
Instruction Hours / week: L: 4 T: 0 P: 0

Marks: Internal: 40 External: 60 Total: 100

End Semester Exam: 3 Hours

Course Objectives

- To impart knowledge on classification of microbes, function and biochemical reaction going on inside the microbial cell.

Course Outcomes (CO's)

1. Students will gain rigorous foundation in various methods to cultivate the microbes and maintenance of the microorganism.

UNIT-I Fundamentals, History, Scope and Evolution of Microbiology:

Classification of microorganisms: Microbial taxonomy, criteria used including molecular approaches, Microbial phylogeny and current classification of bacteria.

UNIT-II Microbial Diversity:

Distribution and characterization Prokaryotic and Eukaryotic cells, Morphology and cell structure of major groups of microorganisms eg. Bacteria, Algae, Fungi, Protozoa and Unique features of viruses.

UNIT-III Cultivation and Maintenance of microorganisms:

Nutritional categories of micro-organisms, Media, Types of media, Methods of isolation, Stating and types, Purification and preservation.

UNIT-IV Microbial growth:

Growth curve, Microbial growth kinetics, batch and continuous culture, Measurement of growth, growth factors, factors affecting growth of bacteria. Bacterial Reproduction: Transformation, Transduction and Conjugation. Endospores and sporulation in bacteria.

UNIT-V Water Microbiology:

Bacterial pollutants of water, coliforms and non coliforms. Sewage composition and its disposal. Food Microbiology: Important microorganism in food Microbiology: Moulds, Yeasts, bacteria. Major food born infections and intoxications, Preservation of various types of foods. Fermented Foods.

SUGGESTED READINGS

1. Aneja KR, and Mehrotra RS. (2015). An Introduction to Mycology. 2nd edition. New Age International.
2. Jay JM, Loessner MJ, and Golden DA. (2005). Modern Food Microbiology. 7th edition. CBS Publishers and Distributors. Delhi: India.
3. Robert Edward Lee, (2008). Phycology. 4th edition. Cambridge University Press.
4. Madigan MT, Martinko JM, and Parker J. (2010). Brock Biology of Microorganisms. 13th edition. Pearson/Benjamin Cummings.
5. Willey JM, Sherwood LM, and Woolverton CJ. (2008). Prescott, Harley and Klein's Microbiology. 7th edition. McGraw Hill Higher Education.
6. Tortora GJ, Funke BR, and Case CL. (2008). Microbiology: An Introduction. 9th edition. Pearson Education.
7. Stanier RY, Ingraham JL, Wheelis ML, and Painter PR. (2005). General Microbiology. 5th edition. McMillan.
8. Pelczar MJ, Chan ECS, and Krieg NR. (1993). Microbiology. 5th edition. McGraw Hill Book Company.



KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University Established Under Section 3 of UGC Act 1956)

Coimbatore – 641 021.

LECTURE PLAN DEPARTMENT OF BIOTECHNOLOGY

STAFF NAME: Dr. Archana.G

SUBJECT NAME: General Microbiology

SEMESTER: II

SUB.CODE:19BTU203

CLASS: I B.Sc. (BT)

S.No	Lecture Duration Period	Topics to be Covered	Support Material/Page Nos
UNIT-I			
1	1	Fundamentals of Microbiology	R1: Pg: 3
2	1	History and Scope of Microbiology	R1: Pg:4
3	1	Evolution of Microbiology	R1: Pg:5
4	1	Classification of Microbiology	R1: Pg: 37-39
5	1	Microbial Taxonomy	R1: Pg: 40 -42
6	1	Molecular Approaches of Microbial Taxonomy	R1: Pg: 43-45
7	1	Microbial Phylogeny	R1: pg: 46-47
		Current Classification of Bacteria	R1: pg: 48
		Genome Sequencing of Microbes	R1: Pg: 49
8	1	Microbial Databases	R1: Pg: 50
		Recapitulation and Discussion of Important Questions	
	Total No of Hours Planned For Unit 1=8		
UNIT-II			
1	1	Microbial Diversity	R1: Pg: 262
2	1	Prokaryotes - Distribution	R1: Pg:265-280
3	1	Prokaryotes - Characterization	R1: Pg:281-295
4	1	Morphology of Cell structure	R2: Pg: 554-563

5	1	Bacteria- types- cell structure	R2: Pg: 538-551
6	1	Algae-types- cell structure	R2: Pg: 568-574
7	1	Fungi-types-cell structure	R2: pg: 575-590
		Protozoa-types-cell structure	R2: Pg: 388-395
		Viruses Introduction	R2: Pg: 396-406
8	1	Viruses-types-structure	R2: Pg: 508-514
		Recapitulation and Discussion of Important Questions	
	Total No of Hours Planned For Unit II=8		
UNIT-III			
1	1	Cultivation and Maintenance of Microbes	R3: Pg: 144-148
2	1	Nutritional Requirement of Bacteria	R1: Pg: 99-103
3	1	Media-types-significance	R1: Pg: 104-106
4	1	Methods of sterilization	R3: Pg: 168
5	1	Techniques of Pure culture	R3: Pg: 190-205
6	1	Isolation Methods	R1: Pg: 136-140
7	1	Staining types-Methods	R3: Pg: 173 -174
		Simple staining –Gram Staining	R1: Pg: 66-67
		Simple staining – Negative staining	R1: pg: 69
8	1	Purification and Preservation of Microbes	R1:pg: 142 -147
		Recapitulation and Discussion of Important Questions	
	Total No of Hours Planned For Unit III=8		
UNIT-IV			
1	1	Microbial Growth	R1: Pg: 117-150
2	1	Factors affecting Microbial Growth	R2: Pg: 107-113
3	1	Microbial Growth Kinetics	R3: Pg: 160-163
4	1	Measurement of Microbial growth	R1: Pg: 119-123

5	1	Batch and Continuous Culture	T2: Pg: 94-105
6	1	Bacterial Reproduction	T1:Pg: 108
7	1	Bacterial transformation	T1: Pg: 109-111
		Bacterial Conjugation	T1: Pg: 112-117
8	1	Bacterial Transduction	T1: Pg: 117-124
		Endospores and Sporulation in Bacteria	T1: Pg: 127-130
		Recapitulation And Discussion Of Important Questions	
	Total No of Hours Planned For Unit IV=8		
UNIT-V			
1	1	Water Microbiology	R1: Pg: 882-884
2	1	Bacterial Pollutants of Water	R1: Pg: 885-890
3	1	Coliforms & Non –Coliforms	R2: Pg: 907-908
4	1	Sewage Composition and Disposal	T2: Pg: 133-157
5	1	Food Microbiology	T2: Pg: 159
6	1	Moulds	T2: Pg: 160-183
		Bacteria & Yeast –Fermentation	T2: Pg: 184-200
		Food Borne Infections and Intoxications	T2: Pg: 200-210
		Preservation of Foods	T2: Pg: 210-235
7	1	Fermented foods	T2: Pg: 236-247
		Recapitulation and Discussion of important Questions	
		Discussion of Previous ESE Question Papers.	
8	1	Discussion of Previous ESE Question Papers.	
		Discussion of Previous ESE Question Papers.	
	Total No of Hours Planned for unit V=8		
Total Planned Hours	40		

References/Text Books:

1. **R1:** PELZER, M.J; 2003, MICROBIOLOGY.TATAMCGRAW. HILL PUBLISHING COMPANY Ltd., NEW YORK
2. **R2:** PRESCOTT, L.M; 2008; MICROBIOLOG, 7th Edition BROWN PUBLISHERS- LONDON
3. **R3:** ALBERT GMOAT, JOHN FOSTER, MICAEL D., SPECTOR 2002. MICROBIAL PHYSIOLOGY, 4th Edition JOHN WILEY SONS PUBLICATIONS

Unit I – Fundamentals, History, Scope and Evolution of Microbiology

Unit I

SYLLABUS

Classification of microorganisms: Microbial taxonomy, criteria used including molecular approaches, Microbial phylogeny and current classification of bacteria.

MICROBIOLOGY is a specialized area of biology (Gr. *bios*-life+ *logos*-to study) that concerns with the study of microbes ordinarily too small to be seen without magnification. Microorganisms are microscopic (Gr. *mikros*-small+ *scopein*-to see) and independently living cells that, like humans, live in communities.

Microorganisms include a large and diverse group of microscopic organisms that exist as single cell or cell clusters (e.g., bacteria, archaea, fungi, algae, protozoa and helminths) and the viruses, which are microscopic but not cellular. While bacteria and archaea are classed as prokaryotes (Gr. *pro*-before+ *karyon*-nucleus) the fungi, algae, protozoa and helminths are eukaryotes (Gr. *eu*-true or good+ *karyon*-nucleus).

Microorganisms are present everywhere on earth, which includes humans, animals, plants and other living creatures, soil, water and atmosphere. Microorganisms are relevant to all of our lives in a multitude of ways. Sometimes, the influence of microorganisms on human life is beneficial, whereas at other times, it is detrimental. For example, microorganisms are required for the production of bread, cheese, yogurt, alcohol, wine, beer, antibiotics (e.g., penicillin, streptomycin, and chloramphenicol), vaccines, vitamins, enzymes and many more important products. Many

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products of microbes contribute to public health as aids to nutrition, other products are used to interrupt the spread of disease, still others hold promise for improving the quality of life in the year's ahead.

CONCEPTS

Microbiology is the study of organisms that are usually too small to be seen by the unaided eye; it employs techniques—such as sterilization and the use of culture media—that are required to isolate and grow these microorganisms.

- Microorganisms are not spontaneously generated from inanimate matter but arise from other microorganisms.
- Many diseases result from viral, bacterial, fungal, or protozoan infections. Koch's postulates may be used to establish a causal link between the suspected microorganism and a disease.
- The development of microbiology as a scientific discipline has depended on the availability of the microscope and the ability to isolate and grow pure cultures of microorganisms.
- Microorganisms are responsible for many of the changes observed in organic and inorganic matter (e.g., fermentation and the carbon, nitrogen, and sulfur cycles that occur in nature).
- Microorganisms have two fundamentally different types of cells—prokaryotic and eukaryotic—and are distributed among several kingdoms or domains.
- Microbiology is a large discipline, which has a great impact on other areas of biology and general human welfare.

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The 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 (figure 1.1*b*). 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 and made bacteria.

clearly visible (figure 1.1*c*). 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.

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Early history and developments of microbiology.

Historians are unsure who made the first observations of microorganisms, but the microscope was available during the mid-1600s, and an English scientist named **Robert Hooke** made key observations. He is reputed to have observed strands of fungi among the specimens of cells he viewed. In the 1670s and the decades thereafter, a Dutch merchant named **Anton van Leeuwenhoek** made careful observations of microscopic organisms, which he called **animalcules**. Until his death in 1723, van Leeuwenhoek revealed the microscopic world to scientists of the day and is regarded as one of the first to provide accurate descriptions of protozoa, fungi, and bacteria.

After van Leeuwenhoek died, the study of microbiology did not develop rapidly because microscopes were rare and the interest in microorganisms was not high. In those years, scientists debated the theory of **spontaneous generation**, which stated that microorganisms arise from lifeless matter such as beef broth. This theory was disputed by **Francesco Redi**, who showed that fly maggots do not arise from decaying meat (as others believed) if the meat is covered to prevent the entry of flies. An English cleric named **John Needham** advanced spontaneous generation, but **Lazzaro Spallanzani** disputed the theory by showing that boiled broth would not give rise to microscopic forms of life.

The Conflict over 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

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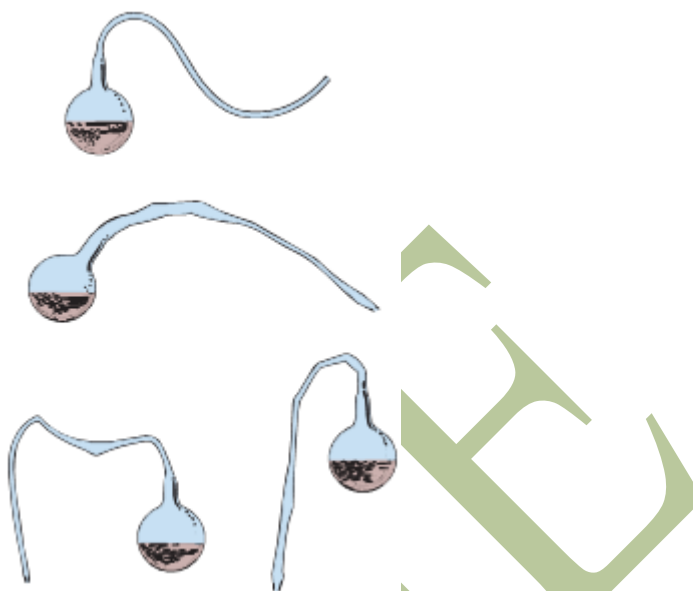
simpler invertebrates could arise by spontaneous generation. This view finally was challenged by the Italian physician Francesco Redi (1626–1697), who carried out a series of experiments on decaying meat and its ability to produce maggots spontaneously. Redi placed meat in three containers. One was uncovered, a second was covered with paper, and the third was covered with a 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

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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.

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The Spontaneous Generation Experiment. Pasteur's swan neck flasks used in his experiments on the spontaneous generation of microorganisms.

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

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forms of bacteria. Working independently, the German botanist Ferdinand Cohn (1828–1898) discovered the existence of heat-resistant bacterial endospores.

Louis Pasteur and the germ theory

Louis Pasteur worked in the middle and late 1800s. He performed numerous experiments to discover why wine and dairy products became sour, and he found that bacteria were to blame. Pasteur called attention to the importance of microorganisms in everyday life and stirred scientists to think that if bacteria could make the wine “sick,” then perhaps they could cause human illness.

Pasteur had to disprove spontaneous generation to sustain his theory, and he therefore devised a series of **swan-necked flasks** filled with broth. He left the flasks of broth open to the air, but the flasks had a curve in the neck so that microorganisms would fall into the neck, not the broth. The flasks did not become contaminated (as he predicted they would not), and Pasteur's experiments put to rest the notion of spontaneous generation. His work also encouraged the belief that microorganisms were in the air and could cause disease. Pasteur postulated the **germ theory of disease**, which states that microorganisms are the causes of infectious disease.

Pasteur's attempts to prove the germ theory were unsuccessful. However, the German scientist **Robert Koch** provided the proof by cultivating anthrax bacteria apart from any other type of organism. He then injected pure cultures of the bacilli into mice and showed that the bacilli invariably caused anthrax. The procedures used by Koch came to be known as **Koch's postulates**. They provided a set of principles whereby other microorganisms could be related to other diseases.

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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

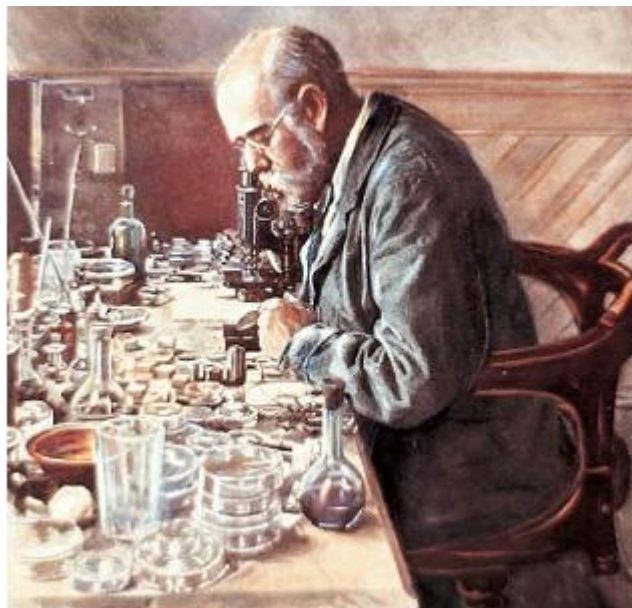
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the study of fermentation, Pasteur was asked by the French government to investigate the pébrine disease of silkworms that was disrupting the silk industry. After several years of work, he showed that the disease was due to a protozoan parasite. The 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 by the German physician Robert Koch (1843–1910).

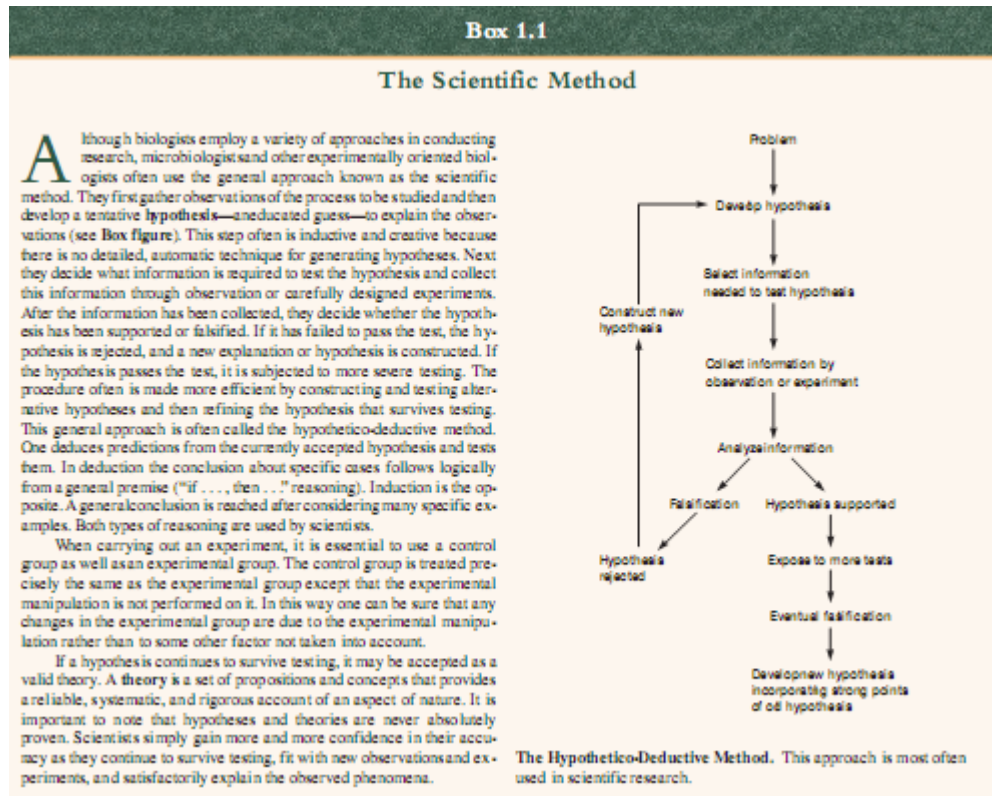
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Robert Koch (1843–1910) examining a specimen in his laboratory

Koch 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 (**Box 1.1** briefly discusses the scientific method).

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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.

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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.

Although the criteria that Koch developed for proving a causal relationship between a microorganism and a specific disease have been of immense importance in medical microbiology, it is not always possible to apply them in studying human diseases. For example, some pathogens cannot be grown in pure culture outside the host; because other pathogens grow only in humans, their study would require experimentation on people. The identification, isolation, and cloning of genes responsible for pathogen virulence have made possible a new molecular form of Koch's postulates that resolves some of these difficulties. The emphasis is on the virulence genes present in the infectious agent rather than on the agent itself. The molecular postulates can be briefly summarized as follows:

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1. The virulence trait under study should be associated much more with pathogenic strains of the species than with nonpathogenic strains.
2. Inactivation of the gene or genes associated with the suspected virulence trait should substantially decrease pathogenicity.
3. Replacement of the mutated gene with the normal wild-type gene should fully restore pathogenicity.
4. The gene should be expressed at some point during the infection and disease process.
5. Antibodies or immune system cells directed against the gene products should protect the host.

The molecular approach cannot always be applied because of problems such as the lack of an appropriate animal system. It also is difficult to employ the molecular postulates when the pathogen is not well characterized genetically.

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

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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 sometime. 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 (table 1.1). 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

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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 honour 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

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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 [Greek *phagein*, eating].

Tindalization /Tyndallization named after John Tyndall is a lengthy process designed to reduce the level of activity of sporulating bacteria that are left by a simple boiling water method. The process involves boiling for a period (typically 20 minutes) at atmospheric pressure, cooling, incubating for a day, boiling, cooling, incubating for a day, boiling, cooling, incubating for a day, and finally boiling again. The three incubation periods are to allow heat-resistant spores surviving the previous boiling period to germinate to form the heat-sensitive vegetative (growing) stage, which can be killed by the next boiling step. This is effective because many spores are stimulated to grow by the heat shock. The procedure only works for media that can support bacterial growth - it will not sterilize plain water. Tindalization/tyndallization is ineffective against prions.

The development and scope 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.

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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

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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.

Two Australians, **Barry J. Marshall** and **Robin Warren** won the 2005 Nobel Prize for showing that bacterial infections of *Helicobacter pylori* (= *Campylobacter pylori*) and not the stress, is responsible for painful ulcers in the stomach and intestine. The 1982 discovery transformed **peptic ulcer disease** from a chronic, frequently disabling condition to one that can be cured by a short regimen of antibiotics and medicines. At the same time, nucleic acid sequencing methods were developed which left its impact in all the areas of biology. Sequencing technology helped microbiologists to reveal phylogenetic evolutionary relationships among prokaryotes, which led to evolutionary new concepts in the field biological classification. The field of **Genomics** is also a contribution of sequencing technology, in which the **comparative analysis of the genes of different organisms** is carried out. The huge amounts genomic information now in hand are leading to major advances in medicine, microbial ecology, industrial microbiology, and many other areas of biology. The genomics era has given birth to a new subdiscipline, **Proteomics**. The proteomics is defined as **the study of protein expression in cells**. The significance of such developments in molecular biology to all of biology is understood by the fact that numerous Nobel Prizes have been awarded to researchers for their work in this field

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Concepts

1. Light microscopes use glass lenses to bend and focus light rays and produce enlarged images of small objects. The resolution of a light microscope is determined by the numerical aperture of its lens system and by the wavelength of the light it employs; maximum resolution is about 0.2 μ m.
2. The most common types of light microscopes are the bright-field, darkfield, phase-contrast, and fluorescence microscopes. Each yields a distinctive image and may be used to observe different aspects of microbial morphology.
3. Because most microorganisms are colorless and therefore not easily seen in the bright-field microscope, they are usually fixed and stained before observation. Either simple or differential staining can be used to enhance contrast. Specific bacterial structures such as capsules, endospores, and flagella also can be selectively stained.
4. The transmission electron microscope achieves great resolution (about 0.5 nm) by using electron beams of very short wavelength rather than visible light. Although one can prepare microorganisms for observation in other ways, one normally views thin sections of plastic-embedded specimens treated with heavy metals to improve contrast.
5. External features can be observed in great detail with the scanning electron microscope, which generates an image by scanning a fine electron beam over the surface of specimens rather than projecting electrons through them.

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6. New forms of microscopy are improving our ability to observe microorganisms and molecules. Two examples are the confocal scanning laser microscope and the scanning probe microscope.

Living organisms are fascinating by its diversity whether it is plants, animals or microbes. A handful of soil is populated with more than the human population on earth. They play important essential roles in nature. So if we arrange these microbes in order or hierarchy by based on its similarity or differences in any characteristics, we can easily get to know and get easy access to all the microbes. So it is desirable to determine the classification. Greek Philosopher Aristotle who is the one classified the living things as plants and animals around 2000 years ago. So in this lecture, we will learn about taxonomy, how is it classified? What methods are available to classify them? And then brief description about microbial evolution and diversity and its phylogeny.

