonomy the sub sps. that differ physiologically is a called___	Morpho var
The bacterium that comes under actinomycetes is _____	*Rhodospirillum r*
Similarity co-efficient is calculated from which of the following formulae ?	ab/a+b
The study evolutionary history of microorganisms is called _____	Fossil
A formal system for organizing,classifying and naming living thing is clled	nomenclature
Which classification is the one based on mutual similarity	phenetic
Phenetic classification are not based on _____	morphology
Nomenclature stands for _____	Naming
Binomial means_____ names.	1
Stromatolites means _____ rocks.	Strong
Curd isa _____	Pathogens
Probiotic bacteria _____.	*Lacto bacilli*
Single cell protein _____.	Cabbage
Modern microbiology deals at _____ level.	Molecular

Polymerase chain reaction basically consists of	two steps
The template for PCR is	RNA
The G+C content was calculated by	settle plate metho
which content in DNA reflects the base sequence?	A+T

Opt 2	Opt 3	Opt 4	Opt 5	Opt 6
melting temperature	high temperature	moderate temperature		
gas chromatography	affinity chromatograhy	column chromatography		
carotene	histone	proline		
immunologic techniques	biochemical test	physiological test		
physiological test	sds page	electrophoretic mobility		
ultra centrifugation	cooling centrifuge	benchtop centrifugation		
no relatedness between t	two strains are closely re	two strains are related to each oth		
nucleic acid sequencing	nucleic acid base compo	nucleic acid hybridization		
sum of absent character	sum of association coeff	sum of the density		
Comparsion of Proteins	nucleic acid hybridizatio	Ecological Characters		
1S	70S	50S		
DNA-DNA hybridizatio	DNA-RNA hybridizatio	Western Blot		
DNA-DNA hybridizatio	DNA-RNA hybridizatio	Western Blot		
Finding the salt toleranc	Finding the resistance	Finding the temperature		
RFLP	Disc diffusion	ELISA		
Sanger's method	Venogradsky Method	Avery's method		
Protein Content	Hydrogen bond	Melting Temperature		
Mid point	Peak point	Starting point		
Environment	Nutrient Type	Temperature		
Buchanan	Haeckel	Koch		
Serovar	Chemovar	Biovar		
Azospirilllum lipoferum	*Mycobacterium tubercul*	*P.flourescens*		
a/a+b+c	a+b+c/a	a/abc		
Evolution	Phylogeny	Phenetic		
numerical value	taxonomy	identification		
phylum	phlogenetic	mutual		
shape	size	phylogenetic analysis		
Dividing	Segregation	Allocation		
2	3	4		
Sandy	sedimentation	salt		
probiotic	Toxin	Toxoid		
E.coli	*Klebsiella*	*Archea*		
Fish	Mushroom	Pearl		
Basic	Numerical	Statistical		

three steps	four steps	five steps		
single stranded DNA	double stranded DNA	mRNA		
mtcl method	genomic method	buoyant density method		
G+A	G+C	C+T		

Answer
melting temperature
HPLC
histone
immunologic techniques
electrophoretic mobility
caesium chloride
two strains are closely related
nucleic acid sequencing
association coefficient
nucleic acid hybridization
32P
DNA-DNA hybridization
DNA-RNA hybridization
Finding the temperature
PCR
Sanger's method
Melting Temperature
Mid point
Nutrient Type
Haeckel
Biovar
Azospirilllum lipoferum
a/a+b+c
Phylogeny
taxonomy
phenetic
phylogenetic analysis
Naming
2
sedimentation
probiotic
Lacto bacilli
Mushroom
Molecular

three steps
double stranded DNA

buoyant density method
G+C

DEPARTMENT OF MICROBIOLOGY
KARPAGAM ACADEMY OF HIGHER EDUCATION
(Established Under Section 3 of UGC Act, 1956)
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I M.Sc MICROBIOLOGY - 2017 – 2019 Batch

FUNDAMENTALS OF MICROBIOLOGY AND CLASSIFICATION (17MBP101)

SEMESTER – I **4H – 4C**

POSSIBLE QUESTION

Unit V

TWO MARK

1. Define molecular taxonomy.
2. What is meant by phylogenetic tree?
3. What is meant by probiotics?
4. What is meant by prebiotics?
5. Expand MFC.