Taxonomy

Taxonomy [Greek *taxis*, arrangement, and *nomos*, law, or *nemein*, to distribute] is defined as the science of biological classification. **In simple term, taxonomy is orderly arranging organisms under study into groups of larger units.** It consists of *three* interrelated parts namely

- 1. Classification** is the *arrangement of organisms* into groups or **taxa** (s., **taxon**) based on mutual similarity or evolutionary relatedness.
- 2. Nomenclature** is 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. **(So in short Identify-Naming them and classify them)**

Classification

It is bringing order to the diverse variety of organisms present in nature. So there are two general ways the classification can be constructed. First one is based on the morphological characters (phenetic classification) and second is based on evolutionary relationship (phylogenetic classification)

Phenetic classification - Grouping organisms together based on the mutual similarity of their phenotypic characteristics. It does not provide information about phylogenetic relations.

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Phylogenetic classification- These are systems based on evolutionary relationships rather than external appearance (the term phylogeny [Greek *phylon*, tribe or race, and *genesis*, generation or origin] refers to the evolutionary development of a species). It is based on the direct comparison of genetic materials and/or gene product.

Nomenclature (Binomial system)

Biologists in the middle ages used to follow polynomial system, i.e naming organisms with many names (*poly* -many, *nomo* - name). For example name for the European honeybee, was *Apis pubescens, thorace subgriseo, abdomine fusco, pedibus posticis glabris utrinque margine ciliatis* (just for example no need to be memorized). Later Binomial systems were developed by Swedish biologist Carolus Linnaeus (1707–1778) based on the anatomical characteristics of plants and animals. Nomenclature in microbiology is developed based on the principals established for the plant and Animal kingdom by Linnaeus. The first word in the binomial is the genus name and is always capitalized. The second word is species name and never capitalized. For example honeybee, *Apis mellifera*

Taxonomic ranks:

In prokaryotic taxonomy the most commonly used levels or ranks (in ascending order) are species, genera, families, orders, classes, phyla, kingdom or domain. In order to remember the seven categories of the taxonomic hierarchy in their proper order, it may be useful to memorize a phrase such as “**k** indly **p** ay **c** ash **o** r **f** urnish **g** ood **s** ecurity” (**k** ingdom– **p** hylum– **c** lass– **o** rder– **f** amily– **g** enus– **s** pecies). The basic taxonomic group in microbial taxonomy is the **species**.

A **species** is a collection of strains that have a similar G+C composition and 70% or greater similarity as judged by DNA hybridization. Ideally a species also should be *phenotypically distinguishable* from other similar species. An example of hierarchy in taxonomy is given below.

Rank	Example
Domain	<i>Bacteria</i>
Phylum	<i>Proteobacteria</i>
Class	<i>γ- Proteobacteria</i>
Order	<i>Enterobacteriales</i>
Family	<i>Enterobacteriaceae</i>
Genus	<i>Shigella</i>
Species	<i>S.dysenteriae</i>

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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 prokaryotic 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 but this strain can be considered as reference strain and can be compared with other strains. 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.

Techniques for identifying or determining taxonomical characters

In order to identify and classify microorganisms, we need to know about their characteristics. There are two ways to determine the taxonomical characters; *classical* and *molecular characters*

Classical characteristics:- This approach uses morphological, biochemical, physiological, ecological and genetic characteristics. It is mainly used in microbial taxonomy.

1. **Morphology:-** Morphology is the one which can be easily studied and analyzed. Structural features (*cell shape, size, colony morphology, appendages, and etc.*) depend on the expression of many genes, are usually genetically stable.

2. **Physiology and metabolism:-** Organisms are classified based on *the requirements for growth* characters like carbon and nitrogen sources, cell wall constituents, general nutritional type, energy sources, optimum growth temperature, Motility.

3. **Ecology:-** These are taxonomically valuable because even very closely related microorganisms can differ considerably with respect to ecological characteristics. *The ability to cause disease in a particular host; and habitat preferences* such as requirements for temperature, pH, oxygen, and osmotic concentration are examples of ecological characteristics.

4. **Genetic analysis:-** The study of chromosomal gene exchange between species through transformation and conjugation (in Enteric bacteria) is sometimes useful in their classification. Most bacteria are harboring plasmids, classification based on plasmid is also an important part of classification.

Molecular characteristics :- This is the most powerful approaches to study taxonomy by analyzing 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.

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- 1. *Comparing amino acid sequences*:- Comparison of amino acid sequences of proteins from different organisms reveals its taxonomic relations. The most direct approach is to determine the amino acid sequence of proteins with the same function. If *the sequences* of proteins with the same function *are similar*, the organisms possessing them are *probably closely related*. The *electrophoretic mobility of proteins* 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.
- 2. *Nucleic acid composition*:- By direct comparison of microbial genomes and based on the G+C content of different organisms (*Escherichia coli* 48-52 %). And genomic fingerprinting (RFLP, AFLP) reveals its relatedness with others.
- 3. *Nucleic acid hybridization*:- It uses the property of complementarities in double stranded DNA. More distantly related organism can be identified based on DNA-RNA hybridization
- 4. *Nucleic acid sequencing* :- Techniques are now available to sequence both DNA and RNA. 5S and 16S RNA (prokaryotes), 18S (fungi) analysis of microorganisms can reveal their relatedness because of its *functional role is same in all ribosomes and slow structural changes with time*.

Microbial evolution and Diversity

It has been estimated that our planet is about 4.6 billion years old. Around 3.5 to 3.8 billion years old fossilized remains of prokaryotic cells have been discovered in sedimentary rocks. Thus earlier prokaryotes were anaerobic and arose shortly after the earth cooled. Cyanobacteria and oxygen-producing photosynthesis probably developed 2.5 to 3.0 billion or more years ago.

It appears likely that modern eukaryotic cells arose from prokaryotes about 1.4 billion years ago.

Two hypotheses for the evolution of eukaryotic cells

1. Organelles arose within prokaryotes from the invagination of the plasma membrane

2. Endosymbiotic hypothesis

Fusion of ancient true bacteria and archaea to form a nucleus. They proposed that the eukaryotic line diverged from the *Archaea* and then the nucleus formed, possibly from the Golgi apparatus

Mitochondria and chloroplasts develop later from a permanent symbiotic relationship with other bacteria, e.g., cyanelle (cyanobacterium) living inside the protist *Cyanophora paradoxa*

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Cyanobacteria have been considered the most likely ancestors of chloroplasts. More recently *Prochloron* has become the favorite candidate. 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 eukaryotic and bacteria with aerobic respiration (possibly an ancestor of three modern groups: *Agrobacterium*, *Rhizobium*, and *Rickettsia*).

Divisions of Life

Kingdom systems of classification

- **Five-kingdom system** (Whittaker, 1960s) - based upon cell type, organization, and the means of nutrient acquisition (Monera, Protista, Fungi, Plantae, Animalia)
- **Six-kingdom system** - differs from five-kingdom system by dividing prokaryotes into bacteria and archaea (Bacteria, Archaea, Protista, Fungi, Plantae, Animalia)
- **Eight-kingdom system** (Cavalier-Smith) - further division of the protists using rRNA data and grouping organisms into two empires (Eucaryota and Bacteria) containing a total of eight kingdoms [(Bacteria, Archaea), (Archezoa, Protista, Plantae, Chromista, Fungi, Animalia)]

Domains

Advances in genomic DNA sequencing of the microorganisms, biologists are increasingly adapting the classification of living organisms that recognizes **three domains**, a taxonomic level higher than kingdom. Archaeobacteria are in one domain, eubacteria in a second, and eukaryotes in the third. Domain Eukarya is subdivided into four kingdoms plants, animals, fungi, protists.

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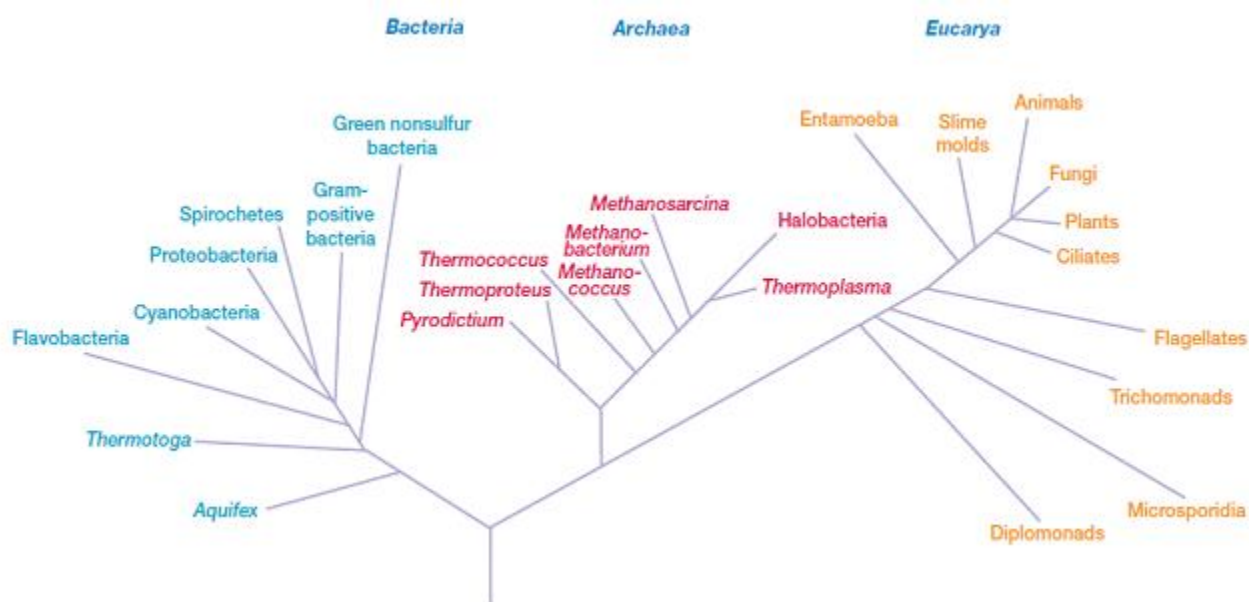


Fig. Three domains based on Woese rRNA sequence analysis

Domain- Archaeobacteria

The term *archaeobacteria* (Greek, *archaio*, ancient) refers to the ancient origin of this group of bacteria, which seem to have diverged very early from the eubacteria. They are inhabited mostly in extreme environments. The archaeobacteria are grouped (based primarily on the environments in which they live) into three general categories methanogens, extremophiles and non extreme Archaeobacteria.



Fig. - Universal Phylogenetic Tree

Domain- Bacteria

The Eubacteria are the most abundant organisms on earth. It plays critical roles like cycling carbon and sulfur. Much of the world's photosynthesis is carried out by eubacteria. However, certain groups of eubacteria are also responsible for many forms of disease.

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Domain- Eukarya

It consists of four kingdoms. The first of which is protista, mostly unicellular organism like amoeba. The other three kingdoms are plants, fungi, animals. Multicellularity and sexuality are the two unique characters that differentiate from prokaryote and eukaryotes.

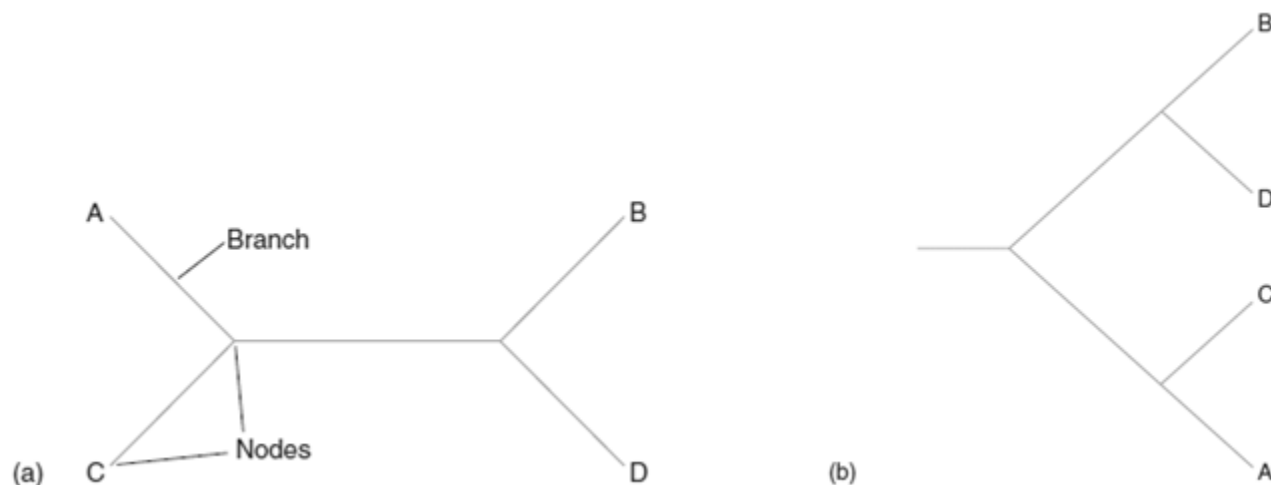


Fig. Phylogenetic tree. a) unrooted tree, b) rooted tree.

Classification of bacteria on the basis of shape:

In the year 1872 scientist Cohn classified bacteria to 4 major types depending on their shapes are as follow: –

1) **Cocci:** These types of bacteria are unicellular, spherical or elliptical shape. Either they may remain as a single cell or may aggregate together for various configurations. They are as follow:

i) **Monococcus:** - they are also called micrococcus and represented by single, discrete round cell. Example: *Micrococcus flavus*. ii) **Diplococcus:** - the cell of the Diplococcus divides ones in a particular plane and after division, the cells remain attached to each other. Example: – *Diplococcus pneumonia*.

iii) **Streptococcus:** - here the cells divide repeatedly in one plane to form chain of cells. Example: – *Streptococcus pyogenes*.

iv) **Tetracoccus:** - this consists of four round cells, which divided in two planes at a right angles to one another. Example: – *Gaffkya tetragena*.

v) **Staphylococcus:** - here the cells divided into three planes forming a structured like bunches of grapes giving and irregular configuration. Example: – *Staphylococcus aureus*.

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vi) Sarcina: -in this case this cells divide in three planes but they form a cube like configuration consisting of eight or sixteen cells but they have a regular shape. Example: –Sarcina lutea.

2) Bacilli: - this are rod shaped or cylindrical bacteria which either remain singly or in pairs. Example: –Bacillus cereus.

3) Vibrio: -the vibrio are the curved, comma shaped bacteria and represented by a single genus. Example: – Vibrio cholerae.

4) Spirilla: - this type of bacteria are spiral or spring like with multiple curvature and terminal flagella. Example: –Spirillum volutans.

Classification of bacteria on the basis of nutrition:

On the basis of nutrition bacteria are classified as following:

- 1) **Autotrophic bacteria:** - these bacteria are nonpathogenic, free living, self sustaining in nature, which prepare their own food by utilisation of solar energy and inorganic components like carbon dioxide, nitrogen etc. They are of two types: i) **Photoautotrophs:** - these bacteria contain bacteriochlorophyll and bacterioviridin and can prepare their own food by fixing carbon dioxide the nature by the utilisation of solar energy. ii) **Chemoautotrophs:** -these are the bacteria which prepare they are food by deriving the energy from oxidation of inorganic substances like nitrogen dioxide, carbon dioxide etc. and they can also fix carbon dioxide and water for their nutrition.
- 2) **Heterotrophic bacteria:** – this type of bacteria cannot fix inorganic Carbone but rather depend on external organic Carbone for their nourishment. They also can be classified on the basis of presence and absence of flight and on the basis of the media on which the bacteria are growing.

Classification of bacteria on the basis of cell wall:

Depending upon the staining reactions by Gram stain bacteria can be classified into two types, those are: – i) **Gram positive:** -this type of bacteria retains the crystal fire lit or gram stain which appear violate. Example: – Streptococcus. ii) **Gram negative:** – they do not retain the gram stain, but they take up the red colour of the counter stain. Example: – Saffranin (Escherichia coli).

Classification on the basis of temperature response:

Bacteria can be classified into four major types on the basis of their temperatures response as indicated below: - i) **Psychrophilic bacteria:** -These type of bacteria grows just above the freezing temperature, they can cause contamination of food stored in the refrigerator. Example: - Pseudomonas. ii) **Mesophilic bacteria:** -These bacteria grow at normal temperature in the water bodies, food products, liberate gas and cause change in texture. Example: -Lactobacillus. iii) **Thermophilic bacteria:** - These types of bacteria can survive at higher temperature and can withstand the pasteurization temperature. Example: - Clostridium, Bacillus. iv) **Thermophilic**

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bacteria: - These types of bacteria can survive pasteurization but cannot grow at the pasteurization temperature. Example: - Micrococcus, Streptococcus.

Example: - Micrococcus, Streptococcus.

Classification of bacteria on the basis of number of flagella:

On the basis of flagella the bacteria can be classified: - i) **Atrichos:** - These bacteria have no flagella. Example: - Corynebacterium diphtheriae. ii) **Monotrichous:** - One flagellum is attached to one end of the bacteria cell. Example: - Vibrio cholera. iii) **Lophotrichous:** - Bunch of flagella is attached to one end of the bacteria cell. Example: - Pseudomonas. iv) **Amphitrichous:** - Bunch of flagella arising from both ends of the bacteria cell. Example: - Rhodospirillum rubrum. v) **Peritrichous:** - The flagella are evenly distributed surrounding the entire bacterial cell. Example: - Bacillus.

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Possible Questions

2 marks

1. Define autotrophs.
2. Define chemotrophs.
3. Define Microbial taxonomy.
4. How bacteria were classified based on temperature?
5. What are facultative anaerobes?
6. What are phototrophs?
7. Define germ theory.

6 marks

1. Write in detail about the history and scope of microbiology.
2. Explain in detail about classification of microorganisms
3. Write in detail about milestones in microbiology.
4. Explain in detail about microbial taxonomy.
5. What is microbiology? Elaborate the application of microbiology.
6. Elaborate bacterial classification with examples.
7. a. Describe in detail: i) History of microbiology ii) Scope of microbiology
8. Discuss in detail about molecular approach in microbial classification
9. Describe in detail about the development of microbiology.
10. Explain about the theory of spontaneous generation.

Unit II – Microbial Diversity

Unit II

SYLLABUS

Distribution and characterization Prokaryotic and Eukaryotic cells, Morphology and cell structure of major groups of microorganisms eg. Bacteria, Algae, Fungi, Protozoa and Unique features of viruses.

Cell

The cell is the basic unit of organization or structure of all living matter.

History:

- The cell was discovered by Robert Hooke in 1665.
- He examined very thin slices of cork and saw a multitude of tiny pores.
 - He remarked that it looked like the walled compartments of a honeycomb, so he called them cells.
 - However, Hooke did not know their real structure or function.
 - His cell observations gave no indication of the nucleus and other organelles found in most living cells.

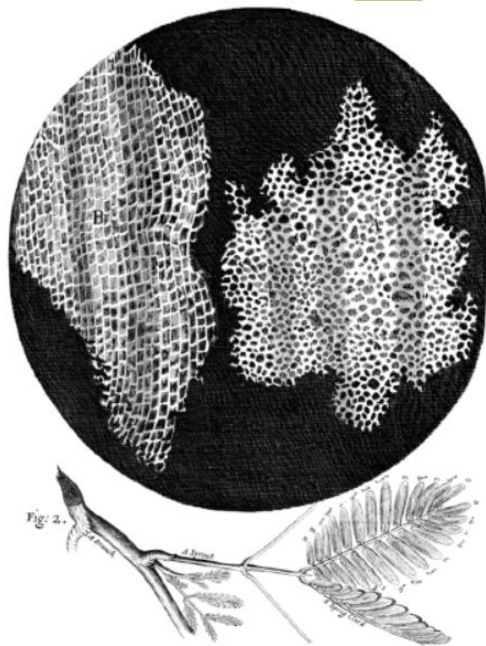


Fig: Drawing of the structure of cork by Robert Hooke that appeared in Micrographia.

Unit II – Microbial Diversity

"The cell is the fundamental element of organization"

The observations of Hooke, Leeuwenhoek, Schleiden, Schwann, Virchow, and others led to the development of the cell theory.

The cell theory states:

- All living things or organisms are made of cells.
- New cells are created by old cells dividing into two.
- Cells are the basic building units of life.

Classification of cells

1. Prokaryotes :

- The prokaryotic (*Greek; pro = primitive or before; karyon = nucleus*) are small, simple and most primitive.
- Prokaryotes lack a nucleus (*though they do have circular DNA*) and other membrane-bound organelles (*though they do contain ribosomes*).
- Bacteria and Archaea are two domains of prokaryotes.

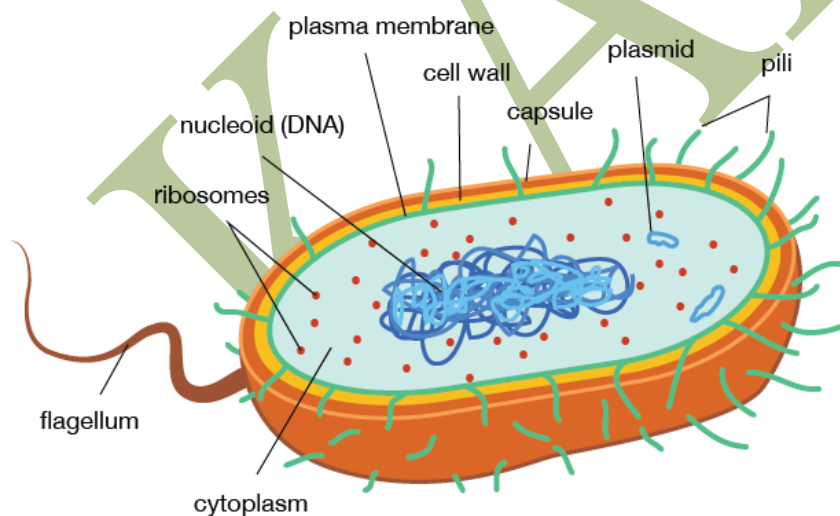


Fig: Schematic diagram of a prokaryotic cell.

Flagella:

- Long, whip-like protrusion found in most prokaryotes that aids in cellular locomotion.

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- It also often functions as a sensory organelle, being sensitive to chemicals and temperatures outside the cell.

Capsule:

- It is found in some bacterial cells.
- This additional outer covering protects the cell when it is engulfed by phagocytes and by viruses.
- Assists in retaining moisture, and helps the cell stick to surfaces and nutrients.
- The capsule is found most commonly among Gram-negative bacteria.
- Examples- *Escherichia coli* (*E.coli*), *Salmonella* etc.
- Examples of Gram positive bacteria -*Streptococcus pneumoniae*, *Streptococcus pyogenes* etc.

Cell wall:

- It is the outermost layer - protects the bacterial cell and gives it shape.
- One exception - Mycoplasma lacks cell wall.
- Bacterial cell walls are made of peptidoglycan which is made from polysaccharide chains cross-linked by unusual peptides containing D-amino acids.
- The antibiotic penicillin is able to kill bacteria by preventing the cross-linking of peptidoglycan and this causes the cell wall to weaken.
- There are two different types of cell wall in bacteria, called Gram-positive and Gram-negative. The names originate from the reaction of cells to the Gram stain, a test long-employed for the classification of bacterial species.
- Gram-positive bacteria possess a thick cell wall containing many layers of peptidoglycan and teichoic acids.
- Gram-negative bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan surrounded by a second lipid membrane containing lipopolysaccharides and lipoproteins.

Cell membrane:

Cell membrane surrounds the cell's cytoplasm and regulates the flow of substances in and out of the cell.

Cytoplasm:

The cytoplasm of a cell is a fluid in nature that fills the cell and is composed mainly of 80% water that also contains enzymes, salts, cell organelles, and various organic molecules.

Cytosol:

(Gel like fluid other than nucleoid)

- The plasma membrane is followed by the colloidal organic fluid called *matrix* or *cytosol*.

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- The cytosol is the aqueous portion of the **cytoplasm**(the extra-nuclear protoplasm) and of the **nucleoplasm**(the nuclear protoplasm).
- It fills all the spaces of the cell and constitutes its true **internal milieu**.
- Cytosol is particularly rich in differentiating cells and many fundamental properties of cell are because of this part of the cytoplasm.
- The cytosol serves to dissolve or suspend the great variety of small molecules concerned with cellular metabolism, *e.g.*, glucose, amino acids, nucleotides, vitamins, minerals, oxygen and ions.

Ribosomes:

Ribosomes are the organelles of the cell responsible for protein synthesis.

Nucleoid Region:

- The nucleoid region is possessed by a prokaryotic bacterial cell.
- It is the area of the cytoplasm that contains the bacterial DNA molecule.

Plasmids:

(The term *plasmid* was first introduced by the American molecular biologist **Joshua Lederberg** in 1952.)

- Many species of bacteria also may carry extrachromosomal genetic elements in the form of small, circular and closed DNA molecules
- Plasmids usually occur naturally in bacteria, but are sometimes found in eukaryotic organisms. Their sizes vary from 1 to over 1,000 kbp.

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2. *Eukaryotes*:

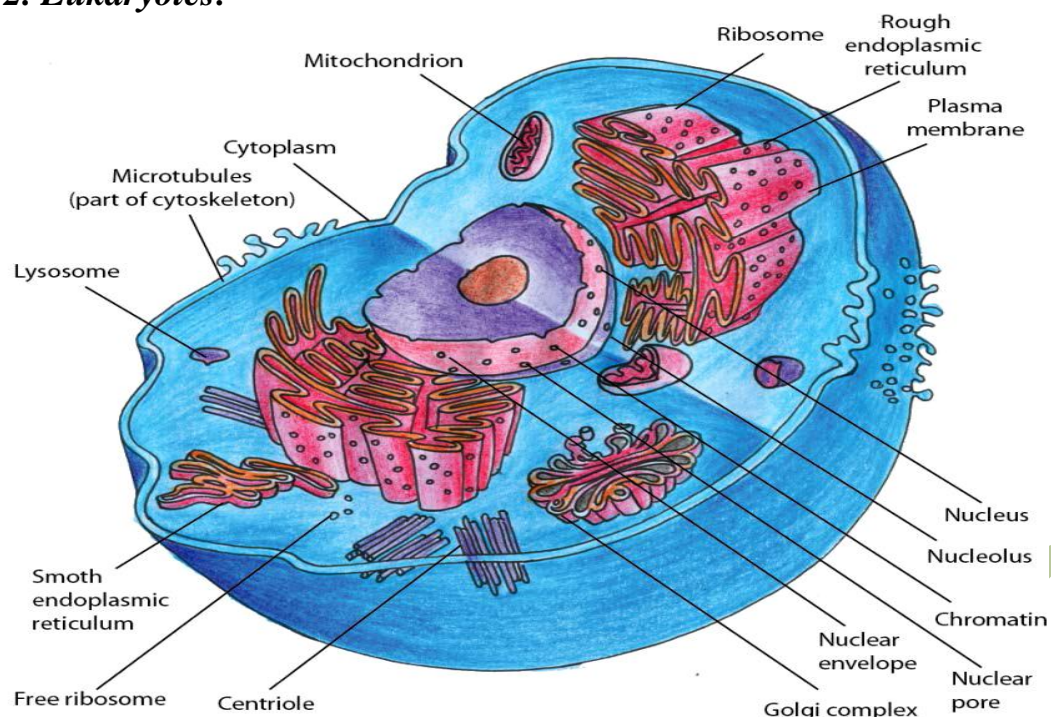


Fig:Eukaryotic cell.

- The eukaryotic cells (*Greek; eu=good, karyotic=nucleated*).
- Eukaryotes, on the other hand, have distinct nuclei bound by a nuclear membrane and membrane-bound organelles (*mitochondria, chloroplasts, lysosomes, rough and smooth endoplasmic reticulum, vacuoles*).
- In addition, they possess organized chromosomes which store genetic material.

Difference between prokaryotes and eukaryotes:

Characteristic	Prokaryotes	Eukaryotes
Size of cell	Typically 0.2-2.0 m m in diameter	Typically 10-100 m m in diameter
Nucleus	No nuclear membrane or nucleoli	True nucleus, consisting of nuclear

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	(nucleoid)	membrane & nucleoli
Membrane-enclosed organelles	Absent	Present; examples include lysosomes, Golgi complex, endoplasmic reticulum, mitochondria & chloroplasts.
Flagella	Consist of two protein building blocks	Complex; consist of multiple microtubules
Glycocalyx	Present as a capsule or slime layer	Present in some cells that lack a cell wall
Cell wall	Usually present; chemically complex (typical bacterial cell wall includes peptidoglycan)	When present, chemically simple
Plasma membrane	No carbohydrates and generally lacks sterols	Sterols and carbohydrates that serve as receptors present
Cytoplasm	No cytoskeleton or cytoplasmic streaming	Cytoskeleton; cytoplasmic streaming
Ribosomes	Smaller size (70S)	Larger size (80S); smaller size (70S) in organelles
Chromosome (DNA) arrangement	Single circular chromosome; lacks histones	Multiple linear chromosomes with histones
Cell division	Binary fission	Mitosis
Sexual	No meiosis; transfer of DNA	Involves Meiosis

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reproduction	fragments only (conjugation)	
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Compartmentalisation of Eukaryotes :

- In Eukaryotes, cells are arranged into compartments (*as it is bound on all the sides by a cell membrane*).
- It separates the protoplasm within the cell from the surrounding environment.
- Intracellular membrane systems, creates enclosed compartments that are separate from Cytosol.
- As a result, the cell is able to retain specific molecules and carry out certain reactions in orderly manner.
- Prokaryotes evolved to form Eukaryotes, in the process *Cytosol compartmentalised* to form *Cytoplasm*.
(The cell cytoplasm contains *cytoplasm, cell organelles, and fluids - Cytosol*).

Plant cells

Plant cells are eukaryotic cells that differ in several key aspects from the cells of other eukaryotic organisms. Their distinctive features include the following organelles:

1. Vacuole:

- It is present at the centre and is water-filled volume enclosed by a membrane known as the tonoplast.
- The function is to maintain the cell's turgor, pressure by controlling movement of molecules between the cytosol and sap, stores useful material and digests waste proteins and organelles.

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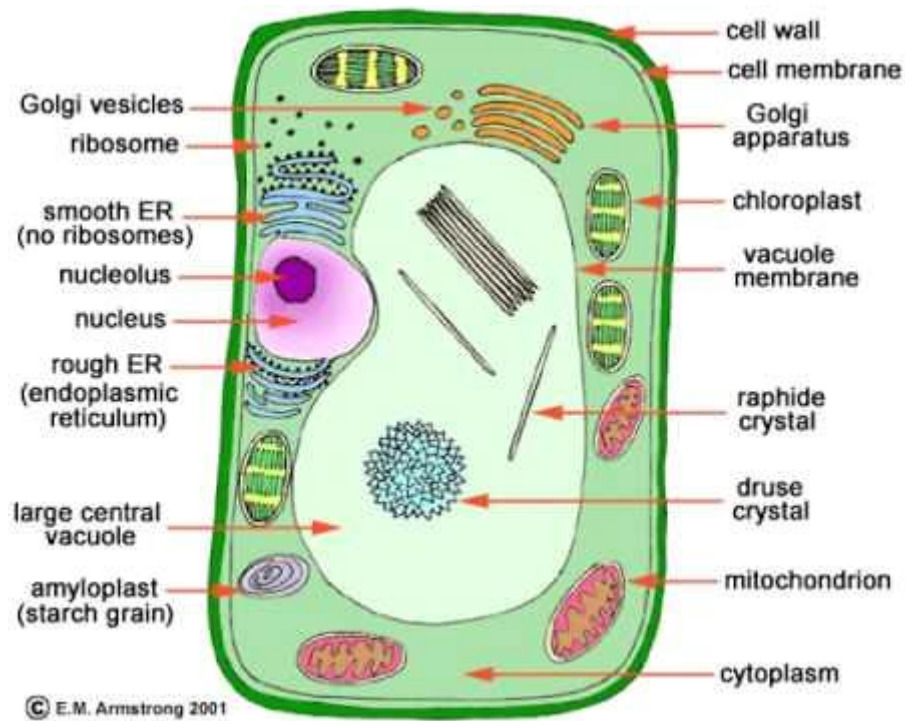


Fig:Anatomy of Plant Cell.

2. Cell Wall:

- It is the extracellular structure surrounding plasma membrane.
- The cell wall is composed of cellulose, hemicellulose, pectin and in many cases lignin, is secreted by the protoplast on the outside of the cell membrane.
- This contrasts with the cell walls of fungi (which are made of chitin), and of bacteria, which are made of peptidoglycan.

3. Plasmodesmata:

Pores in the primary cell wall through which the plasmalemma and endoplasmic reticulum of adjacent cells are continuous.

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4. Plastids:

- The plastids are chloroplasts, which contain chlorophyll and the biochemical systems for light harvesting and photosynthesis.
- A typical plant cell (e.g., in the palisade layer of a leaf) might contain as many as 50 chloroplasts.

Morphology and cell structure of major groups of microorganisms**Bacteria**

Bacteria are microscopic unicellular, prokaryotic organisms. The study of bacteria is called Bacteriology. Ehrenberg (1829) established the genus bacterium. Bacteria are present everywhere, in the water, in the soil, in the air, on our body and in our body. Eg. E.coli, lactobacillus, streptococcus, etc.

Major Features of Bacteria

The following are the major features of bacteria:

1. They exist everywhere.
2. They are unicellular. some exist as colonies.
3. They are prokaryotic.
4. They range in size from 0.5micron to 3micron.
5. They are in the form of rods, spheres, spirals or filaments.
6. The cell is enclosed in a cell envelope made up of a capsule, a cell wall and a plasma membrane.
7. Nuclear material is represented by a nucleoid without nuclear membrane.
8. An extra chromosomal DNA called plasmid is usually present in the cytoplasm.

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9. Cell organelles include 70S ribosomes and mesosomes others organelles such as mitochondria, lysosomes, golgi body, endoplasmic reticulum, centrioles, etc. are absent.
10. Appendages like flagella, pili are present.
11. They are either Gram positive or Gram negative.
12. They show absorptive mode of nutrition.
13. They multiply by binary fission.
14. Some produce endospores.

Structure of Bacteria

- Bacteria are unicellular, microscopic, prokaryotic organisms lacking chlorophyll.
- Bacteria were omnipresent. They range in size from 0.5 micro meter to 600 micro meter.
- The bacteria are either spherical or rod shaped or spiral or curved,
- The spherical bacterium is called coccus, coccus means a berry.
- The individual spherical bacterium is called micrococcus. Some spherical bacteria are arranged in pairs and they are called diplococci (sl. Diplococcus).
- When the cocci are arranged in chains, they are called tetrads. When the cocci are arranged in chains, they are called streptococci. When the cocci are arranged in clusters like a bunch of grapes, they are called staphylococci.
- The rod shaped bacteria are called bacilli (sl. bacillus). The bacillus may be found individually or in pairs or in the form of chains or in the form of a bunch of grapes.
- A chain of is called streptobacillus. A bunch of bacilli is called *Staphylo bacillus*.
- The spiral bacteria are spirally curved. They may be slightly curved like a comma eg. *Vibrio* or spirally coiled eg. *Spirillum*. In addition there are filamentous bacteria and fungus like bacteria. They are multicellular.

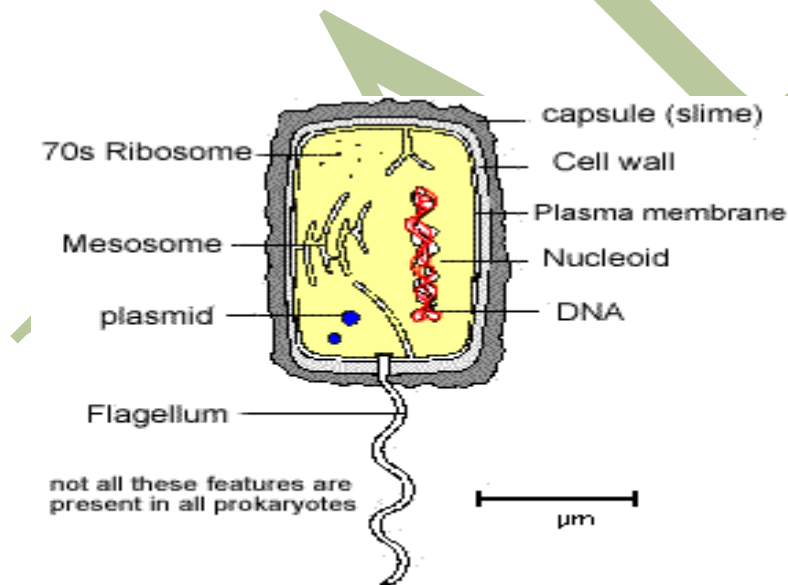
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- There are two types of bacteria. They are Gram positive bacteria and Gram negative bacteria. Gram positive bacteria retain violet colour on Gram staining Gram negative bacteria appear in red colour.
- The bacteria are motile or non-motile. They may or may not contain flagella.
- When the flagellum is absent, the bacterium is called atrichous. When the bacterium contains only one flagellum at the end, it is called monotrichous. When the flagellum is present at both the ends, the bacterium is called amphitrichous. When there is a bunch of flagella at one end, the bacterium is called lophotrichous. In some bacteria, the flagella are present all over the cell, these bacteria are called peritrichous.
- The flagella are present in bacilli and spiral bacteria. They are absent from cocci. They are whip-like. Each flagellum has three parts, namely basal body, a hook and a shaft. The flagella are used for locomotion.
- The bacteria may be motile or non-motile. The bacilli and spirilla are motile. The cocci are non-motile. Motility is brought about by a flagella.
- Some hair-like structures are present on the bacteria. These are called pili or fimbriae. They are used for attachment. Some pili are longer in some bacteria and they are called sex pili.
- A bacterial cell is protected by a cell envelope. The cell envelope is made up of a capsule, a cell wall and a plasma membrane.
- In some cells a slimy cover is present instead of a capsule.
- In some other cells an outer plasma membrane is present between the capsule and the cell wall.
- The bacteria covered by a capsule are called capsulated bacteria. The bacteria which do not contain a capsule are called non-capsulated bacteria.
- The cell envelope encloses the cytoplasm. It is colloidal in nature. It does not exhibit streaming movement. It contains ribosomes and mesosomes. Golgi bodies, endoplasmic reticulum and mitochondria are absent. The ribosomes are 70S type.

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- Mesosomes are pocket-like structures formed by the invagination of plasma membrane.
- The general size of a prokaryotic cell is about 1-2 μm .
- Note the absence of membrane bound organelles
- There is no true nucleus with a nuclear membrane
- The ribosome's are smaller than eukaryotic cells
- The slime capsule is used as a means of attachment to a surface
- Only flagellate bacteria have the flagellum

Plasmids are very small circular pieces of DNA that maybe transferred from one bacteria to another



4.1. 3 Structure and function of bacteria

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Structure	Function of bacteria
Cell Wall	<ul style="list-style-type: none">• Made of murein (not cellulose), which is a glycoprotein or peptidoglycan (i.e. a protein/carbohydrate complex).• There are two kinds of bacterial cell wall, which are identified by the Gram Stain technique when observed under the microscope. Gram positive bacteria stain purple, while Gram negative bacteria stain pink. The technique is still used today to identify and classify bacteria. We now know that the different staining is due to two types of cell wall
Plasma membrane	<ul style="list-style-type: none">• Controls the entry and exit of substances, pumping some of them in by active transport
Mesosome	<ul style="list-style-type: none">• A tightly-folded region of the cell membrane containing all the membrane-bound proteins required for respiration and photosynthesis.• Can also be associated with the nucleoid.• This is now thought to be an artifact of the electron microscope and not a real structure.
Cytoplasm	<ul style="list-style-type: none">• Contains all the enzymes needed for all metabolic reactions, since there are no organelles
Ribosome's	<ul style="list-style-type: none">• The smaller (70 S) type are all free in the cytoplasm, not attached to membranes (like RER). They are used in protein synthesis which is part of gene expression.
Naked DNA	<ul style="list-style-type: none">• Nucleoid is the region of the cytoplasm that contains DNA. It is not surrounded by a nuclear membrane. DNA is always circular (i.e. a closed loop), and not associated with any proteins to form chromatin. Sometimes confusingly referred to as the bacterial chromosome

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- | | |
|------------------|---|
| Slime
Capsule | <ul style="list-style-type: none">• A thick polysaccharide layer outside of the cell wall, like the glycocalyx of eukaryotes. Used for sticking cells together, as a food reserve, as protection against desiccation and chemicals, and as protection against phagocytosis. In some species the capsules of many cells in a colony fuse together forming a mass of sticky cells called a biofilm. Dental plaque is an example of a biofilm. |
|------------------|---|

Classification of bacteria

Bacteria have a large range of different metabolic reactions at their disposal, far more than in the eukaryotes, confined to just respiration or photosynthesis.

- | | |
|---------------|--|
| Fermentation: | <ul style="list-style-type: none">• sometimes called organotrophs these bacteria oxidise organic molecules like glucose. In many instances they metabolise as far as lactic acid or alcohol molecules making them useful to fermentation industry. |
|---------------|--|

- | | |
|----------------|--|
| Photosynthesis | <ul style="list-style-type: none">• sometimes called Phototrophs obtain their energy from sunlight. Many bacteria are photosynthetic and use the same process of photosynthesis as plants. These phototrophic bacteria were some of the earliest forms of life on the planet, and their metabolic reactions increased the oxygen content of the atmosphere from 1% to 20%. |
|----------------|--|

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**Nitrogen
Fixing**

- Lithotrophs obtain their energy by oxidising inorganic compounds like ammonia, nitrite, methane or hydrogen sulphide. These bacteria use a variety of unusual metabolic reactions and many are able to synthesise carbohydrates from carbon dioxide – the chemosynthetic bacteria. There are whole eco-systems on the deep ocean floor with no light, based on lithotrophic bacteria as producers. Although rare, lithotrophic species are enormously important in ecology, as they are responsible for much of the cycling of matter (e.g. the nitrifying bacteria). They could also be useful in biotechnology as they can synthesise useful organic compounds from waste inorganic ones (e.g. methylomonas can make carbohydrates from methane).

Fungi

Fungus is a Latin word which means Mushrooms. The study of mushroom is known as Mycology. Fungi are Non-vascular plants without chlorophyll. Their mode of nutrition is heterotrophic.

They live as saprophytes or parasites or symbionts. They are found in soil, water, air and in our food stuffs.

Characteristics:

- Fungi are eukaryotes (i.e., their cell possesses a true nucleus)
- They are non-green plants.
- The body of fungus is known as thallus. It consists of a single cell as in yeast or it consists of filaments (Mould)
- They do not possess stems, roots, leaves or vascular system.
- They do not show division of labour.

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- In fungus the growth take place at the tip or apex of the filament. This type of growth is known as apical growth or terminal growth.
- Fungi are chemoorganotrophic microorganism.
- They reproduce by means of spores. spores are sexual or asexual. At the same time any part of the filament sufficient to start a new individual organism.
- Their reproductive structure are differentiated from somatic structure.
- The reproductive structure are important in classification and identification of fungi.
- The somatic structure of any fungus resemble those any other fungi.
- The optimum temperature for the growth of fungus between 20°C and 30°C
- Fungi can withstand extremely low temperature as low as 195°C for at least few hours.
- Fungi prefer an acid medium for growth (pH 6)
- Although light is not essential for growth, some light is essential for sporulation in many species.

General structure

Fungi are non-vascular plants lacking chlorophyll. They are eukaryotic protists. It includes yeast, moulds, and mushrooms. The body of fungus is not differentiated into roots, stem and leaves.

The body of fungus is called thallus. Hence it include under the group Thallophyta, which also include algae. The study of fungus is called mycology and the scientists who are studying fungi are called Mycologists.

The fungi of microbiological importance are yeast, penicillium, Agaricus, Rhizopus, Puccinia etc. Fungi are either unicellular or multicellular forms. The yeast are unicellular. The moulds and mushrooms are multicellular.

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The multicellular in the form of filament. The filaments body is called mycelium. Each filament of the mycelium is called a hypha.

Mycelium It can be divided into vegetarian mycelium which grows into the medium and the aerial mycelium which projects from the surface.

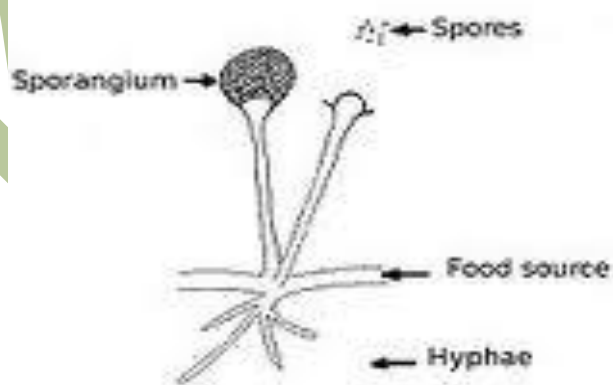
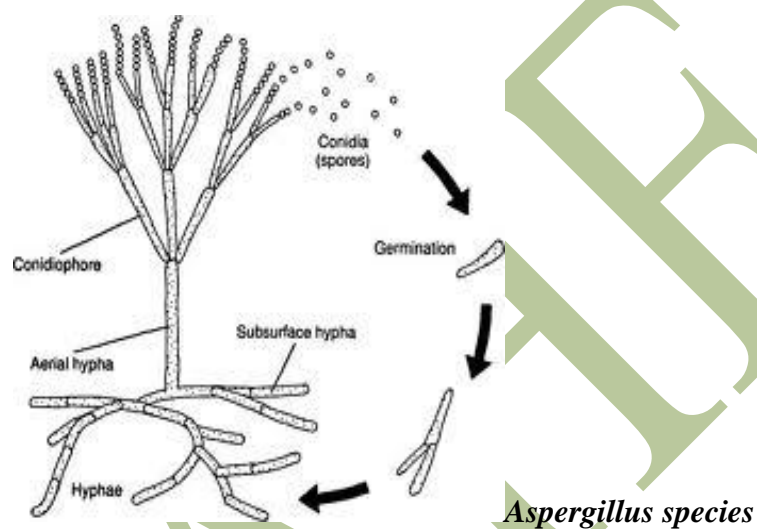
Hypha They are tubular in nature and its consists of cytoplasm enclosed by plasma membrane and the cellwall. The cell wall consists of nucleus. The hyphae are of two types, namely aseptate hypha and septae hypha. The aseptate hypha, cellwalls are absent and nuclei are scattered in the continous mass of cytoplasm. The septate hypha crosswalls are absent and it may be uninucleate or multinucleate.