EIGHT MARKS

1. Discuss about the modern microbiology.
2. Describe the 16s rRNA sequencing.
3. Outline the protocol of 18s rRNA sequencing.
4. Explain the molecular tool in assessing microbial diversity.
5. Write in detail about the importance of Probiotics.
6. Comment on Prebiotics and its importance.
7. Narrate the properties of Microbial fuel cells.

Reg. No. : ----------

[17MBP101]

KARPAGAM ACADEMY OF HIGHER EDUCATION

KARPAGAM UNIVERSITY

(Under Section 3 of UGC Act 1956)

COIMBATORE – 641 021

FIRST INTERNAL ASSESSMENT, AUGUST 2017

FIRST SEMESTER

MICROBIOLOGY

FUNDAMENTALS OF MICROBIOLOGY AND CLASSIFICATION

Time: 2 hours **Maximum: 50 marks**

Date: 28 /08/2017 [FN] **Class: I M.Sc. MB**

PART A – (20 x 1 = 20 marks)

Answer all the questions

1. Who first described microorganisms_____.
   a. Louis Pasteur b. Robert Koch
   c. Fannie Hesse d. Leeuwenhoek
2. _____disease is otherwise known as hydrophobia.
   a. Chicken pox b. Anthrax
   c. Rabies d. Cholera
3. The first bacterium shown to cause human disease_____.
   a. Anthrax b. Mycobacterium
   c. Diphtheria d. Leprosy
4. The petridish was invented by _____.
   a. Hook b. RichardPetri
   c. Pasteur d. Fannie Hesse
5. The Swan necked flask was introduced by _____.
   a. Spallan Zani b. Francois Appert
   c. Louis Pasteur d. Robert Koch
6. Who defined numerical taxonomy _____?
   a. Carl linnaeus b. Sneath & Sokal
   c. Robert koch d. koch & Hooke
7. QA stands for _____.
   a. Quality assurance b. Quality association
   c. Quality acceptance d. Quality abolision.
8. Five Kingdom concept was devised by_____.
   a. Carl linnaeus b. Carl woose
   c. Whittaker d. Charles
9. Tyndallisation is the process of _____ heating.
   a. Continuous b. Discontinuous
   c. Semidry d. Wet
10. The process of colony purification on solid media was developed by _____.
    a. Louis Pasteur b. Robert Koch
    c. Fannie Hesse d. Leeuwenhoek

11. _____microscopy, shows varying degrees of darkness within the cell.
    a. Dark-field b. Bright field
    c. Phase contrast d. Electron
12. _____of the following is a supravital fluorescent stain.
    a. Acridine orange b. Congored
    c. Neutral red d. Safarinin
13. Magnification of the microscope depend on_____.
    a. Light source b. Magnifying power of the eyepiece
    c. Wire d. Body tube
14. Stains are used to _____.
    a. Store cultures b. Reveals their shape and size
    c. Pathogen d. Antiseptic
15. Spore staining involves the use of _____ .
    a. Malachite green and safranin b. Lactophenol cotton blue
    c. Crystal violet and safranin d. Methylene blue
16. In Mac conkey's Medium, lactose fermenters produce _____
    a. Pink b. Yellow
    c. Black d. Green
17. The source of electron is a hot _____ filament in an electron gun.
    a. Copper b. Tungsten
    c. Zinc d. Mercury
18. The term algae was originally used to simple _____.
    a. Marine plants b. Aquatic plants
    c. Fresh water plants d. Hybrid Plants
19. Phycocyanin is a _____.
    a. Red pigment b. Blue pigment
    c. Brown pigment d. Yellow pigment
20. _____ is the study of algae.
    a. Phycology b. Physiology
    c. Mycology d. Zoology

PART B – (3 x 02 = 06 marks)

Answer all questions

21. Define microbiology and microscope.
22. What is magnification?
23. Define Phycology.

PART B – (3 x 08 = 24 marks)

Answer all questions choosing either a or b. (All questions carry equal marks)

24. a. Outline the history and scope of Microbiology. OR

    b. Explain the Whittaker's five kingdom concept.