The mycelium without septa is called aseptate mycelium. The mycelium with septum is called a septate mycelium.

The fungi are sedentary and they are immobile. However, the motile cells appear in their life cycles. The motile cells have flagella. Each flagellum has a central axoneme and a cytoplasmic sheath. The axoneme has 9+2 fibrils.

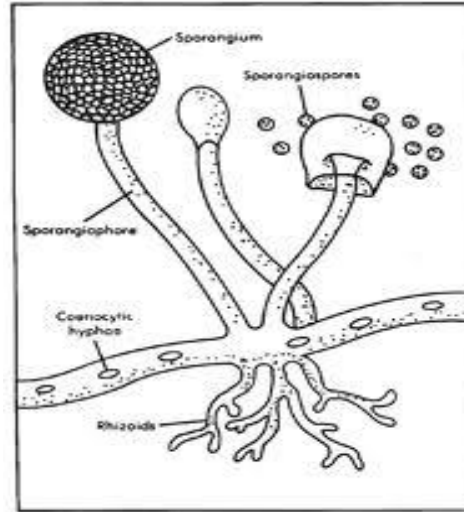
The nutrition in fungus is heterotrophic. They live as saprophytes or parasites or symbionts. Reproduction occurs by asexual and sexual methods.

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Fungus

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Asexual fruiting structure

Economic importance of fungi

Medicines: Antibiotics are obtained from fungi.

- Penicillium is obtained from *Penicillium notatum*.
- Streptomycin is obtained from *Streptomyces griseus*.

Fungal food: There are 200 species of edible fungus. Eg-Mushrooms

Alcoholic fermentation: Yeasts bring about alcoholic fermentation, baking industry:

Yeasts are extensively used in baking industry. They cause the dough to rise and make the bread light and spongy.

Enzymes: Various enzymes are produced by fungi. Eg. *Aspergillus flavus* produce digestion.

Growth hormones: The fungus, *Gibberella fujikuroi* produce gibberelin. It is a plant hormone.

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It is used to accelerate the growth of several crops.

Soil fertility: Soil fungi maintain the fertility of soil

Plant disease: Fungi cause about 30.000 diseases in agricultural plants. Eg-Red root disease of sugar cane.

General studies: The fungus *Neurospora* is used for general studies.

Human fungal disease: Fungus cause the following diseases in man: *Aspergillosis*, athlete's foot, ring worm, thrush etc.

Algae

Phycology or algology 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

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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. This classical classification is based on cellular, not organismal, properties. Some more important properties include: (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.

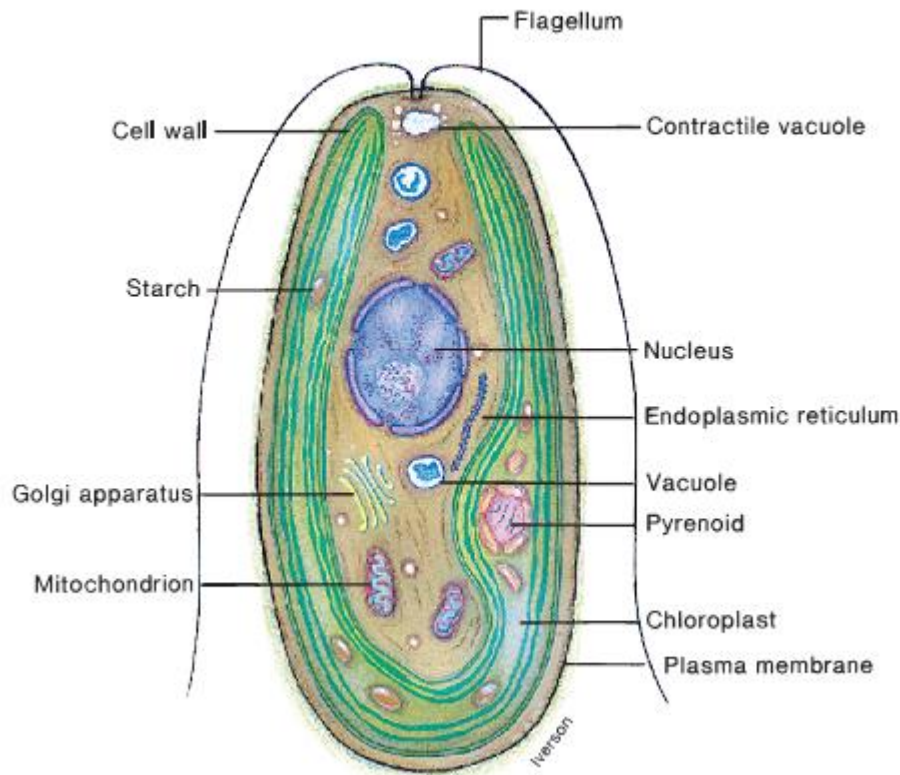
Classical Classification of Algae^a

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

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Ultrastructure of the Algal Cell

The eucaryotic algal cell 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. 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.

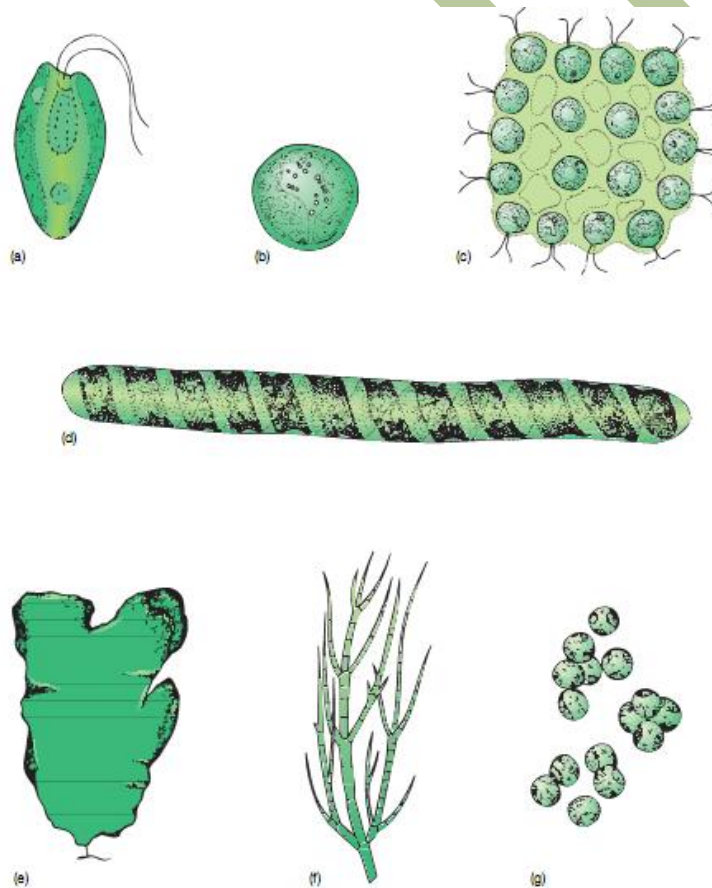


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Algal Morphology. Schematic drawing of a typical eucaryotic algal cell showing some of its organelles and other structures.

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, colonial, filamentous, membranous and bladelike or tubular.



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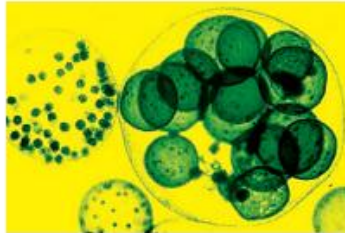
Diagrammatic Algal Bodies: (a) unicellular, motile, *Cryptomonas*; (b) unicellular, nonmotile, almellopsis; (c) colonial, *Gonium*; (d) filamentous, *Spirotaenia*; (e) bladelike kelp, *Monostroma*; (f) leafy tubular axis, branched tufts or plumes, *Stigeoclonium*; (g) unicellular, nonmotile, *Chrysocapsa*.

Characteristics of the Algal Divisions**Chlorophyta (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.



(a)



(b)



(c)



(d)



(e)



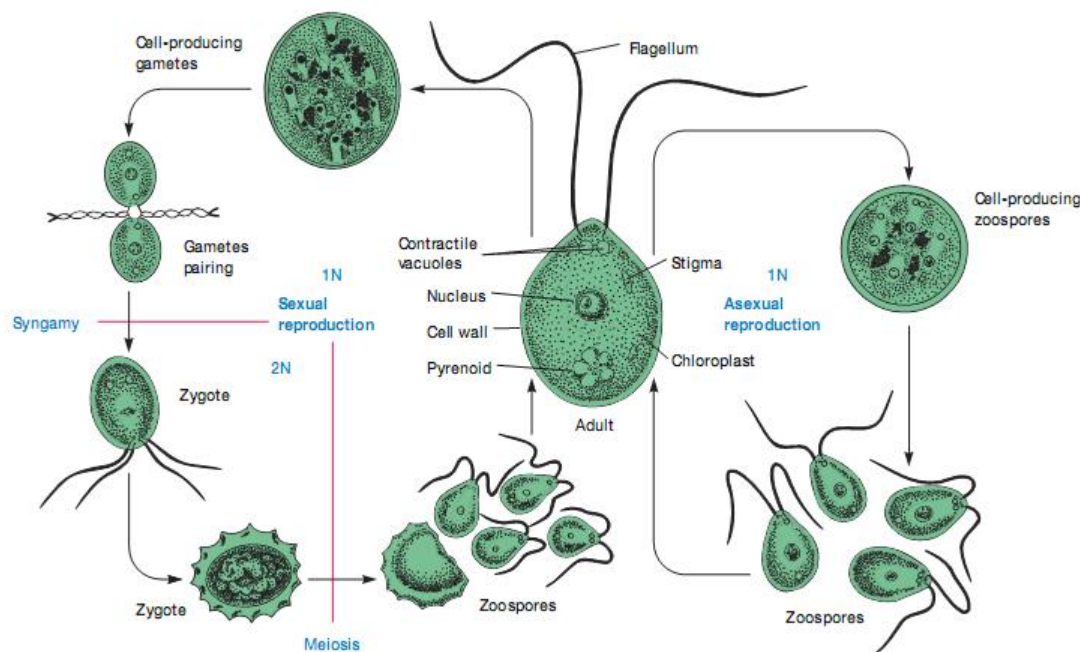
(f)

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

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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 (figure 26.4). 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.



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.

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Charophyta (Stoneworts/Brittleworts)

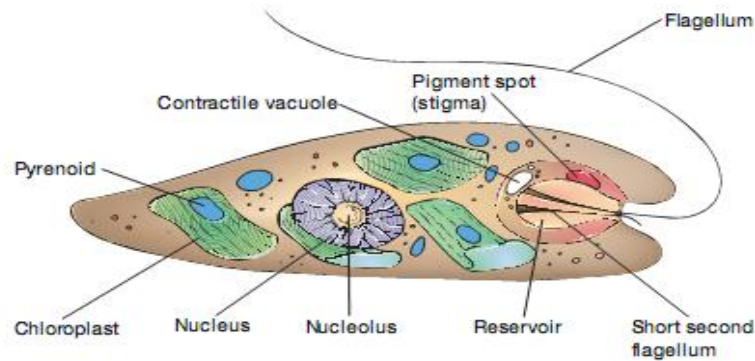
The stoneworts are abundant in fresh to brackish waters and have a worldwide distribution. Often they appear as a dense covering on the bottom of shallow ponds. Some species precipitate calcium and magnesium carbonate from the water to form a limestone covering, thus giving the Charophyta their common names of stoneworts or brittleworts.

Euglenophyta (Euglenoids)

The euglenoids share with the Chlorophyta and Charophyta the presence of chlorophylls a and b in their chloroplasts. The primary storage product is paramylon (a polysaccharide composed of β -1,3 linked glucose molecules), which is unique to euglenoids. They occur in fresh, brackish, and marine waters and on moist soils; they often form water blooms in ponds and cattle water tanks. In molecular classification schemes, euglenoids are associated with the amoeboflagellates (flagellated protozoa) and kinetoplastids because all members have related rRNA sequences and mitochondria with discoid cristae at some stage in their life cycle.

The representative genus is *Euglena*. A typical *Euglena* cell is elongated and bounded by a plasma membrane. Inside the plasma membrane is a structure called the pellicle, which is composed of articulated proteinaceous strips lying side by side. The pellicle is elastic enough to enable turning and flexing of the cell, yet rigid enough to prevent excessive alterations in shape. The several chloroplasts contain chlorophylls a and b together with carotenoids. The large nucleus contains a prominent nucleolus. The stigma is located near an anterior reservoir. A large contractile vacuole near the reservoir continuously collects water from the cell and empties it into the reservoir, thus regulating the osmotic pressure within the organism. Two flagella arise from the base of the reservoir, although only one emerges from the canal and actively beats to move the cell. Reproduction in euglenoids is by longitudinal mitotic cell division.

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Euglena. A Diagram Illustrating the Principal Structures Found in This Euglenoid. Notice that a short second flagellum does not emerge from the anterior invagination. In some euglenoids both flagella are emergent.

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

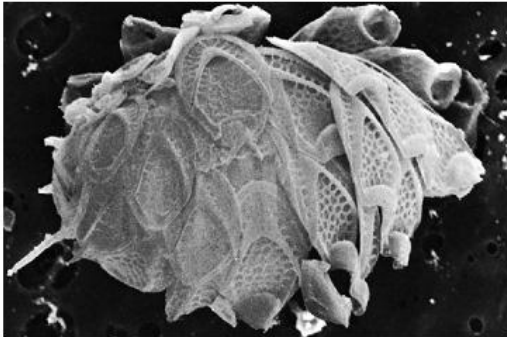
The division Chrysophyta is quite diversified with respect to pigment composition, cell wall, and type of flagellated cells. In molecular classification schemes, these algae are associated with the stramenopiles and have mitochondria with tubular cristae. The division is divided into three major classes: golden-brown algae [Greek *chrysos*, gold], yellow-green algae, and diatoms. The major photosynthetic pigments are usually chlorophylls *a* and *c1/c2*, and the carotenoid fucoxanthin. When fucoxanthin is the dominant pigment, the cells have a golden-brown color. The major carbohydrate reserve in the Chrysophyta is chrysolaminarin (a polysaccharide storage product composed principally of α -1,3 linked glucose residues).

Some Chrysophyta lack cell walls; others have intricately patterned coverings external to the plasma membrane, such as scales (figure 26.6a), walls, and plates. Diatoms have a distinctive two-piece wall of silica, called a frustule. Two anteriorly attached flagella of unequal length are common among Chrysophyta (figure 26.6b), but some species have no flagella, and others have either one flagellum or two that are of equal length. Most Chrysophyta are unicellular or colonial.

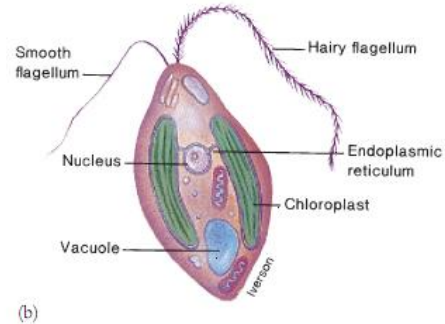
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Reproduction usually is asexual but occasionally sexual. Although some marine forms are known, most of the yellow-green and golden-brown algae live in fresh water. Blooms of some species produce unpleasant odors and tastes in drinking water. The diatoms (figure 26.6c,d; see also figure 4.1b) 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 (Box 26.1). 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 sexually, 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 an auxospore, which increases in size again and forms a new wall. The mature auxospore eventually divides mitotically to produce vegetative cells with normal frustules.

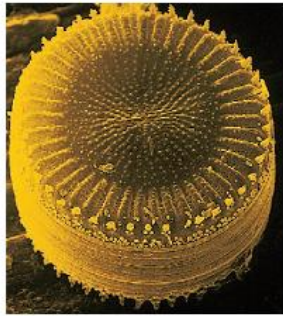
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(a)



(b)



(c)



(d)

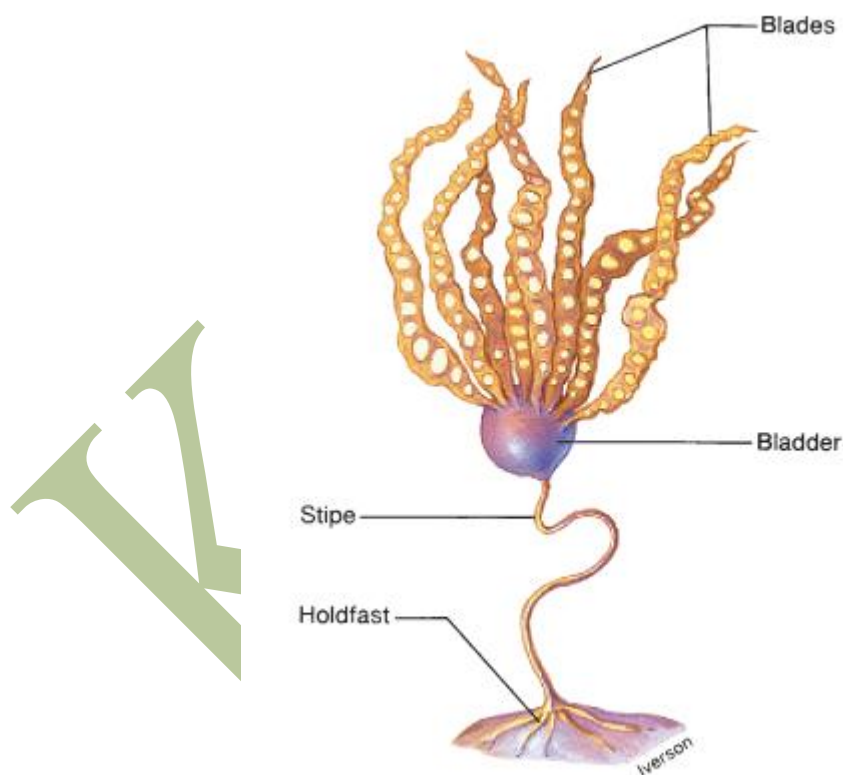
Chrysophyta (Yellow-Green and Golden-Brown Algae; Diatoms). (a) Scanning electron micrograph of Mallomonas, a chrysophyte, showing its silica scales. The scales are embedded in the pectin wall but synthesized within the Golgi apparatus and transported to the cell surface in vesicles (9,000). (b) Ochromonas, 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

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



Phaeophyta (Brown Algae). Diagram of the parts of the brown alga, *Nereocystis*. Due to the holdfast organ, the heaviest tidal action and surf seldom dislodge brown algae from their substratum. The stipe is a stalk that varies in length; the bladder is a gas-filled float.

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Rhodophyta (Red Algae)

The division Rhodophyta, the red algae [Greek rhodon, rose], includes most of the seaweeds (figure 26.8). 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 floridean starch (composed of α -1,4 and α -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 undergoes 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.

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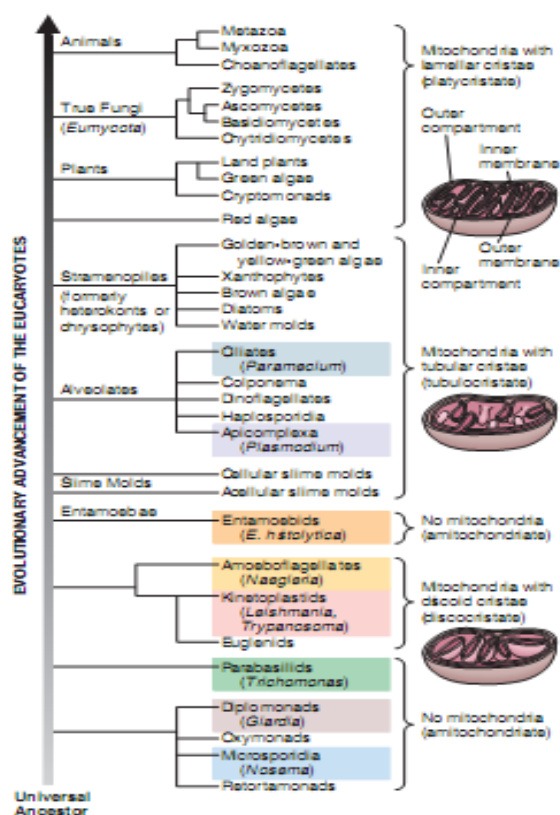


Rhodophyta (Red Algae). These algae (e.g., *Corallina gracilis*) are much smaller and more delicate than the brown algae. Most red algae have a filamentous, branched morphology.

Protozoan

The microorganisms called protozoa [s., protozoan; Greek protos, first, and zoon, animal] are studied in the discipline called protozoology. A protozoan can be defined as a usually motile eucaryotic unicellular protist. Protozoa are directly related only on the basis of a single negative characteristic—they are not multicellular. All, however, demonstrate the basic body plan of a single protistan eucaryotic cell.

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Phylogenetic Diagram: Tentative Phylogeny of the Protozoan-Like Eucaryotes Based on 18S rRNA Sequence Comparisons. Recent molecular phylogeny of the nuclear SSU rRNA indicates that these eucaryotes are highly polyphyletic (protozoan groups are highlighted by different colors). Thus, like the algae, the protozoa do not represent a monophyletic group and the taxon “Protozoa” should not be used in classification schemes that seek to represent true molecular evolutionary histories. The word protozoa can still be used (as it is in this chapter) to denote a nonrelated polyphyletic group of eucaryotic organisms that share some morphological, reproductive, ecological, and biochemical characteristics.

Protozoa play a significant role in the economy of nature. For example, they make up a large part of plankton—small, freefloating organisms that are an important link in the many aquatic food chains and food webs of aquatic environments. A food chain is a series of organisms, each feeding on the

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preceding one. A food web is a complex interlocking series of food chains. Protozoa are also useful in biochemical and molecular biological studies. Many biochemical pathways used by protozoa are present in all eucaryotic cells. Finally, some of the most important diseases of human and animals are caused by protozoa.

Pathogenic Protozoa That Cause Major Diseases of Domestic Animals				
Protozoan Group ^a	Genus	Host	Preferred Site of Infection	Disease
Amoebae	<i>Entamoeba</i>	Mammals	Intestine	Amebiasis
	<i>Iodamoeba</i>	Swine	Intestine	Enteritis
Sporozoa	<i>Babesia</i>	Cattle	Blood cells	Babesiosis
	<i>Theileria</i>	Cattle, sheep, goats	Blood cells	Theileriasis
	<i>Sarcocystis</i>	Mammals, birds	Muscles	Sarcosporidiosis
	<i>Toxoplasma</i>	Cats	Intestine	Toxoplasmosis
	<i>Isospora</i>	Dogs	Intestine	Coccidiosis
	<i>Eimeria</i>	Cattle, cats, chickens, swine	Intestine	Coccidiosis
	<i>Plasmodium</i>	Many animals	Bloodstream, liver	Malaria
	<i>Leucocytozoon</i>	Birds	Spleen, lungs, blood	Leucocytozoonosis
	<i>Cryptosporidium</i>	Mammals	Intestine	Cryptosporidiosis
	<i>Balantidium</i>	Swine	Large intestine	Balantidiasis
Ciliates	<i>Leishmania</i>	Dogs, cats, horses, sheep, cattle	Spleen, bone marrow, mucous membranes	Leishmaniasis
Flagellates	<i>Trypanosoma</i>	Most animals	Blood	Trypanosomiasis
	<i>Trichomonas</i>	Horses, cattle	Genital tract	Trichomoniasis (abortion)
	<i>Histomonas</i>	Birds	Intestine	Blackhead disease
	<i>Giardia</i>	Mammals	Intestine	Giardiasis

Morphology

Because protozoa are eucaryotic cells, in many respects their morphology and physiology are the same as the cells of multicellular animals (see figures 4.2 and 4.3). However, because all of life's various functions must be performed within the individual protozoan, some morphological and physiological features are unique to protozoan cells. In some species the cytoplasm immediately under the plasma membrane is semisolid or gelatinous, giving some rigidity to the cell body. It is termed the ectoplasm. The bases of the flagella or cilia and their associated fibrillar structures are embedded in the ectoplasm. The plasma

membrane and structures immediately beneath it are called the pellicle. Inside the ectoplasm is the area referred to as the endoplasm, which is more fluid and granular in composition and contains most of the organelles. Some protozoa have one nucleus, others have two or more identical nuclei.

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Still other protozoa have two distinct types of nuclei—a macronucleus and one or more micronuclei. The macronucleus, when present, is typically larger and associated with trophic activities and regeneration processes. The micronucleus is diploid and involved in both genetic recombination during reproduction and the regeneration of the macronucleus.

One or more vacuoles are usually present in the cytoplasm of protozoa. These are differentiated into contractile, secretory, and food vacuoles. Contractile vacuoles function as osmoregulatory organelles in those protozoa that live in a hypotonic environment, such as a freshwater lake. Osmotic balance is maintained by continuous water expulsion. Most marine protozoa and parasitic species are isotonic to their environment and lack such vacuoles. Phagocytic vacuoles are conspicuous in holozoic and parasitic species and are the sites of food digestion. Secretory vacuoles usually contain specific enzymes that perform various functions (such as excystation). Most anaerobic protozoa (such as *Trichonympha*, which lives in the gut of termites; see figure 28.26) have no mitochondria, no cytochromes, and an incomplete tricarboxylic acid cycle. However, some do have small, membrane-delimited organelles termed hydrogenosomes. These structures contain a unique electron transfer pathway in which hydrogenase transfers electrons to protons (which act as the terminal electron acceptors), and molecular hydrogen is formed. Other protozoa have mitochondria with discoid cristae (trypanosomes), tubular mitochondrial cristae (ciliates, sporozoa), and lamellar cristae (foraminiferans).

Classification

Many protozoan taxonomists regard the Protozoa as a subkingdom, which contains seven of the 14 phyla found within the kingdom Protista (table 27.2). The phylum Sarcomastigophora consists of flagellates and amoebae with a single type of nucleus. The phyla Labyrinthomorpha, Apicomplexa, Microspora, Ascetospora, and Myxozoa have either saprozoic or parasitic species. The phylum Ciliophora has ciliated protozoa with two types of nuclei. The classification of this subkingdom into

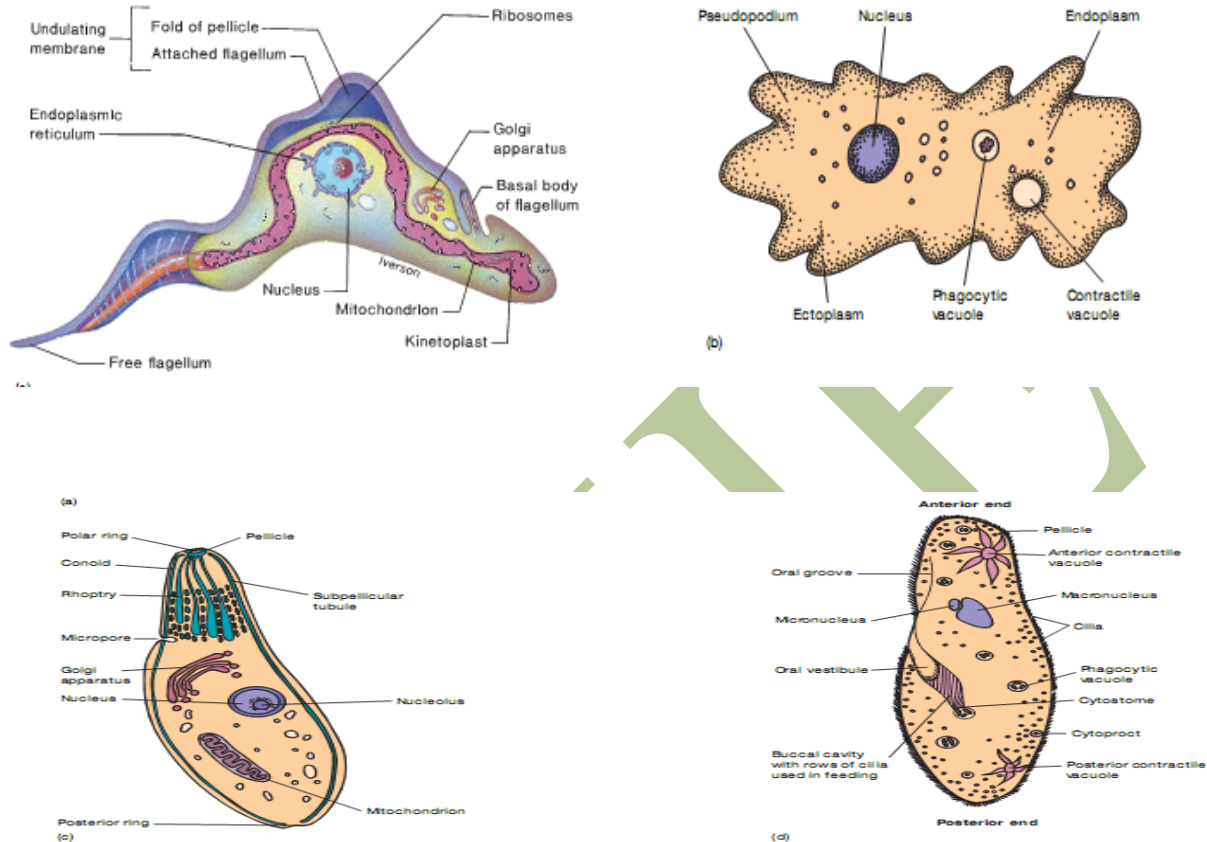
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phyla is based primarily on types of nuclei, mode of reproduction, and mechanism of locomotion. More recent classifications are quite different. In 1993 T. Cavalier-Smith proposed that the protozoa be elevated to kingdom status with 18 phyla based on the structure of mitochondrial cristae and other characteristics (see section 19.7). The acceptance of this new classification by protozoologists, however, remains to be determined. In recent molecular classification schemes, the protozoa do not exist as a discrete taxon. Protozoan-like eucaryotes are found at all evolutionary levels.

Abbreviated Classification of the Subkingdom *Protozoa*^a

Taxonomic Group	Characteristics	Examples
Phylum: <i>Sarcomastigophora</i>	Locomotion by flagella, pseudopodia, or both; when present, sexual reproduction is essentially syngamy (union of gametes external to the parents); single type of nucleus	
Subphylum: <i>Mastigophora</i>	One or more flagella; division by longitudinal binary fission; sexual reproduction in some groups	
Class: <i>Zoomastigophorea</i>	Chromatophores absent; one to many flagella; amoeboid forms, with or without flagella; sexuality known in some groups; mainly parasitic	<i>Trypanosoma</i> <i>Giardia</i> <i>Trichomonas</i> <i>Leishmania</i> <i>Trichonympha</i>
Subphylum: <i>Sarcodina</i>	Locomotion primarily by pseudopodia; shells (tests) often present; flagella restricted to reproductive stages when present; asexual reproduction by fission; mostly free living	
Superclass: <i>Rhizopoda</i>	Locomotion by pseudopodia or by protoplasmic flow with discrete pseudopodia; some contain tests	<i>Amoeba</i> <i>Elphidium</i> <i>Coccolithus</i> <i>Labyrinthula</i>
Phylum: <i>Labyrinthomorpha</i>	Spindle-shaped cells capable of producing mucous tracks; trophic stage as ectoplasmic network; nonamoeboid cells; saprozoic and parasitic on algae and seagrass	
Phylum: <i>Apicomplexa</i>	All members have a spore-forming stage in their life cycle; contain an apical complex; sexuality by syngamy; all species parasitic; cysts often present; cilia absent; often called the Sporozoa	<i>Plasmodium</i> <i>Toxoplasma</i> <i>Eimeria</i> <i>Cryptosporidium</i> <i>Nosema</i> <i>Haplosporidium</i>
Phylum: <i>Microspora</i>	Unicellular spores with spiroplasm containing polar filaments; obligatory intracellular parasites	
Phylum: <i>Ascetospora</i>	Spore with one or more spiroplasm; no polar capsules or polar filaments; all parasitic in invertebrates	
Phylum: <i>Myxozoa</i>	Spores of multicellular origin; one or more polar capsules; all parasitic, especially in fish	<i>Myxosoma</i>
Phylum: <i>Ciliophora</i>	Simple cilia or compound ciliary organelles in at least one stage in the life cycle; two types of nuclei; contractile vacuole present; binary fission transverse; sexuality involving conjugation; most species free living, but many commensal, some parasitic	<i>Didinium</i> <i>Stentor</i> <i>Vorticella</i> <i>Tetrahymena</i> <i>Paramecium</i> <i>Tokophrya</i> <i>Entodinium</i> <i>Nyctotherus</i> <i>Balantidium</i> <i>Ichthyophthirius</i>

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Drawings of Some Representative Protozoa. (a) Structure of the flagellate, *Trypanosoma brucei rhodesiense*. (b) The structure of the amoeboid protist, *Amoeba proteus*. (c) Structure of an *apicomplexan sporozoite*. (d) Structure of the ciliate *Paramecium caudatum*.

Types of Protozoan

Phylum Sarcomastigophora

Protists that have a single type of nucleus and possess flagella (subphylum Mastigophora) or pseudopodia (subphylum Sarcodina) are placed in the phylum Sarcomastigophora. Both sexual and asexual reproduction are seen in this phylum. The subphylum Mastigophora contains both phytoflagellates, chloroplast-bearing flagellates and close relatives, and zooflagellates. Zooflagellates do not have chlorophyll and are either holozoic, saprozoic, or symbiotic. Asexual

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reproduction occurs by longitudinal binary fission along the major body axis. Sexual reproduction is known for a few species, and encystment is common. Zooflagellates are characterized by the presence of one or more flagella. Most members are uninucleate. One major group, the kinetoplastids, has its mitochondrial DNA in a special region called the kinetoplast.

Phylum Labyrinthomorpha

The very small phylum Labyrinthomorpha consists of protists that have spindle-shaped or spherical nonamoeboid vegetative cells. In some genera, amoeboid cells move within a network of mucous tracks using a typical gliding motion. Most members are marine and either saprozoic or parasitic on algae. Several years ago *Labyrinthula* killed most of the “eel grass” on the Atlantic coast, depriving ducks of their food and starving many of them.

Phylum Apicomplexa

The apicomplexans, often collectively called the sporozoans, have a spore-forming stage in their life cycle and lack special locomotory organelles (except in the male gametes, and the zygote or ookinete). They are either intra- or intercellular parasites of animals and are distinguished by a unique arrangement of fibrils, microtubules, vacuoles, and other organelles, collectively called the apical complex, which is located at one end of the cell.

The apical complex contains several components. One or two polar rings are at the apical end. The conoid consists of a cone of spirally arranged fibers lying next to the polar rings. Subpellicular microtubules radiate from the polar rings and probably serve as support elements. Two or more rhoptries extend to the plasma membrane and secrete their contents at the cell surface. These secretions aid in the penetration of the host cell. One or more micropores are thought to function in the intake of nutrients.

Apicomplexans have complex life cycles in which certain stages occur in one host (the mammal) and other stages in a different host (often a mosquito). The life cycle has both asexual and sexual phases and is characterized by an alternation of haploid and diploid generations. At some point an asexual reproduction process called schizogony occurs. Schizogony is a rapid series of mitotic

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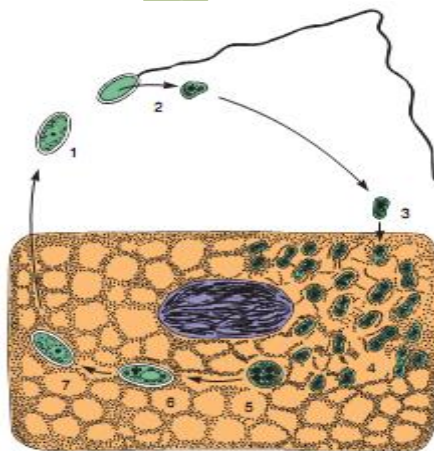
events producing many small infective organisms through the formation of uninuclear buds. Sexual reproduction involves the fertilization of a large female macrogamete by a small, flagellated male gamete. The resulting zygote becomes a thickwalled cyst called an oocyst. Within the oocyst, meiotic divisions produce infective haploid spores.

Phylum Microspora

The small microsporans (3 to 6 μm) are obligatory intracellular parasites lacking mitochondria. The infective stage is transmitted from host to host as a resistant spore. Included in these protozoa are several species of some economic importance because they

parasitize beneficial insects. *Nosema bombycis* parasitizes silk worms causing the disease pebrine, and *Nosema apis* causes serious dysentery (foul brood) in honeybees. There has been an increased interest in these parasites because of their possible role as biological control agents for certain insects. For example, *Nosema locustae* has been approved and registered by the United States Environmental Protection Agency for use in

long-lasting control of rangeland grasshoppers. Recently seven microsporidian genera (*Nosema*, *Encephalitozoon*, *Pleistophora*, *Microsporidium*, *Vittaforma*, *Trachipleistophora*, and *Enterocytozoon*) have been implicated in human diseases in immunosuppressed and AIDS patients.



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The Microsporean *Nosema bombycis*, Which is Fatal to Silkworms. (1) A typical spore with one coiled filament. (2) When ingested, it extrudes the filament. (3) The parasite enters an epithelial cell in the intestine of the silkworm and (4) divides many times to form small amoebae that eventually fill the cell and kill it. During this phase, some of the amoebae with four nuclei become spores (5, 6, 7). Silkworms are infected by eating leaves contaminated by the feces of infected worms.

Phylum Ascomycota

Ascomycota is a relatively small phylum that consists exclusively of parasitic protists characterized by spores lacking polar caps or polar filaments. Ascomycotans such as *Haplosporidium* are parasitic primarily in the cells, tissues, and body cavities of mollusks.

Phylum Myxozoa

The myxozoans are all parasitic, most on freshwater and marine fish. They have a resistant spore with one to six coiled polar filaments. The most economically important myxozoan is *Myxosoma cerebralis*, which infects the nervous system and auditory organ of trout and salmon (salmonids). Infected fish lose their sense of balance and tumble erratically—thus the name whirling or tumbling disease. Proliferative kidney disease, caused by an unclassified myxozoan, has become one of the most important diseases of cultured salmon throughout the world.

Phylum Ciliophora

The phylum Ciliophora is the largest of the seven protozoan phyla. There are about 8,000 species of these unicellular, heterotrophic protists that range from about 10 to 3,000 μm long. As their name implies, ciliates employ many cilia as locomotory organelles. The cilia are generally arranged either in longitudinal rows (figure 27.3d; see also figure 4.24) or in spirals around the body of the organism. They beat with an oblique stroke; therefore the protist revolves as it swims. Coordination of ciliary beating is so precise that the protist can go either forward or backward.

There is great variation in ciliate shape, and most do not look like the slipper-shaped *Paramecium* (see figures 2.8e and 4.1a). In some species (*Vorticella*) the protozoan attaches itself to the substrate

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by a long stalk. Stentor attaches to a substrate and stretches out in a trumpet shape to feed (see figure 4.1e). A few species have tentacles for the capture of prey. Some can discharge toxic threadlike darts called toxicysts, which are used in capturing prey.

A most striking feature of ciliates is their ability to capture many particles in a short time by the action of the cilia around the buccal cavity. Food first enters the cytostome and passes into phagocytic vacuoles that fuse with lysosomes after detachment from the cytostome. A vacuole's contents are digested when the vacuole is acidified and lysosomes release digestive enzymes into it. After the digested material has been absorbed into the cytoplasm, the vacuole fuses with a special region of the pellicle called the cytoproct and empties its waste material to the outside.

Contractile vacuoles are used for osmoregulation and are present chiefly in freshwater species. Most ciliates have two types of nuclei: a large macronucleus and a smaller micronucleus. The micronucleus is diploid and contains the normal somatic chromosomes. It divides by mitosis and transmits genetic information through meiosis and sexual reproduction. Macronuclei are derived from micronuclei by a complex series of steps. Within the macronucleus are many chromatin bodies, each containing many copies of only one or two genes. Macronuclei are thus polyploid and divide by elongating and then by constricting. They produce mRNA to direct protein synthesis, maintain routine cellular functions, and control normal cell metabolism.

Viruses

Viruses are defined as 'sub-microscopic, self producing particles capable of being introduced into living cells and reproducing inside such cells only'

Virus means poison in Latin. Viruses are certainly not cells (acellular microorganisms). But they do possess some properties of cells. They are intermediate between living and non-living things, they are neither prokaryotes nor eukaryotes. They are the simplest form of life. They are parasitic. They live inside the cells and they are active, feed, respire, reproduce, grow and move.

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Discovery

The existence of virus was first proved by Ivanowski in 1892. The first virus was discovered by Ivanowski in 1899.

Characteristics:

Viruses are extremely smaller in size. They are smaller than bacteria. They are invisible under the light microscope. They are only slightly larger than a large protein and a nucleic acid. They range in size from 100 Å to 2,500 Å.

They are potentially infectious.

They have a single nucleic acid either DNA or RNA (except RNA-DNA viruses).

They can be crystallized.

They do not contain information for the production of enzymes in energy cycle.

They do not contain information for the synthesis of ribosomal protein, rRNA and tRNA.

Structure of viruses

Viruses are omnipresent. The viruses living on animals are called animal viruses. The viruses that live on plants are called plant viruses. The viruses that live on bacteria are called bacterial viruses or bacteriophages.

They are ultramicroscopic. They can be seen only by an electron microscope. Viruses are smaller than bacteria. The smallest virus is 10 nm in diameter. (Eg, *parvo virus*) the largest virus is about 250 nm.

Most of the viruses fall into two categories, namely polyhedral or helical.

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The virus consist of two major components , namely a protein coat called capsid and a core made up of nucleic acid. The nucleic acid with the capsid is called a nucleocapsid.

The nucleic acid of virus may be single-stranded or double stranded. Thus there are four types of viruses. They are,

<i>Viruses are single stranded DNA(Ssdna)</i>	<i>Eg-Coliphage</i>
<i>Viruses with double strandedDNA(DsDNA)</i>	<i>Eg-Vaccina</i>
<i>Viruses with single stranded RNA(SsRNA)</i>	<i>Eg-TMV</i>
<i>Viruses with double stranded RNA(DsRNA)</i>	<i>Eg-Reovirus.</i>

The nucleic acid is surrounded by a protein coat called Capsid. The capsid is made up of many smaller units called capsomers. The capsid provides three main function . It gives shape to the virus and protects the nucleic acid. It helps in the attachment of the virus to the host cell (antigen specificity)

The capsomers are made up of polypeptide chains. The capsid provides shape to the virus. There are two main shapes (symmetry). They are icosahedral (cubical) corners or vertices and 20 facets or sides. The shape of each facet is like an equilateral triangle.

The viruses exist in three main shapes. They are spherical or rod shaped or tadpole shaped. Adenovirus and HIV are spherical in shape. TMV is rod shaped. T4 bacteriophage is tadpole shaped.

The viruses do not cause damage to the host cell are called avirulent viruses. They do not cause lysis of the host cell. They exhibit lysogenic life cycle.

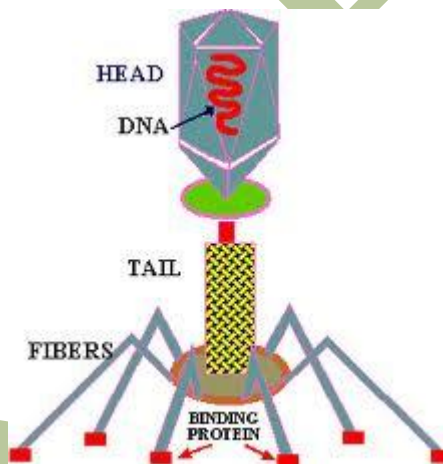
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Structure of virus**Bacteriophage**

They are bacterial virus and it living inside of the bacterial cells. Bacteriophage means bacteria eating agent .It was first described by Twort in 1915.

The common bacteriophage is T4 bacteriophage.It is parasitic on human colon bacteria, *Escherichia coli*. It is also known as coliphage.

The head is polyhedral.It is covered by a protein coat called capsid.The capsid is made of about 2,000 protein subunits called capsomeres.

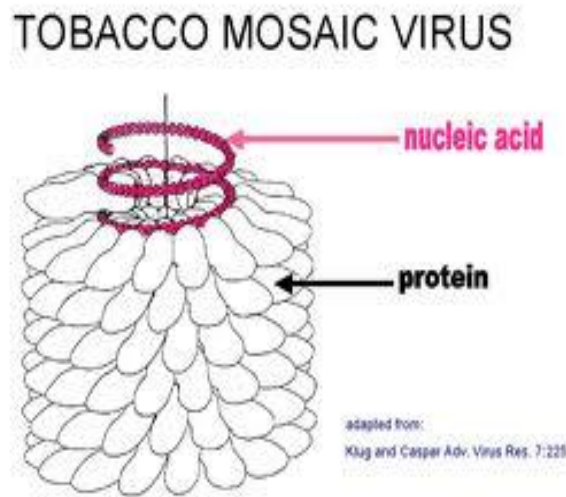
**T4 bacteriophage**

The neck is very short and its contain collar.It connects the head with the tail. The tail consists of a central hollow core tube. Through this core tube the DNA of the phage can pass into the bacterial cell.

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The bacteriophage has a complex symmetry .It exhibits a combination of icosahedral and helical symmetry. The head is icosahedral in symmetry and the tail is helical in symmetry.

The bacteriophage has 244 capsomers .of these, 2000 capsomers are present in the head and 144 capsomers are in the tail. The 2000 capsomers of the head are arranged in the form of an icosahedron having 20 triangular sides called facets and 12 corners called vertices. There are two types of capsomers, namely pentons and hexons. Pentons are in 12 number and are located on the head. The hexons are located on the facets. The 144 capsid of the tail are helically arranged from the core tube.



Shape of the viruses

Viruses vary widely in their shape. They may be spherical, Polyhedral, helical, cylindrical or rod-like.

Viruses occur in three main shapes, namely

A, Polyhedral

B, Helical

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C,Complex

A, Polyhedral

In polyhedral viruse,the capsids are many sided. They are of three types, namely tetrahedral, octahedral and isohedral.

In tetrahedral viruses, the capsid has 4 sides. In octahedral viruses,the capsid has 8 sides.In icosahedral viruses,the capsid has 8 sides.IN icosahedral viruses,the capsid has 20 triangular facets and 12 corners.Most of the viruses are icohederal.

The number of capsomers will be either 12, 32, 42, 72, 92, 162, 252, 362, 492, 642 or 812.

B, Helical

The capsid and the nucleic acid are helically coiled. The monomers curve into helix.Eg-Tobacco mosaic virus,Mumps virus etc.