25. a. Discuss about the various parts of Microscope and its application. OR

    b. Write about the grams staining technique with a neat sketch?

26. a. Describe the structure and properties of Clamydomonas. OR

    b. Comment on Bergeys manual and its importance.

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PART B – (3 x 02 = 06 marks)

Answer all questions

21. Define microbiology and microscope.
    Microbiology – Branch of science- Microorganism – can't be seen through naked eye – Instrument- observing- tiny organism- enlarged images of small objects
22. What is magnification?
    Microscope -magnifying lens- magnifying power – objective lens – ocular lens – Magnification power.
23. Define Phycology.
    Algology - Branch of science- algae – Properties.

PART B – (3 x 08 = 24 marks)

Answer all questions choosing either a or b. (All questions carry equal marks)

24. a. Outline the history and scope of Microbiology. OR

    Microbiology – definition, terms, Beginning of Microbiology- microbes-microbiology is an infant science, Historical Events of Microbiology - Leeuwenhoek (1673) ,Biogenesis, Francesco Redi, John Needham, Lazzaro Spallanzani, Louis Pasteur, Aseptic Techniques, Bassi (1830s-40s), Semmelweiss (1840s), Joseph Lister (1860s), Robert Koch (1876), Jenner (1796), Fleming (1928, Chain and Florey (1941), Watson and Crick,R.H. Whittaker (1969).

    b. Explain the Whittaker's five kingdom concept.

    R.H. Whittaker (1969) - comprehensive classification- five kingdoms of living beings - Kingdom Monera - Kingdom Protista - Kingdom Fungi - Kingdom Animalia - Kingdom Plantae - five major groups of microorganisms-viruses, bacteria, fungi, algae and protozoa.

25. a. Discuss about the various parts of Microscope and its application. OR

    Microscope – definitions – magnifications properties – Eye piece – coarse adjustment- fine adjustment - arm – objectives – stage- diaphragm – oil immersion – base.

    b. Write about the grams staining technique with a neat sketch?

    Christian Gram – Bacterial cell wall – properties – smear preparation – primary stain – crystal violet – Grams iodine – mordant – decolorizing agent- 95% ethyl alcohol – counter stain - safranin – observation report.

26. a. Describe the structure and properties of Clamydomonas. OR

    Chlorophyta or green algae - grow in fresh and salt water - chlorophylls a and b- carotenoids - store carbohydrates as starch - unicellular to colonial, filamentous, membranous or sheetlike, and tubular types – pyrenoid and a stigma (eyespot) - cell in phototactic - osmoregulatory organelles - reproduces asexually by producing zoospores through cell division.

    b. Comment on Bergeys manual and its importance.

Classifcation of Bacteria (Bergey's Manual) - 1923, David Bergey- identification of bacterial species - the Bergey's Manual of Determinative Bacteriology - The First Edition of *Bergey's Manual of Systematic Bacteriology* - phylogenetic relationships and phonetic- 33 sections in the four volumes- Gram-negative bacteria of general, medical, or industrial importance; Gram-positive bacteria other than actinomycetes - Gram-negative bacteria with distinctive properties, cyanobacteria, and archaea; and Actinomycetes (gram-positive filamentous bacteria).

Reg. No. : ----------

[17MBP101]

KARPAGAM ACADEMY OF HIGHER EDUCATION

KARPAGAM UNIVERSITY

(Under Section 3 of UGC Act 1956)

COIMBATORE – 641 021

SECOND INTERNAL ASSESSMENT, OCTOBER 2017

FIRST SEMESTER

MICROBIOLOGY

FUNDAMENTALS OF MICROBIOLOGY AND CLASSIFICATION

Time: 2 hours **Maximum: 50 marks**

Date: 23/10/2017 [FN] **Class: I M.Sc. MB**

PART A – (20 x 1 = 20 marks)