The tobacco mosaic virus is rod-shaped.

The capsid is made up of 2,130 monomers.The monomers are arranged in 130 turns.

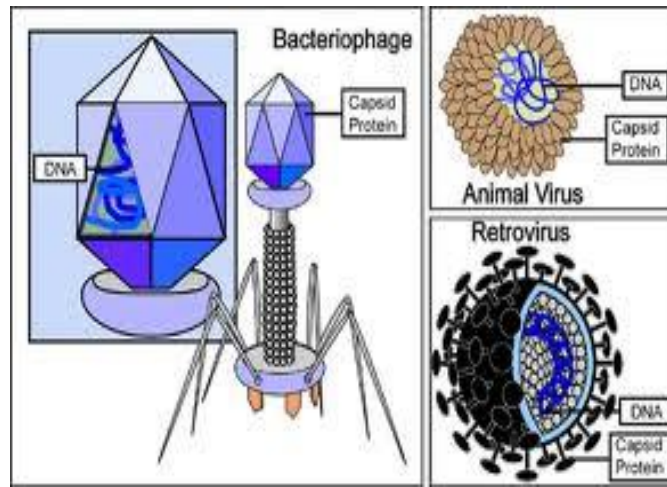
C, Complex

The capsid may be absent or it may be attached with additional structures

.Eg- Vaccina virus.

The T4 bacteriophage shows a combination of icohedral symmetry and helical symmetry.

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Classification

Viruses are grouped under Acaryota, Holmes(1948) included virus under the order Virales.

Viruses are mainly divided in to three groups.They are the following;

Animal viruses- Zoophaginae

Plant viruses- Phytophaginae

Bacterial viruses- Phaginae (Bacteriophage)

Animal viruses

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Viruses that infect animals are called animal viruses. They are included in the group Zoophaginae. Animal viruses contain DNA. Some animal viruses contain RNA. The nucleic acid may be single stranded or double stranded.

The animal viruses cause dangerous diseases in human beings and domestic animals. The following are the common animal viruses :

<i>Poliovirus</i>	<i>yellow fever virus</i>
<i>Vaccinia virus</i>	<i>rabies virus</i>
<i>Adeno virus</i>	<i>mumps virus</i>
<i>Herpes virus</i>	<i>Measles virus</i>
<i>Reovirus</i>	<i>Influenza virus</i>
<i>Dengue virus</i>	<i>HIV</i>

Plant viruses

Viruses that infect plant cells are called plant viruses. About 170 plant viruses have been identified. Most of plant viruses contain RNA as the genetic material. But only one group of plant virus contains DNA. The RNA may be single stranded or double stranded.

The plant viruses have been divided into nineteen groups. The following are the important plant viruses:

mosaic virus (TMV)

Cucurbit virus

Tobacco

Tomato mosaic virus

Tobacco necrosis virus

Potato virus

Alfalfa mosaic virus

Papaya mosaic virus

cauliflower mosaic virus

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Bacterial viruses

Viruses that infect bacterial cells are called bacterial viruses or bacteriophages. Phage means to eat. Bacteriophage means Viruses eating bacteria.

Bacteriophage was first described by Twort in 1915.

The genetic material is either DNA or RNA. They may be single stranded or double stranded.

The common bacteriophage is T4 bacteriophage. It infects the human colon bacterium, Escherichia coli. 'T' stands for the type. Bacteriophages are numbered from 1 to 7.

Multiplication of Bacteriophages

Bacteriophages are capable of multiplication only within the bacteria. They remain inactive outside the bacterium. They are host specific. For example, T4 bacteriophage infects and multiplies only inside the bacterium E. Coli. The multiplication of phages take place in the following steps;

1. Attachment of phages on Bacterium

When the phage and bacterium come close together by random collision, the phage attaches itself to the surface of the bacterium. The tail fibres select specific sites on the bacterial cellwall and attach with it. Then the tail fibres bend to keep the end plaque in contact with the bacterial cellwall.

2. Injection of phage DNA into the Bacterium

The tail secrete the enzyme lysozyme which dissolves the bacterial cellwall to make a hole. The contractile sheath contracts and this force the tail core into the bacterial cellwall through the hole. The phage DNA is then injected into the bacterium through the tail core. The protein coat does not enter in; it remains outside the bacterium.

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1. Disruption of Bacterial Metabolism

The phage DNA disrupts the bacterial metabolism and synthesis phage DNAs and proteins. The synthesis of normal bacterial proteins stops. Mean time, the phage DNA synthesis phage protein using the host protein synthetic machinery. The viral DNA produces mRNA which later forms phage proteins. The bacterial DNA is degraded into free nucleotides by the action of viral deoxyribonuclease. Therefore the synthesis of bacterial DNA stops. At the same time, the phage DNA replicates by using the free nucleotides and produce many copies of phage DNA. These biochemical reactions is infected bacterium last for 4-10 minutes after the entry of phage DNA.

5. Assembly of phage particles

The phage proteins from sub-units of capsids, tail sheath, tail fibres and end plate. The subunits from head. Each phage DNA copy then enter the head and beomes tightly packed. The tail sheath and end plate join together to form a tail. This tail then attached to the head. Thereafter, the tail fibres join with the end plate to form a new phage particle. Thus about 150-300 phages are produced in a bacterium. The duration between injection of viral DNA and the formation of the first phage is called eclipse period. It takes place between 10 and 20 minutes after infection.

6. Lysis of Bacterium

As many phages have been produced in a bacterium, the bacterial cellwall rupture suddenly. The new phage particles are released free. It takes place in 20 minutes after infection.

Life cycle of phages

Bacteriophages exhibit two types of life cycles.

They are

- Lytic cycle(virulent cycle)
- Lysogenic cycle(temperature cycle)

Lytic cycle

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All the T-series phages exhibit lytic cycle. These viruses infect bacteria and on the completion of life cycle, they cause lysis or rupture of the bacterium. Hence the cycle is called lytic cycle. It involves,

Infection takes place by random collision between the phage and the bacterium.

The tail fibres select specific site on the surface of the bacterium.

The spikes anchor firmly

A hole is made in the cell wall of bacterium by the enzyme lysozyme secreted by the tail.

The sheath contracts and this causes the tail core to penetrate the cell wall

The DNA of the head is discharged into the cell through the tail core.

The protein coat does not enter in it remains outside the cell

The host DNA is degraded by the viral deoxyribonuclease

Viral DNA is transcribed into mRNA

The mRNA translates protein which are utilized for the synthesis of viral protein

Viral DNA also replicates to produce hundreds of copies.

Each DNA copy is enclosed by a protein coat producing new virus

Soon the bacterial wall ruptures and the new virus particle is released

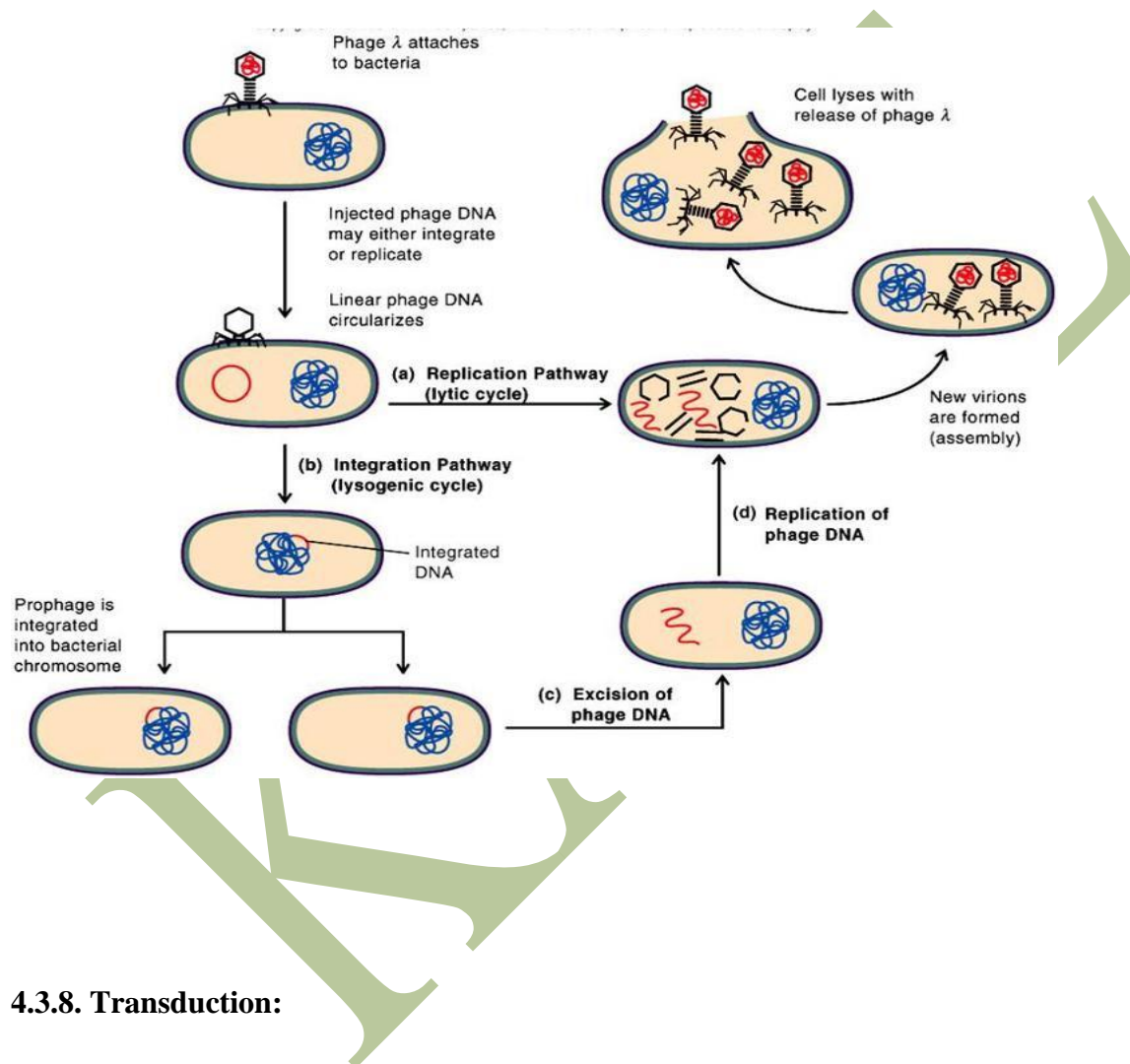
A single phage can produce about 200 phages twenty minutes after infection.

Lysogenic cycle

All the lambda series bacteriophages exhibit lysogenic cycle. In lysogenic cycle the bacterium is not lysed. As in the lytic cycle the viral DNA enters the bacterial cell. But the viral DNA does not take

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over the protein synthesizing machinery of the host. Instead it gets integrated with the bacterial chromosome. At this stage, the virus is called prophage bacterium with the prophage to other phages. The viral DNA replicates along with the bacterial chromosome.. Rarely, the prophage DNA can disintegrate and enter into lytic cycle.

**4.3.8. Transduction:**

Transfer of genetic material from one bacterium to another through bacteriophages is called transduction. As in the lysogenic cycle, the viral DNA gets integrated with the bacterial chromosomes to become prophage. Then the prophage DNA integrates. During this process, the prophage DNA gets a fragment of bacterial DNA. These are released by the rupture of bacterial

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cells. These phages contain a portion of bacterial DNA are called Transducing phages. These phages now infect new hosts and the DNA integrated with the bacterial chromosomes. When the viral DNA, deintegrates from the bacterial DNA it leaves the bacterial DNA drawn from the first bacterium. Thus the bacterium gets a fragment of DNA from the previous bacterium through bacteriophages.

Replication of Viruses :**Adsorption**

The first step in infection of a cell is attachment to the cell surface. Attachment is via ionic interactions which are temperature-independent. The viral attachment protein recognizes specific receptors, which may be protein, carbohydrate or lipid, on the outside of the cell. Cells without the appropriate receptors are not susceptible to the virus.

Penetration

The virus enters the cell in a variety of ways according to the nature of the virus.

Enveloped viruses

(A) **Entry by fusing with the plasma membrane.** Some enveloped viruses fuse directly with the plasma membrane. Thus, the internal components of the virion are immediately delivered to the cytoplasm of the cell .

(B) **Entry via endosomes at the cell surface** Some enveloped viruses require an acid pH for fusion to occur and are unable to fuse directly with the plasma membrane. These viruses are taken up by invagination of the membrane into endosomes. As the endosomes become acidified, the latent fusion activity of the virus proteins becomes activated by the fall in pH and the virion membrane fuses with the endosome membrane. This results in delivery of the internal components of the virus to the cytoplasm of the cell

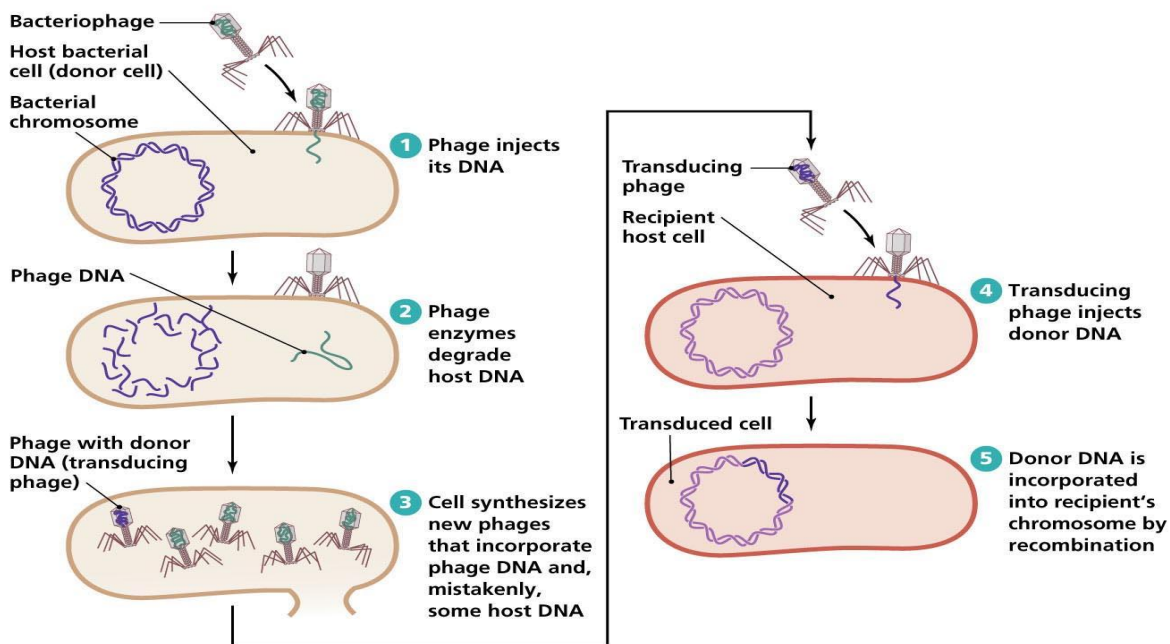
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Non-enveloped viruses

Non-enveloped viruses may cross the plasma membrane directly or may be taken up into endosomes. They then cross (or destroy) the endosomal membrane.

Uncoating

Nucleic acid has to be sufficiently uncoated that virus replication can begin at this stage. When the nucleic acid is uncoated, infectious virus particles cannot be recovered from the cell - this is the start of the ECLIPSE phase - which lasts until new infectious virions are made.

Synthesis of viral nucleic acid and protein.

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Assembly/maturation

New virus particles are assembled. There may be a maturation step that follows the initial assembly process.

Release

Virus may be released due to cell lysis, or, if enveloped, may bud from the cell. Budding viruses do not necessarily kill the cell. Thus, some budding viruses may be able to set up persistent infections. Not all released viral particles are infectious. The ratio of non-infectious to infectious particles varies with the virus and the growth conditions.

Structural**Versus****Non-Structural****Proteins**

All proteins in a mature virus particle are said to be structural proteins - even if they make no contribution to the morphology or rigidity of the virion - non-structural proteins are those viral proteins found in the cell but not packaged into the virion.

effect (CPE)**4.3,10.1 Cytopathic**

The presence of the virus often gives rise to morphological changes in the host cell. Any detectable changes in the host cell due to infection are known as a cytopathic effect

Cytopathic effects (CPE) may consist of cell rounding, disorientation, swelling or shrinking, death, detachment from the surface, etc.

Many viruses induce apoptosis (programmed cell death) in infected cells. This can be an important part of the host cell defense against a virus - cell death before the completion of the viral replication cycle may limit the number of progeny and the spread of infection. (Some viruses delay or prevent apoptosis - thus giving themselves a chance to replicate more virions.)

Some viruses affect the regulation of expression of the host cell genes which this can have important results both for the virus's ability to grow, and in terms of the effect on the host cell.

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The cytopathic effects produced by different viruses depend on the virus and the cells on which it is grown. This can be used in the clinical virology laboratory to aid in identification of a virus isolate.

Assays for plaque-forming units

The CPE effect can be used to quantitate infectious virus particles by the plaque-forming unit assay

Cells are grown on a flat surface until they form a monolayer of cells covering a plastic bottle or dish. They are then infected with the virus. The liquid growth medium is replaced with a semi-solid one so that any virus particles produced as the result of an infection cannot move far from the site of their production. A plaque is produced when a virus particle infects a cell, replicates, and then kills that cell. Surrounding cells are infected by the newly replicated virus and they too are killed. This process may repeat several times. The cells are then stained with a dye which stains only living cells. The dead cells in the plaque do not stain and appear as unstained areas on a colored background. Each plaque is the result of infection of one cell by one virus followed by replication and spreading of that virus. However, viruses that do not kill cells may not produce plaques.

Possible Questions**2 marks**

1. Draw the diagram of a prokaryotic cell.
2. Write short notes on Prokaryotes.
3. Write notes on Protozoa.
4. What are bacteriophages?
5. Write about the classification of fungi.
6. Name the cell organelles of animal cell.
7. Differentiate lytic cycle and lysogenic cycle.

6 marks

1. Illustrate about the unique features of viruses.
2. Explain in detail about morphology of fungi with diagram.
3. Explain in detail about the structure of bacteria.

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4. Explain in detail about the structure and reproduction of algae.
5. Give a detailed account on fungi classification.
6. Explain about lytic and lysogenic cycle of bacteriophage.
7. Write in detail about protozoa.
8. List out the differences between prokaryotes and eukaryotes.
9. Diagrammatically explain the structure of eukaryotic cell.
10. Comment on the structure of bacteriophage.

KAHE

Unit III– Cultivation and Maintenance of microorganisms

UNIT III

Nutritional categories of micro-organisms, Media, Types of media, Methods of isolation, Stating and types, Purification and preservation.

3.1 The Common Nutritional Requirements

Analysis of microbial cell composition shows that over 95% of cell dry weight is made up of a few major elements: carbon, oxygen hydrogen, nitrogen, sulfur, phosphorous, potassium, Calcium, magnesium and iron. These are called macro elements or macronutrients because they are required by microorganism in relatively large amounts. The first six are components of carbohydrates, proteins ,lipids and nucleic acids. The remaining four macronutrients exist in the cell as cat ions and play a variety of roles. For example potassium (K⁺) is required for activity for a number of enzymes including some of those involved in protein synthesis. Calcium, among other functions, contributes to the heat réistance bacterial endospores. Magnesium serves as cofactor for many enzymes, complexes with ATP, and stabilizes ribosomes and cell membranes. Iron is a part of cytochromes and cofactor for enzymes and electron carrying proteins.

All organisms , including microorganisms, require several micronutrients or trace elements besides macro elements. The micronutrients- manganese, cobalt, zinc, molybdenum, nickel, and copper- are needed by most cells. However, cells require regular media components are often adequate for growth. Therefore it is very difficult to demonstrate a micronutrient requirement. In nature ,micronutrients are ubiquitous and probably do not usually limit growth. Micronutrients are normally a part of enzymes and cofactors , and they aid in the catalysis of reactions and maintenance of protein structure. For example zinc is present at the active sites of some enzymes but is also involved in the regulatory and catalytic subunits of E.coli aspartate carbamoyltransferase.

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Besides the common macroelements and trace elements, microorganisms may have particular requirements that affect the nature of their morphology or environment. Diatoms require silicic acid to construct their beautiful cell walls of silica. Although most bacteria do not require large amounts of sodium, many bacteria growing in saline lakes and oceans depend on the presence of high concentrations of sodium ions.

Finally it must be emphasized that microorganisms require a balanced mixture of nutrients. If an essential nutrient is in short supply, microbial growth will be limited regardless of concentration of other nutrients.

3.2 Growth Factors

Microorganisms often grow and reproduce when minerals and sources of energy, carbon nitrogen, phosphorous and sulfur are supplied. These organisms have the enzymes and pathways necessary to synthesize all cell components required for their well being. Many microorganisms on the other hand, lack one or more essential enzymes. Therefore they cannot manufacture all indispensable constituents but must obtain them or their precursors from the environment. Organic components required because they are essential cell components or precursors of such components and cannot be synthesized by the organism are called growth factors. There are three major classes of growth factors: (1) amino acids (2) purines and pyrimidines, and (3) vitamins.

Amino acids are required for protein synthesis, purines and pyrimidines for nucleic acid synthesis. Vitamins are small organic molecules that usually make up all or part of enzyme cofactors and only very small amounts sustain growth. Some microorganisms require many vitamins for growth. Other growth factors are also seen heme (from hemoglobin) is required by *Haemophilus influenzae*, and some mycoplasmas need cholesterol.

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Knowledge of the specific growth factor requirements of many microorganisms makes possible quantitative growth-response assays for a variety of substances. For example species from the bacterial genera *Lactobacillus* and *Streptococcus* can be used in microbiological assays of mostly vitamins and amino acids. The appropriate bacterium is grown in a series of culture vessels, each containing medium with an excess amount of all required components except the growth factors to be assayed. A different amount of growth factor is added to each vessel. The standard curve is prepared by plotting the growth factor quantity or concentration against the total extent of bacterial growth. Ideally the amount of growth resulting is directly proportional to the quantity of growth factor present : if the growth factor concentration doubles the final extent of bacterial growth doubles.

3.3 Impact of environmental factors on growth

3.3.1 Nutrients

Nutrients such as carbohydrates, fats, proteins, vitamins, minerals and water, required by, man are also needed by microorganisms to grow. Microbes differ in their abilities to use substrates as nutrient sources. Their enzyme systems are made available according to their genetic code. They vary in ability to use nitrogen sources to produce amino acids and, therefore, proteins. Some require amino acids to be supplied by the substrate. When organisms need special materials provided by their environment, we refer to them as fastidious. Difference in the utilization of nutrients and the waste products they produce are important in differentiating between organisms.

3.3.2. Oxygen

Microbes also differ in their needs for free oxygen. Aerobic organisms must grow in the presence of free oxygen and anaerobic organisms must grow in the absence of free

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oxygen. Facultative organisms can grow with or without oxygen, while microaerophilic organisms grow in the presence of small quantities of oxygen.

3.3.3. Water

Water is necessary for microbes to grow, but microbes cannot grow in pure water. Some water is not available. A measurement of the availability of water is a_w or water activity. The a_w of pure water is 1.0 while that of a saturated salt solution is 0.75. Most spoilage bacteria require a minimum a_w of 0.90. Some bacteria can tolerate an a_w above 0.75 as can some yeasts and most molds. Most yeasts require 0.87 water activity. An a_w of 0.85 or less suppresses the growth of organisms of public health significance.

3.3.4. Temperature

Microorganisms can grow in a wide range of temperatures. Since they depend on water as a solvent for nutrients, frozen water or boiling water inhibits their growth. General terms are applied to organisms based on their growth at different temperatures. Most organisms grow best at or near room and body temperature. These are mesophiles. Those growing above 40°C (105°F) are called thermophiles while those growing below 25°C (75°F) are called psychrotrophs.

3.3.5. Acidity

The nature of a solution based on its acidity or alkalinity is described as pH. The pH scale ranges from 0, strongly acidic, to 14, strongly basic. Neutral solutions are pH 7, the pH of pure water. Most bacteria require near neutral conditions for optimal growth with minimums and maximums between 4 and 9. Many organisms change the pH of their

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substrate by producing by-products during growth. They can change conditions such that the environment can no longer support their growth. Yeasts and molds are more tolerant of lower pH than the bacteria and may outgrow them under those conditions.

3.3.6. Light & Chemicals

Ultraviolet light and the presence of chemical inhibitors may also affect the growth of organisms. Many treatments such as hydrogen peroxide and chlorine can kill or injure microbes. Under certain conditions those given a sublethal treatment are injured, but can recover.

3.3.7 Growth

Characteristic growth patterns can be illustrated on a graph. There is a selected portion of the normal growth curve which is referred to as the logarithmic growth phase or the log phase. When cells begin to grow, we usually observe a period of no apparent growth which we refer to as the lag phase. This occurs because cells are making necessary adjustments to adapt. Next we experience the rapid growth or the log phase previously described. As cell mass becomes large, nutrients are exhausted and metabolic byproducts collect. Growth tapers off and the population remains constant for a time. This is referred to as the stationary phase of growth. With no intervention in the system the population will enter a death phase and total numbers of organisms will decline.

3.3.7.1 Microbial Growth curve**Principle**

Bacterial population growth studies require inoculation of viable cells into a sterile broth medium and incubation of the culture under optimum temperature, pH, and gases conditions. Under these conditions, the cells will reproduce rapidly and the dynamics of the microbial growth can be charted by means of a population growth curve, which is constructed by plotting the increase in cell number versus time of incubation. The curve can be used to delineate stages of the growth cycle. It also facilitates measurement of cell numbers and the rate of growth of a particular organism under standardized conditions as expressed by its generation time, the time required for a microbial population to double.

1. **Lag phase :** During this stage the cells are adjusting to their new environment. Cellular metabolism is accelerated, resulting in rapid biosynthesis of cellular macromolecules, primarily enzymes, in preparation for the next phase of the cycle. Although the cells are increasing in size, there is no cell division and therefore no increase in number.
2. **Logarithmic (log) phase :** Under optimum nutrition and physical conditions the physiologically robust cells reproduce at a uniform and rapid rate by binary fission. Thus there is a rapid exponential increase in population, which doubles regularly until a maximum number of cells is reached. The time required for the

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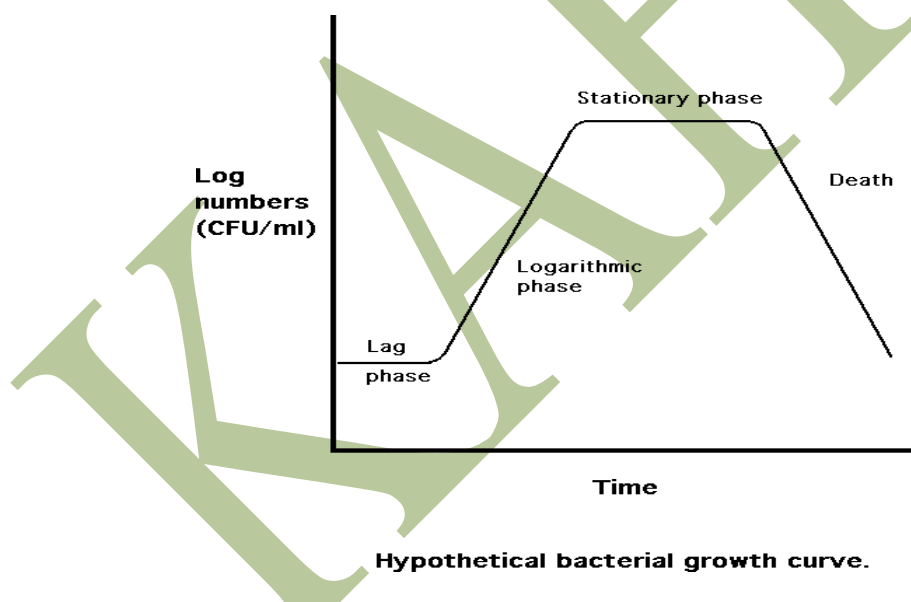
population to doubles is the generation time. The length of the log phase varies, depending on the organism composition of the medium . the average may be estimated to last 6 to 12 hours .

3. **Decline, or death /phase :** because of the continuing depletion of nutrients and buildup of metabolic wastes, the micro organism die at a rapid and uniform rate . the decrease in population closely parallels its increase during the log phase . theoretically, the entire population should die during a time interval equal should die during a time interval equal to that of the log phase. This does not occur, however, since a small number of highly resistant organism persist for an indeterminate length of time.

Construction of a complete Bacteria growth curve require that aliquots of a 24-4 hours shake –flask culture the inoculation period .such a procedure does not lend itself to a regular laboratory session. Therefore this experiment follows a modified procedure designed to demonstrate only the lag and log phase. The curve will be plotted on semilog paper by using two values for the measurement of growth. the direct method requires enumeration of viable cells in serially diluted sample of the test culture taken at 30-minute intervals as described in Experiment 19. the indirect method uses spectrophotometric measurement developing turbidity at the same 30- minute intervals, as an index of increasing cells mass.

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Determination of generation time with indirect and direct methods by using data on the growth curve indirect determination is made by simple extrapolation from the log phase..Select two points On the optical density scale , such as 0.2 and 0.4 that represent doubling of turbidity. Using a ruler ; extrapolate by drawing line between each of the selected optical densities on the ordinate (x axis) and the plotted line of the growth curve to their respective time intervals on the abscissa (y axis). With this information, determine the generation time as follows.

**Principle:**

The increase in the cell size and cell mass during the development of an organism is termed as growth. It is the unique characteristics of all organisms. The organism must

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require certain basic parameters for their energy generation and cellular biosynthesis. The growth of the organism is affected by both physical and Nutritional factors. The physical factors include the pH, temperature, Osmotic pressure, Hydrostatic pressure, and Moisture content of the medium in which the organism is growing. The nutritional factors include the amount of Carbon, nitrogen, Sulphur, phosphorous, and other trace elements provided in the growth medium. Bacteria are unicellular (single cell) organisms. When the bacteria reach a certain size, they divide by binary fission, in which the one cell divides into two, two into four and continue the process in a geometric fashion. The bacterium is then known to be in an actively growing phase. To study the bacterial growth population, the viable cells of the bacterium should be inoculated on to the sterile broth and incubated under optimal growth conditions. The bacterium starts utilising the components of the media and it will increase in its size and cellular mass. The dynamics of the bacterial growth can be studied by plotting the cell growth (absorbance) versus the incubation time or log of cell number versus time. The curve thus obtained is a sigmoid curve and is known as a standard growth curve. The increase in the cell mass of the organism is measured by using the Spectrophotometer. The Spectrophotometer measures the turbidity or Optical density which is the measure of the amount of light absorbed by a bacterial suspension. The degree of turbidity in the broth culture is directly related to the number of microorganism present, either viable or dead cells, and is a convenient and rapid method of measuring cell growth rate of an organism. Thus the increasing the turbidity of the broth medium indicates increase of the microbial cell mass (Fig 1) .The

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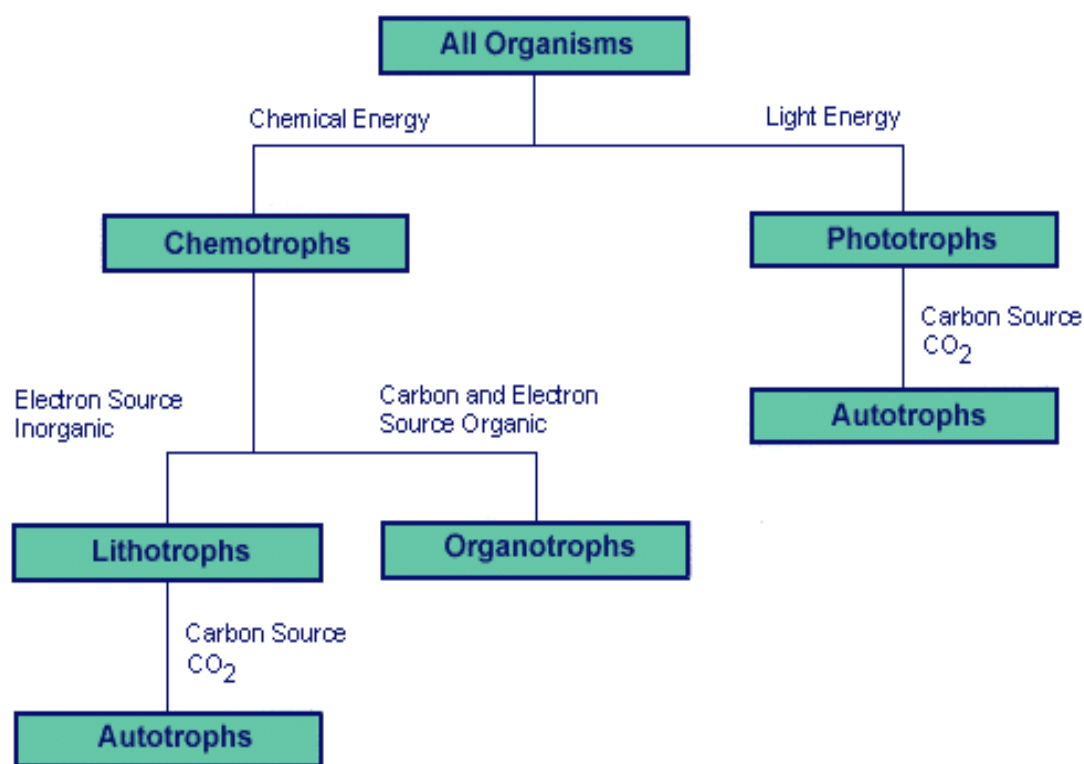
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amount of transmitted light through turbid broth decreases with subsequent increase in the absorbance value.

3.4. Nutritional Classification of microbes : energy production, oxidation, reduction reactions

3.5. Microorganisms are classified on the basis of their utilisation of nutrients as an energy source and as a carbon source. The major level of classification is into chemotrophs and phototrophs. The former derive energy from chemicals and the latter depend primarily on radiant energy.

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These two major groups are further classified on the basis of the carbon sources utilised.

3.4.1 Chemoautotrophs (Chemolithotrophs) - use inorganic substrates as sources of energy and CO₂ as the main source of carbon.

3.4.2 Chemoheterotrophs (Chemorganotrophs) - utilise organic substrates for both needs.

3.4.3 Photoautotrophs (Photolithotrophs) - use light as energy source and CO₂ as carbon source.

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3.4.4 Photoheterotrophs (photoorganotrophs) - use light as energy source and organic carbon sources.

Not all bacteria can be so precisely categorised, for example, some phototrophic bacteria can also grow as chemotrophs.

Rhodospirillum rubrum exists as a photoheterotroph in anaerobic conditions but when oxygen is available, and in the dark, it grows as a chemoheterotroph. This important metabolic switch is also reflected in changes in internal cell structure. The vesicles which contain the photosynthetic pigments become disorganised when the organism grows as a chemoheterotroph. Chemoheterotrophs show a wide diversity with respect to the sources of organic nutrients that are essential for their growth. At the two extremes are the saprotrophs (saprophytes), which utilise nonliving organic matter, and the biotrophs (parasites) which may be totally dependent on another living organism for their nutrition and cause disease (pathogens). In between there are several types of association between this group of microorganisms and another organism. These include mutualistic or symbiotic associations whereby both partners gain benefit, organisms that live as commensals and under some circumstances cause disease, and finally obligate biotrophs that can live both a saprotrophic and biotrophic existence.

Bacteria exhibit an extremely wide variety of metabolic types. The distribution of metabolic traits within a group of bacteria has traditionally been used to define their taxonomy, but these traits often do not correspond with modern genetic

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classifications. Bacterial metabolism is classified into nutritional groups on the basis of three major criteria: the kind of energy used for growth, the source of carbon, and the electron donors used for growth. An additional criterion of respiratory microorganisms is the electron acceptors used for aerobic or anaerobic respiration.

Nutritional types in bacterial metabolism

Nutritional type	Source of energy	Source of carbon	Examples
Phototrophs	Sunlight	Organic compounds (photoheterotrophs) or carbon fixation bacteria (photoautotrophs)	Cyanobacteria, Green sulfur bacteria, Chloroflexi, or Purple bacteria
Lithotrophs	Inorganic compounds	Organic compounds (lithoheterotrophs) or carbon fixation (lithoautotrophs)	Thermodesulfobacteria, <i>Hydrogenophilaceae</i> , or Nitrospirae
Organotrophs	Organic compounds	Organic compounds (chemoheterotrophs) or carbon fixation (chemoautotrophs)	<i>Bacillus</i> , <i>Clostridium</i> or <i>Enterobacteriaceae</i>

Carbon metabolism in bacteria is either *heterotrophic*, where organic carbon compounds are used as carbon sources, or *autotrophic*, meaning that cellular carbon is obtained by fixing carbon dioxide. Heterotrophic bacteria include parasitic types. Typical autotrophic bacteria are phototrophic cyanobacteria, green sulfur-bacteria and some purple bacteria, but also many chemolithotrophic species, such as nitrifying or sulfur-oxidising bacteria. Energy metabolism of bacteria is either based on *phototrophy*,

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the use of light through photosynthesis, or based on *chemotrophy*, the use of chemical substances for energy, which are mostly oxidised at the expense of oxygen or alternative electron acceptors (aerobic/anaerobic respiration).

Bacteria are further divided into *lithotrophs* that use inorganic electron donors and *organotrophs* that use organic compounds as electron donors. Chemotrophic organisms use the respective electron donors for energy conservation (by aerobic/anaerobic respiration or fermentation) and biosynthetic reactions (e.g., carbon dioxide fixation), whereas phototrophic organisms use them only for biosynthetic purposes. Respiratory organisms use chemical compounds as a source of energy by taking electrons from the reduced substrate and transferring them to a terminal electron acceptor in a redox reaction. This reaction releases energy that can be used to synthesise ATP and drive metabolism. In *aerobic organisms*, oxygen is used as the electron acceptor. In *anaerobic organisms* other inorganic compounds, such as nitrate, sulfate or carbon dioxide are used as electron acceptors. This leads to the ecologically important processes of denitrification, sulfate reduction, and acetogenesis, respectively.

Another way of life of chemotrophs in the absence of possible electron acceptors is fermentation, wherein the electrons taken from the reduced substrates are transferred to oxidised intermediates to generate reduced fermentation products (e.g., lactate, ethanol, hydrogen, butyric acid). Fermentation is possible, because the energy content of the substrates is higher than that of the products, which allows the organisms to synthesise ATP and drive their metabolism.

These processes are also important in biological responses to pollution; for example, sulfate-reducing bacteria are largely responsible for the production of the highly toxic forms of mercury (methyl- and dimethylmercury) in the environment. Non-respiratory anaerobes use fermentation to generate energy and reducing power, secreting

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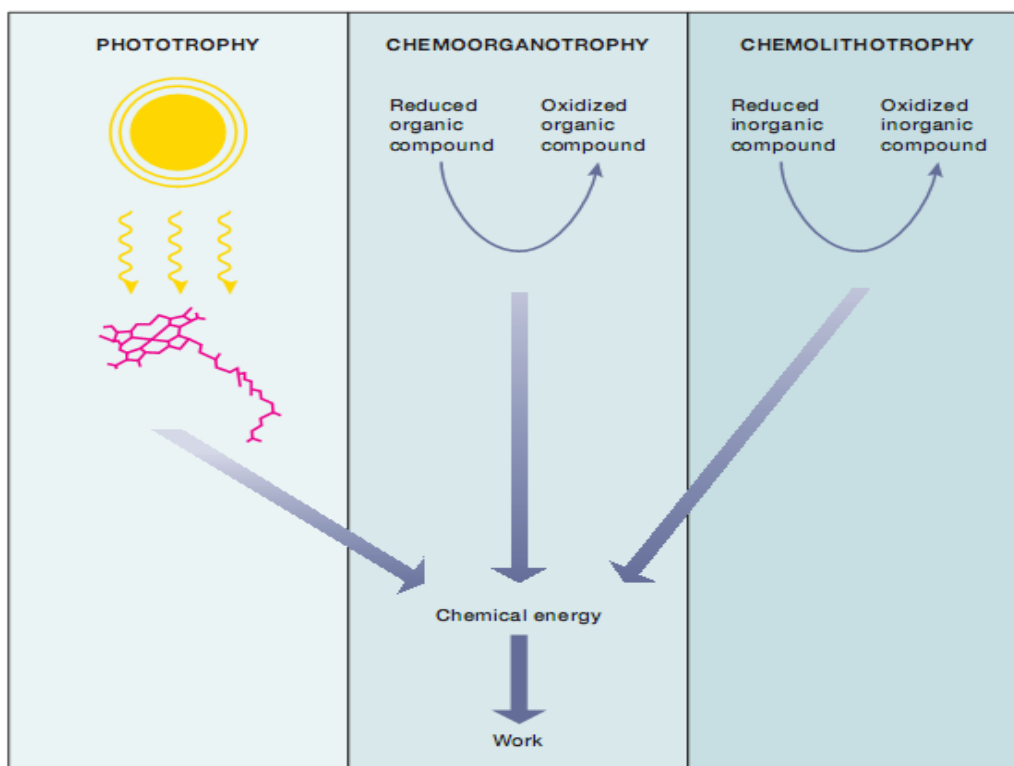
metabolic by-products (such as ethanol in brewing) as waste. Facultative anaerobes can switch between fermentation and different terminal electron acceptors depending on the environmental conditions in which they find themselves.

Lithotrophic bacteria can use inorganic compounds as a source of energy. Common inorganic electron donors are hydrogen, carbon monoxide, ammonia (leading to nitrification), ferrous iron and other reduced metal ions, and several reduced sulfur compounds. In unusual circumstances, the gas methane can be used by methanotrophic bacteria as both a source of electrons and a substrate for carbonanabolism. In both aerobic phototrophy and chemolithotrophy, oxygen is used as a terminal electron acceptor, whereas under anaerobic conditions inorganic compounds are used instead. Most lithotrophic organisms are autotrophic, whereas organotrophic organisms are heterotrophic.

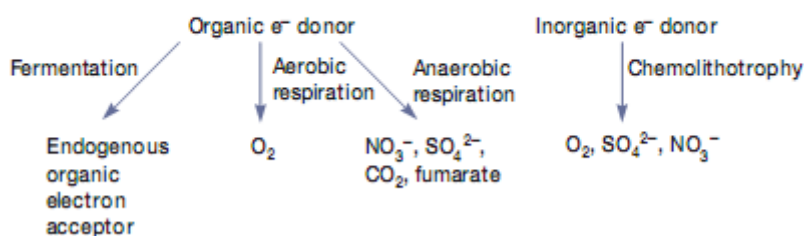
In addition to fixing carbon dioxide in photosynthesis, some bacteria also fix nitrogen gas (nitrogen fixation) using the enzyme nitrogenase. This environmentally important trait can be found in bacteria of nearly all the metabolic types listed above, but is not universal.

Regardless of the type of metabolic process they employ, the majority of bacteria are able to take in raw materials only in the form of relatively small molecules, which enter the cell by diffusion or through molecular channels in cell membranes. The Planctomycetes are the exception (as they are in possessing membranes around their nuclear material). It has recently been shown that *Gemmata obscuriglobus* is able to take in large molecules via a process that in some ways resembles endocytosis, the process used by eukaryotic cells to engulf external items.

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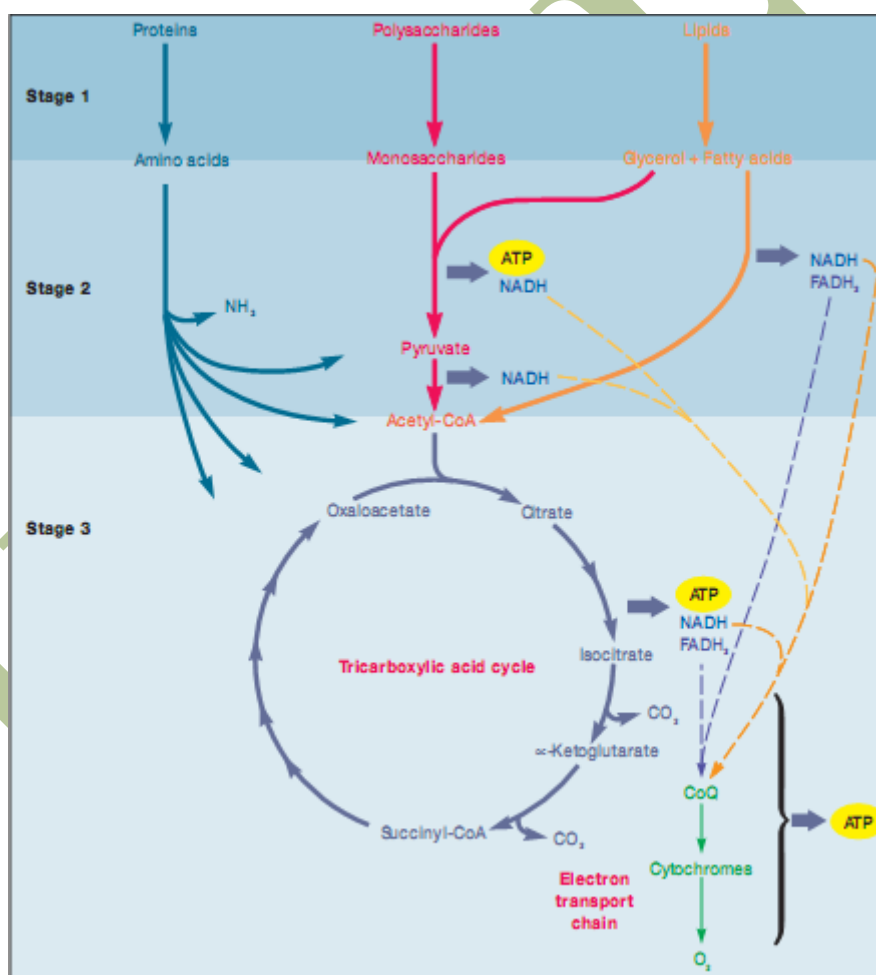


Sources of Energy for Microorganisms. Most microorganisms employ one of three energy sources. Phototrophs trap radiant energy from the sun using pigments such as bacteriochlorophyll and chlorophyll. Chemotrophs oxidize reduced organic and inorganic nutrients to liberate and trap energy.



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Patterns of Energy Release. Fermentation is the energy-yielding process in which an organic electron donor gives electrons to an endogenous acceptor, usually an intermediate derived from catabolism of the nutrient. In respiration, the electrons are donated to an exogenous acceptor, either oxygen (aerobic respiration) or some other acceptor such as nitrate or sulfate (anaerobic respiration). Reduced inorganic compounds also can serve as electron donors in energy production (chemolithotrophy).