Answer all the questions

1. The kingdom fungi contains_____
   a. Prokaryotes with unicellular b. Eukaryotes with multicellular
   c. Prokaryotes with multicellular d. Eukaryotes with unicellular
2. Protozoa are placed in _____
   a. Eukaryotes b. Prokaryotes
   c. Archae d. Animalia
3. Autoclaving is usually done at _____
   a. 100º C 15 lbs 15 min b. 160ºC 15 lbs 60 min
   c. 115º C 15 lbs 15 min d. 121º C 15 lbs 15 min
4. Virus contains_____
   a. Cell membrane b. Cell wall
   c. DNA and RNA d. DNA or RNA
5. The apparatus used to maintain a continuous culture _____
   a. Chemostat b. Autostat
   c. Thermostat d. Photostat
6. Which content in DNA reflects the base sequence for taxonomy?
   a. A+T b. G+A
   c. G+C d. C+T
7. Fractional sterilisation was also called_____.
   a. Tyndallisation b. Pasteurisation
   c. Heating d. Sterilisation.
8. Killing effect of dry heat is due to ____.
   a. Protein denaturation b. Elevated levels of electrolytes
   c. Oxidative demand d. Diffusion Process
9. The process of destroying pathogens in milk is called ____.
   a. Tyndallisation b. Pasteurisation
   c. Disinfection d. Sterilization
10. Protozoans were first observed by _____
   a. Louis Pasteur b. Robert Koch
   c. Fritch d. Leeuwenhoek

11. Microbial Fuel Cell
    a. drives current by using bacteria b. drives current from water
    c. drives current from solid substances d. drives current form gas
12. The part of chromosome used for DNA fingerprinting is_____.
    a. Microsatellite b. Minisatellite
    c. Tandem satellite d. Macrosatellite
13. Assembly of DNA virus occurs in _____ of the host.
    a. Nucleus b. Cytoplasm
    c. Capsid d. Envelope
14. The method in which the cells are frozen dehydrated is called.
    a. Pasteurization b. Dessication
    c. Disinfection d. Lypophilization
15. Example of Anaerobic medium is
    a. Robertson cooked-meat medium b. Nutrient agar
    c. Nutrient broth d. Mac-Conkey's agar
16. Western blotting is technique for hybridization of_____
    a. Antigen-Antibody b. DNA-cDNA
    c. Polyamines d. Monosaccharides
17. The viruses that live as parasites on bacteria are _____.
    a. Fungi b. Commensels
    c. Bacteriophages d. Macrophage
18. Virion means_____
    a. Infectious virus particles b. Non-infectious particles
    c. Incomplete particles d. Defective virus particles
19. Nomenclature stands for _____
    a. Naming b. Dividing
    c. Segregation d. Allocation
20. Phenetic classification are based on _____.
    a. morphology b. shape
    c. size d. phylogenetic analysis

PART B – (3 x 02 = 06 marks)
Answer all questions

21. Define mycology.
22. What is generation time?
23. Explain the term probiotics.

PART B – (3 x 08 = 24 marks)
Answer all questions choosing either a or b. (All questions carry equal marks)

24. a. Explain the classification and taxonomy of fungi. **OR**

    b. Write detailed note on characteristics and lifecycle of *Entameobae histolytica*.

25. a. Discuss about the working principle autoclave and hot air oven. **OR**

    b. Explain in detailed about growth curve and generation time?

26. a. Explain probiotics and their applications. **OR**

    b. Comment on modern microbiology and its importance.

****

PART B – (3 x 02 = 06 marks)
Answer all questions

21. Define mycology.
Branch of science – study of Fungi -Properties

22. What is generation time?
Doubling time - the population will double in number - the increase in population is always 2*n*

23. Explain the term probiotics.
Live bacteria and yeasts - good for health- digestive system - "good" or "helpful" bacteria

PART B – (3 x 08 = 24 marks)
Answer all questions choosing either a or b. (All questions carry equal marks)

24. a. Explain the classification and taxonomy of fungi. **OR**

1990s- classification of fungi- Chytridiomycota and Zygomycota – Zygomycota - phylogenetic classification - monophyletic clade called kingdom - Fungi, comprise seven phyla: Chytridiomycota, Blastocladiomycota, Neocallimastigomycota, Microsporidia, Glomeromycota, Ascomycota, and Basidiomycota - kingdom Fungi, which includes the yeasts, rusts, smuts, mildews, molds, mushrooms, and toadstools.

b. Write detailed note on characteristics and lifecycle of *Entameobae histolytica*.

Entamoeba histolytica was discovered in 1875 by Losch in the dysenteric feces of a patient in St Petersburg, Russia - colonic ulcers at autopsy - world-wide in prevalence - *E. histolytica* occurs in three forms—the trophozoite, precystic and cystic stages - granular endoplasm – ectoplasm and endoplasm - pseudopodium

25. a. Discuss about the working principle autoclave and hot air oven. **OR**

Autoclaving –sterilization-Principle -steam under pressure - Steaming at temperature higher than 100°C is used in autoclaving - atmospheric pressure. 121°C temperatures at 15 ib per sq. inch pressure -15 minutes for sterilization to kill spores - It works like a pressure cooker - Autoclave is a metallic cylindrical vessel- A gauge - A safety valve - pressure stopcock .

Hot Air Oven (Sterilizer) - Glass wares, swab sticks, all-glass syringes, powder and oily substances are sterilized in hot air oven. - temperature of 160°C is maintained (holding) for one hour. Spores are killed at this temperature. It leads to sterilization - double metallic walls and a door -air space between these walls- A thermostat is fitted to maintain a constant temperature of 160°C.

b. Explain in detailed about growth curve and generation time?

Growth Curve - analyzing the growth - cultivated in liquid medium- batch culture - Lag Phase - exponential or log phase - growing and dividing - balanced growth - stationary phase – starvation - death phase - generation time or doubling time

26. a. Explain probiotics and their applications. **OR**

Probiotics are live bacteria and yeasts that are good for your health- your digestive system- Probiotics are often called "good" or "helpful" bacteria because they help keep your gut healthy- Definition -Probiotics are naturally found in your body -foods and supplements - probiotics and their health benefits- Types of Probiotics – Lactobacillus – Bifidobacterium -

b. Comment on modern microbiology and its importance.

Modern microbiology - Phycology, Mycology, Virology, Protozoology, Bacteriology, Medical microbiology, Agricultural microbiology, Industrial microbiology, Food and Dairy microbiology, Aquatic microbiology, Aero microbiology, Environmental microbiology, Geochemical microbiology, Biotechnolog, Immunology, Exo microbiology.

Reg. No.....

[07MBP01]

KARPAGAM ARTS AND SCIENCE COLLEGE

(AUTONOMOUS)

[AFFILIATED TO BHARATHIAR UNIVERSITY]

COIMBATORE – 641 021

(For the candidates admitted from 2007 onwards)

M.Sc. DEGREE EXAMINATION, NOVEMBER 2007

First Semester

MICROBIOLOGY

FUNDAMENTALS OF MICROBIOLOGY

Time : 2.30 hours Maximum : 100 Marks

PART – A

(Question Nos. 1 to 30 Online Examination)

Answer ALL the questions.

PART – B (5 x 8 = 40 Marks)

31. a. Write a detailed account on Recognition of the Microbial Role in Diseases.

Or

b. Explain in detail Whittaker's Five Kingdom concept.

32. a. Explain in detail the types of culture Media used for the cultivation of bacteria.

Or

b. Give an account on the Role of ATP in Metabolism.

33. a. Explain in detail the numerical classification of Microbes.

Or

b. Write a detailed note on Archae bacteria.

34. a. Give an account on Structure, Nutrition and Reproduction of the fungi.

Or

b. Explain in detail Algal structure.

35. a. Explain in detail the Simple Compound Microscope.

Or

b. Give an detailed account on Principle and procedure of Gram's staining.

PART – C (5 X 12 = 60 Marks)

36. a. Discuss the theory of Spontaneous generation and its disproval.

Or

b. Write in detailed on the salient features of bacteria to the Bergey's Manual.

37. a. Give detailed account on physical methods in sterilization.

Or

b. Explain in detail on Serological methods involved in identification of bacteria.

38. a. Discuss in detail the Molecular Based classification of Microbes.

Or

b. Give an account on Actinomycetes and its general properties.

39. a. Give an account on Slime and Water Molds.

Or

b. Write in detail about Algal Reproduction.

40. a. Write a detailed account on Dark field and Phase contrast Microscope

Or

b. Explain in detail about Stains used in bacterial staining

Reg. No. .....

[06MBP01]

KARPAGAM ARTS AND SCIENCE COLLEGE

(AUTONOMOUS)

[AFFILIATED TO BHARATHIAR UNIVERSITY]

COIMBATORE - 641 021.

(For the candidates admitted from 2006 onwards)

M.Sc. DEGREE EXAMINATION, NOVEMBER 2006

First Semester

MICROBIOLOGY

2 COPY

FUNDAMENTALS OF MICROBIOLOGY

Time : 2.30 hours

Maximum : 75 marks

PART - A (25 Marks)
(Question Nos. 1 to 25 Online Examination)

Answer ALL the questions.

PART – B (5 × 3 = 15 Marks)

26. (a) State the contributions of Louis Pasteur.

(OR)

(b) State Koch's postulates and write a note on his contibutions.

27. (a) Describe the process of Lyophilization.

(OR)

(b) Explain the types of media you know.

28. (a) Write a short note on Archaeabacteria.

(OR)

(b) Explain the hierarchial classification of micro organisms.

29. (a) Explain the ultrastructure of Algae.

(OR)

(b) Explain the reproduction of Fungi.

30. (a) Explain the working of Fluorescent microscope.

(OR)

(b) How do you stain the spore of a bacteria?

PART – C (5× 7 = 35 Marks)

31. (a) Explain whittaker's five kingdom concept of classification.

(OR)

(b) What are the major characteristics used in Taxonomy for classification? Explain.

32. (a) Explain moist heat sterilization in detail.

(OR)

(b) Give the principle and working mechanism of Hot air oven.

33. (a) Explain the Diversity and Evolution of microbes in detail.

(OR)

(b) Explain the Universal Phylogenetic tree.

34. (a) Explain the classification of Algae.

(OR)

(b) Describe the classification of Fungi.

35. (a) Explain the techniques involved in Electron Microscopy. Describe SEM in detail.

(OR)

(b) Explain the Differential staining techniques.

Reg. No.....

[10MBP101]

KARPAGAM UNIVERSITY

(Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
(For the candidates admitted from 2010 onwards)

M.Sc. DEGREE EXAMINATION, NOVEMBER 2010

First Semester

MICROBIOLOGY

GENERAL MICROBIOLOGY AND CLASSIFICATION

Time: 3 hours Maximum : 60 marks

PART – A (20X ½ = 10 Marks) (30 Minutes)
(Question Nos. 1 to 20 Online Examinations)

PART B (5 X 4= 20 Marks) (2 ½ Hours)
Answer ALL the Questions

21. a. Describe the Taxonomic ranks of any one micro organism.

Or

b. Give a brief account on classification system.

22. a. Explain the various microscopes resolution index.

Or

b. Draw the structure of dark-field microscope, functions.

23. a. Explain the classification of Bacteria.

Or

b. Briefly explain the reproduction of Volvox.

24. a. Explain the different preservation methods of microorganisms.

Or

b. Briefly explain the basic principles of sterilization.

25. a. Write a short notes on economical importance of fungi.

Or

b. Describe the important characters and structure of Giardia.

PART C (3 x 10 = 30 Marks)
Answer any THREE Questions

26. Give a detailed account on major characters used in classification of microorganism.

27. Write a note on electron microscope, uses and applications with neat diagram.

28. Give detailed account on viral classification.

29. Write an essay on controlling of microorganisms by physical methods.

30. Explain the life cycle, structure and characters of Entamoeba histolytica.

Reg. No.....

[09MBP101

KARPAGAM UNIVERSITY

(Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021

(For the candidates admitted from 2009 onwards)

M.Sc., DEGREE EXAMINATION, NOVEMBER 2009

First Semester

MICROBIOLOGY

GENERAL MICROBIOLOGY AND CLASSIFICATION

Time : 3 hours Maximum : 60 mar

PART – A (10 Marks) (30 Minutes)
(Question Nos. 1 to 20 Online Examination)

PART B (5 X 4 = 20 Marks) (2 ½ Hours)
Answer ALL the Questions

21. a. Comment on Edward Jenner.

Or

b. Bring out the characteristics of microbes.

22. a. Write notes on SEM.

Or

b. Bring out the application of microscopy in research.

23. a. How microorganisms are classified on the basis of cellularity.

Or

b. Briefly write about asexual reproduction in chlanydomonas.

24. a. Define sterilization and its methods.

Or

b. Briefly explain about the maintenance of cultures.

25. a. Comment on Plasmodium.

Or

b. Explain about the reproduction in Protozoa.

PART C (3 X 10 = 30 Marks)
Answer any THREE Questions

26. Give an account of phylogenetic classification.

27. Briefly write about phase contrast microscope.

28. Write an essay on Bergey's classification of Bacteria.

29. Give an account of differential staining technique.

30. Write an essay on economic importance of Fungi.

Reg. No.....

[13MBP101]

KARPAGAM UNIVERSITY

(Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
(For the candidates admitted from 2013 onwards)

M.Sc. DEGREE EXAMINATION, NOVEMBER 2013

First Semester

MICROBIOLOGY

GENERAL MICROBIOLOGY AND CLASSIFICATION

Time: 3 hours Maximum : 60 marks

PART – A (10 x 2 = 20 Marks)
Answer Any TEN Questions

1. Give Koch postulates.
2. What is meant by Spontaneous Generation?
3. Write the germ theory of disease.
4. Write the principles of fluorescence microscope.
5. Describe the method of preparation for electron microscopy.
6. Compare the image formation in bright field microscope with dark field microscope.
7. What is a phase ring?
8. What is an Algae?
9. How will you Classify Virus based on Baltimore classification?
10. What are Saprophytes?
11. Define mycoses.
12. What is meant by a Mycelium?
13. Define a spore.
14. What is meant by Sterilization?
15. Define Staining.

PART B (5 x 8 = 40 Marks)
Answer ALL the Questions

16. a. Explain Whittaker's five Kingdom concept of classification.

Or

b. Give an account of the aspects of numerical taxonomy in microbiology.

17. a. Comment on phase contrast microscope and its advantages.

Or

b. Write the principles of Scanning Electron microscope include its application.

18. a. Add a note on bacterial taxonomy and nomenclature.

Or

b. Give a detailed note on the growth and reproduction of *Chlamydomonas*.

19. a. Discuss in detail about the Capsular staining.

Or

b. Explain various steps involved in Lyophilization with neat diagram.

20. a. Describe the classification, reproduction and importance of *Plasmodium*.

Or

b. Write in detail the economic importance of fungi.

Reg. No.....

[15MBP101]

KARPAGAM UNIVERSITY

Karpagam Academy of Higher Education
(Established Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
(For the candidates admitted from 2015 onwards)

M.Sc., DEGREE EXAMINATION, NOVEMBER 2015

First Semester

MICROBIOLOGY

FUNDAMENTALS OF MICROBIOLOGY AND CLASSIFICATION

Time: 3 hours Maximum : 60 marks

PART – A (20 x 1 = 20 Marks) (30 Minutes)
(Question Nos. 1 to 20 Online Examinations)

(Part - B & C 2 ½ Hours)

PART B (5 x 6 = 30 Marks)
Answer ALL the Questions

21. a. Explain in detail about Koch postulates and his findings.
Or
b. Write about the Whittaker's Five Kingdom classification.

22. a. Outline the working principle of compound microscope.
Or
b. Give the protocol of negative staining.

23. a. Comment on the life cycle of Malarial Parasite
Or
b. Give the economical importance of Fungi.

24. a. Explain the various physical methods of sterilization.
Or
b. Describe the methods of preservation of microbes.

25. a. Explain the 16s rRNA sequence and its importance.
Or
b. Give a detail note on phylogenetic tree with a neat sketch.

PART C (1 x 10 = 10 Marks)
(Compulsory)

26. Explain the working principle of electron microscopy.