The Three Stages of Catabolism. A general diagram of aerobic catabolism in a chemoorganoheterotroph showing the three stages in this process and the central position of the tricarboxylic acid cycle. Although there are many different proteins,

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polysaccharides, and lipids, they are degraded through the activity of a few common metabolic pathways. The dashed lines show the flow of electrons, carried by NADH and FADH₂, to the electron transport chain.

Aerobic& Anaerobic Organism

An **aerobic organism** or **aerobe** is an organism that can survive and grow in an oxygenated environment

TYPES

- **Obligate aerobes** need oxygen to grow. In a process known as cellular respiration, these organisms use oxygen to oxidize substrates (for example sugars and fats) and generate energy.
- **Facultative anaerobes** use oxygen if it is available, but also have **anaerobic** methods of energy production.
- Microaerophiles require oxygen for energy production, but are harmed by atmospheric concentrations of oxygen (21% O₂).

Aerotolerant anaerobes do not use oxygen but are not harmed by it.

Examples

- A good example would be the oxidation of glucose (a monosaccharide) in aerobic respiration.
- $C_6H_{12}O_6 + 6 O_2 + 38 ADP + 38 \text{ phosphate} \rightarrow 6 CO_2 + 6 H_2O + 38 ATP$
- Oxygen is used during the oxidation of glucose and water is produced.
- This equation is a summary of what actually happens in three series of biochemical reactions: glycolysis, the Krebs cycle, and oxidative phosphorylation.

Yeast is an example of a facultative anaerobe. It grows best in the presence of oxygen but does not require it.

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An anaerobic organism or anaerobe is any organism that does not require oxygen for growth. It may react negatively or even die if oxygen is present. An anaerobic organism may be unicellular

eg. protozoans, bacteria¹)ormulticellular(eg. Nereid(worm) polychaetes, juvenile *Trichine lla spiralis* (pork worm) parasites. For practical purposes, there are three categories of anaerobe:

- **Obligate anaerobes**, which are harmed by the presence of oxygen.
- **Aerotolerant organisms**, which cannot use oxygen for growth, but tolerate its presence.
- **Facultative anaerobes**, which can grow without oxygen but use oxygen if

Distribution

In human beings, anaerobic organisms are usually found in the gastrointestinal tract. Some anaerobic bacteria produce clinically important toxins (e.g.tetanus).

Culturing

Obligate anaerobes may use fermentation or anaerobic respiration. Aerotolerant organisms are strictly fermentative. In the presence of oxygen, facultative anaerobes use aerobic respiration; without oxygen, some of them ferment; some use anaerobic respiration.

Fermentation

There are many anaerobic fermentative reactions.

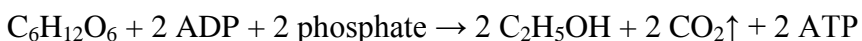
Fermentative anaerobic organisms mostly use the lactic acid fermentation pathway:



The energy released in this equation is approximately 150 kJ per mol, which is conserved in regenerating two ATP from ADP per glucose. This is only 5% of the energy per sugar molecule that the typical aerobic reaction generates.

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Plants and fungi (e.g., yeasts) in general use alcohol (ethanol) fermentation when oxygen becomes limiting:



The energy released is about 180 kJ per mol, which is conserved in regenerating two ATP from ADP per glucose.

Anaerobic bacteria and archaea use these and many other fermentative pathways, e.g., propionic acid fermentation, butyric acid fermentation, solvent fermentation, mixed acid fermentation, butanediol fermentation, Stickland fermentation, acetogenesis, or methanogenesis.

Culturing

Since normal microbial culturing occurs in atmospheric air, which is an aerobic environment, the culturing of anaerobes poses a problem. Therefore, a number of techniques are employed by microbiologists when culturing anaerobic organisms, for example, handling the bacteria in a glovebox filled with nitrogen or the use of other specially sealed containers, or techniques such as injection of the bacteria into a dicot plant, which is an environment with limited oxygen. The GasPak System is an isolated container that achieves an anaerobic environment by the reaction of water with sodium borohydride and sodium bicarbonate tablets to produce hydrogen gas and carbon dioxide. Hydrogen then reacts with oxygen gas on a palladium catalyst to produce more water, thereby removing oxygen gas. The issue with the Gaspak method is that an adverse reaction can take place where the bacteria may die, which is why a thioglycollate medium should be used. The Thioglycollate supplies a medium mimicking that of a Dicot, thus providing not only an anaerobic environment but all the nutrients needed for the bacteria to thrive.

UNIT-IV

SYLLABUS

Microbial growth: Growth curve, Microbial growth kinetics, batch and continuous culture, Measurement of growth, growth factors, factors affecting growth of bacteria. Bacterial Reproduction: Transformation, Transduction and Conjugation. Endospores and sporulation in bacteria

The Bacterial Growth curve

Principle

Bacterial population growth studies require inoculation of viable cells into a sterile broth medium and incubation of the culture under optimum temperature, PH, and gases conditions. under these conditions , the cells will reproduce rapidly and the dynamics of the microbial growth can be charted by means of a population growth curve, which is constructed by plotting the increase in cell number versus time of incubation the curve can be used to delineate stages of the growth cycle. It also facilitates measurement of cell numbers and the rate of growth of a particular organism under standardized conditions as expressed by its generation time the time required for a microbial population to double.

Lag phase :during this stage the cells are adjusting to their new environment cellular metabolism is accelerated , resulting in rapid biosynthesis of cellular macromolecules, primarily enzymes, in preparation for the next phase of the cycle .Although the cells are increasing in size , there is no cell division and therefore no increase in number

Logarithmic (log) phase:under optimum nutrition and physical conditions the physiologically robust cells reproduce at a uniform and rapid rate by binary fission thus there is a rapid exponential increase in population , which doubles regularly until a maximum number of cells is reached .the time required for the population to double is the generation time. The length of the log phase varies, depending on the organism composition of the medium .the average may be estimated to last 6 to 12 hours .

1. **Decline, or death /phase :** because of the continuing depletion of nutrients and buildup of metabolic wastes, the micro organism die at a rapid and uniform rate . the decrease in population closely parallels its increase during the log phase . theoretically, the entire population should die during a time interval equal should die during a time interval equal to that of the log phase. This does not occur, however, since a small number of highly resistant organism persist for an indeterminate length of time.

Construction of a complete Bacteria growth curve require that aliquots of a 24-4 hours shake –flask culture the inoculation period .such a procedure does not lend itself to a regular laboratory session. Therefore this experiment follows a modified procedure designed to demonstrate only the lag and log phase. The curve will be plotted on semilog paper by using two values for the measurement of growth. the direct method requires enumeration of viable cells in serially diluted sample of the test culture taken at 30-minute intervals as described in Experiment 19. the indirect method uses spectrophotometric measurement developing turbidity at the same 30- minute intervals, as an index of increasing cells mass.

Determination of generation time with indirect and direct methods by using data on the growth curve indirect determination is made by simple extrapolation from the log phase..Select two points On the optical density scale , such as 0.2 and 0.4 that represent doubling of turbidity. Using a ruler ; extrapolate by drawing line between each of the selected optical densities on the ordinate (x axis) and the plotted line of the growth curve to their respective time intervals on the abscissa (y axis). With this information, determine the generation time as follows.

Principle:

The increase in the cell size and cell mass during the development of an organism is termed as growth. It is the unique characteristics of all organisms. The organism must require certain basic parameters for their energy generation and cellular biosynthesis.

The growth of the organism is affected by both physical and Nutritional factors. The physical factors include the pH, temperature, Osmotic pressure, Hydrostatic pressure, and Moisture content of the medium in which the organism is growing.

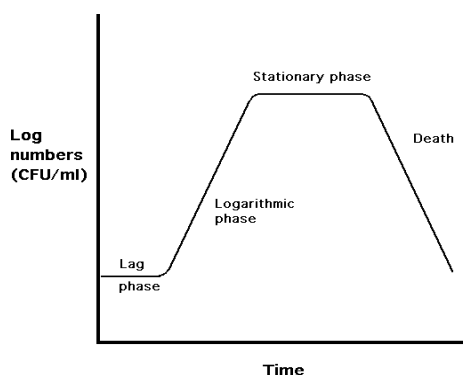
The nutritional factors include the amount of Carbon, nitrogen, Sulphur, phosphorous, and other trace elements provided in the growth medium. Bacteria are unicellular (single cell) organisms. When the bacteria reach a certain size, they divide by binary fission, in which the one cell divides into two, two into four and continue the process in a geometric fashion. The bacterium is then known to be in an actively growing phase.

To study the bacterial growth population, the viable cells of the bacterium should be inoculated on to the sterile broth and incubated under optimal growth conditions. The bacterium starts utilising the components of the media and it will increase in its size and cellular mass. The dynamics of the bacterial growth can be studied by plotting the cell growth (absorbance) versus the incubation time or log of cell number versus time.

The curve thus obtained is a sigmoid curve and is known as a standard growth curve. The increase in the cell mass of the organism is measured by using the Spectrophotometer. The Spectrophotometer measures the turbidity or Optical density which is the measure of the amount of light absorbed by a bacterial suspension.

The degree of turbidity in the broth culture is directly related to the number of microorganism present, either viable or dead cells, and is a convenient and rapid method of measuring cell growth rate of an organism.

Thus the increasing the turbidity of the broth medium indicates increase of the microbial cell mass (Fig 1) .The amount of transmitted light through turbid broth decreases with subsequent increase in the absorbance value.



Hypothetical bacterial growth curve.

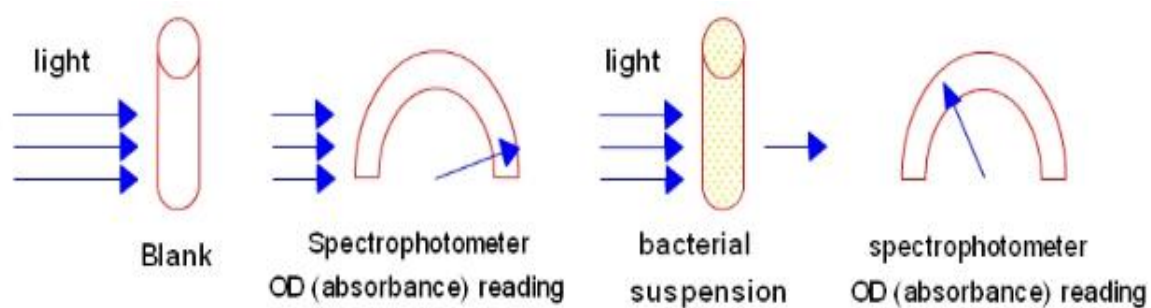
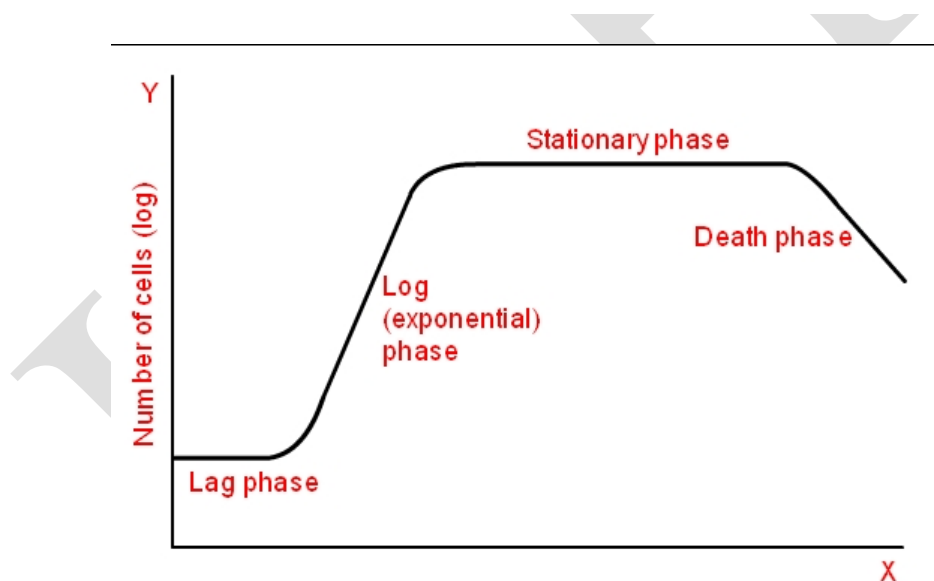


Fig1 . Absorbance Reading of the Bacterial Suspension



The growth curve has four distinct phases (Fig 2)

Lag phase

When a microorganism is introduced into the fresh medium, it takes some time to adjust with the new environment. This phase is termed as Lag phase, in which cellular metabolism is accelerated, cells are increasing in size, but the bacteria are not able to replicate and therefore no increase in cell mass. The length of the lag phase depends directly on the previous growth condition of the organism. When the microorganism growing in a rich medium is inoculated into nutritionally poor medium, the organism will take more time to adapt with the new environment. The organism will start synthesising the necessary proteins, co-enzymes and vitamins needed for their growth and hence there will be a subsequent increase in the lag phase. Similarly when an organism from a nutritionally poor medium is added to a nutritionally rich medium, the organism can easily adapt to the environment, it can start the cell division without any delay, and therefore will have less lag phase it may be absent.

Exponential or Logarithmic (log) phase

During this phase, the microorganisms are in a rapidly growing and dividing state. Their metabolic activity increases and the organism begin the DNA replication by binary fission at a constant rate. The growth medium is exploited at the maximal rate, the culture reaches the maximum growth rate and the number of bacteria increases logarithmically (exponentially) and finally the single cell divide into two, which replicate into four, eight, sixteen, thirty two and so on (That is 2^0 , 2^1 , 2^2 , 2^3 2^n , n is the number of generations) This will result in a balanced growth. The time taken by the bacteria to double in number during a specified time period is known as the generation time. The generation time tends to vary with different organisms. *E.coli* divides in every 20 minutes, hence its generation time is 20 minutes, and for *Staphylococcus aureus* it is 30 minutes.

Stationary phase

As the bacterial population continues to grow, all the nutrients in the growth medium are used up by the microorganism for their rapid multiplication. This result in the accumulation of waste materials, toxic metabolites and inhibitory compounds such as antibiotics in the medium. This shifts the conditions of the medium such as pH and temperature, thereby creating an

unfavourable environment for the bacterial growth. The reproduction rate will slow down, the cells undergoing division is equal to the number of cell death, and finally bacterium stops its division completely. The cell number is not increased and thus the growth rate is stabilised. If a cell taken from the stationary phase is introduced into a fresh medium, the cell can easily move on the exponential phase and is able to perform its metabolic activities as usual.

Decline or Death phase

The depletion of nutrients and the subsequent accumulation of metabolic waste products and other toxic materials in the media will facilitates the bacterium to move on to the Death phase. During this, the bacterium completely loses its ability to reproduce. Individual bacteria begin to die due to the unfavourable conditions and the death is rapid and at uniform rate. The number of dead cells exceeds the number of live cells. Some organisms which can resist this condition can survive in the environment by producing endospores.

Microbial Growth kinetics

I. The Growth Curve in batch culture

- A. Growth is an increase in cell constituents
- B. For most microbes, growth is indicated by an increase in cell # because cell division accompanies growth
- C. Batch culture = cultivation of organisms in 1 batch of liquid medium
- D. Growth curve (Fig. 6-1)
 1. Experimental design
 - a) Introduce small number of microbes into new medium □
 - b) monitor # of viable cells as a function of time by spectrophotometry or by diluting aliquot of culture and plate on agar plates (plate counts)
 - c) plot on semilogarithmic scale: log for # of cells (or OD) and linear for time)
 2. Phases of growth of a population of cells
 - a) Lag

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: I B.Sc

COURSE NAME: General Microbiology

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UNIT: IV Microbial Growth

BATCH-2019-22

(1) No increase in cell # when cells are introduced into fresh media

(2) Reasons

(a) Cells may be depleted of a variety of factors that may need to be resynthesized

(b) Medium may be different than previous one and thus new enzymes may be needed for growth

(c) Cells may be injured and need time to recover

b) Exponential phase

(1) Microbes are growing at maximal rate possible for the particular conditions

(2) Growth rate is constant

(3) Growth is exponential – cell growth doubles every x min (generation time)

time	# of cells
0	1
0.5	2
1	4
1.5	8
2	16
2.5	32
3	64
3.5	128
4	256
4.5	512
5	1024
5.5	2048

c) Stationary phase

(1) No net increase in cell #

(2) Mostly due to cessation of cell division

(a) depletion of nutrients

(b) accumulation of waste

(3) Also due to balance between cell death and cell division

(4) For bacteria typically at 10^9 bacteria/ml

d) Death phase

(1) Decrease in viable cell #

(2) Causes are extended nutrient deprivation and accumulation of toxic waste

E. Generation time

1. Generation time (g) is the time it takes a culture or microbial population to double in number (AKA doubling time)

2. Determination g using mathematics

a) $g = t/n$ where:

(1) t = time of exponential growth

(2) n = # of generations in time t as calculated:

exponential growth = 2^n

N_o = # of cells in population initially N_t = # of cells in population at time t

$$N_t = N_o \times 2^n$$

$$\log N_t = \log N_o + n \log 2$$

$$\log N_t - \log N_o = n \log 2$$

b) For example: What is the generation time if 100 bacterial cells growing logarithmically for 5 hours produced 1.7×10^6 cells?

$$n = \frac{\log(1.7 \times 10^6) - \log 100}{\log 2} = 14 \text{ generations in 5 hours}$$

$$g = 5 \text{ hours} / 14 \text{ generations} = 0.357 \text{ generations/hour}$$

Determination of g using growth curve data

- Plot time on X axis and CFU/ml on Y axis (log scale)
- Pick a point on the Y axis in log growth
- Draw a line from the Y axis point in (b) to the plotted graph and then down to the X axis to determine the time at which the population was at that cell density (b)
- Multiple the point that you picked in (b) by 2 (because we want to know when the population doubles)
- Draw a line from the Y axis point determined in (d) to the plotted graph and then down to the X axis to determine the time at which the population was at that cell density (d)

II. Measurement of microbial growth

A. Total cell number by direct counting

1. Counting chambers (Fig. 6-4): Special slides with a chamber that holds a known volume and contains an etched grid in the bottom for counting □ microbes are counted and normalized per ml based on the chamber volume
 - a) Pros: Easy, inexpensive, and quick
 - b) Cons: Cannot tell live from dead; need population $>10^6$ /ml; precision is difficult to achieve due to small sample
2. Coulter counter (measures resistance as cells are passed through orifice)
 - a) Pros: Easy, inexpensive, and quick
 - b) Cons: Cannot tell live from dead; only for larger microbes
3. Filter and stain systems (Fig. 6-7, 8): Filter aliquot of a sample through a membrane filter which retains the bacteria □ bacteria on filter are stained with fluorescent dye □ bacteria are then counted using a fluorescence microscope (some stains can differentiate live from dead)
- 4.

B. Viable cell counting techniques

1. Plate aliquot of liquid culture on solid media □ count colony forming units (CFU).

Assumption is that each cell in the aliquot can form one CFU on the solid media.

- a) Pros: Easy; high sensitivity
- b) Cons: Have to do several dilutions, many plates; Need correct media; clumps of cells will only give one CFU
- c) Calculation of colonies in sample:

$$\frac{\# \text{ colony forming units}}{(\text{ml plated}) (\text{dilution plated})} = \frac{\text{total CFU/ml}}{\text{total bacteria /ml}}$$

2. Filter techniques: Filter aliquot of a sample through a membrane filter which retains the bacteria □ filter is placed on agar medium □ each cell grows into a colony that can be counted

This technique is frequently used to sample water supplies; agar medium that filter is placed on can be selective for certain kinds of bacteria (Fig. 6-7, 8)

Measurement of cell mass

1. Dry weight – concentrate the culture into a pellet by centrifugation and weigh; useful for larger microbes
2. Turbidity measurement using spectrophotometer (Fig. 6-9)
 - a) The spectrophotometer measures the turbidity of a sample and generates a value called optical density (OD).
 - b) Turbidity is a measure of the light absorption by particles in a sample (i.e. microbes in media). Within limits, the light absorbed by a sample is proportional to the concentration of light absorbing materials.
 - c) Since the OD of a culture increases as the number of organisms increases in a culture, the OD can be used to indirectly calculate the number of microbes.
 - d) Pros: very easy once relationship between # of cells and OD reading is

determined for an organism

- e) Cons: Samples must contain at least $10^7 - 10^9$ bacteria per ml; does not indicate whether cells are viable

3. Measurements of cell components

- a) total protein
- b) chlorophyll
- c) ATP

III. Continuous culture

A. Maintenance of a culture in constant environmental conditions through continual provision of nutrients and removal of wastes. Useful for:

- 1. Study in a certain growth phase
- 2. Study under low nutrient concentrations
- 3. Evolution studies

B. The chemostat

- 1. Apparatus that feeds sterile media into a culture at the same rate in which it is removed
- 2. Essential nutrient is limiting so that flow rate determines growth rate

C. Turbidostat

- 1. Flow rate into the system is adjusted to maintain preset turbidity (cell density).
- 2. No limiting nutrient

IV. Influence of environmental factors on growth

A. General info:

- 1. Most microbes live in moderate environments; however, some called extremophiles live in extremely harsh environments (bacteria of generation X)
- 2. Study of environmental effects on growth important for understanding

ecological distribution of organisms, control of organisms

3. While we are addressing growth here, keep in mind that some organisms may survive (but not grow) under conditions above and below the growth conditions

B. Water activity (and osmolarity)

1. Water activity

- a) Amount of water available to an organism affected by

(1) water interactions with solute molecules (osmotic effect)

(a) Hypertonic solution = osmotic is higher outside water exits the cell

(b) Hypotonic solution = osmotic is lower outside water enters the cell

(2) water adsorption to solid surfaces (matric effect)

b) water activity of a solution = a_w

(1) a_w = vapor pressure of solution

vapor pressure of water

(3) a_w = (% relative humidity of solution) / 100 where relative means relative to water

2. environment with low a_w = high osmotic pressure

- a) effects on cell if placed in such an environment

(1) dehydration

(2) plasmolysis (plasma membrane shrinks from the cell wall)

(3) cessation of metabolism

- b) Bacterial solution to the problem: Compatible solutes - solutes that bacterium takes up to maintain a slightly hypertonic state; are compatible with metabolism and growth of the organism when at high a_w inside

(1) bacteria: choline, betaine, potassium, some amino acids

(2) algae/fungi: sucrose and polyols

(3) Halobacterium: potassium ions

(4) mycoplasmas are the exception – they do not have compatible solutes;

instead they maintain their cytoplasm at the same osmolarity as the environment using a Na pump

- c) Halophiles actually require high levels of NaCl in the environment to survive.

Adaptations include:

- (1) modified cell wall structure that has adapted to high NaCl: acidic glycoprotein cell wall is stabilized by sodium ions binding and shielding negative charge. When NaCl is removed, acidic proteins repel each other resulting in cell lysis.

- (2) K accumulation to remain hypertonic to their high salt environment

- (3) acidic proteins that have adapted to high K ions in the cytoplasm

- d) environments with low a_w /high osmotic pressure: dried foods, salt lakes, salted foods, preserves

3. high a_w = low osmotic pressure

- a) effects on cells: cells will take up water

- b) bacterial solution to the problem

- (1) cell wall provides protection against lysis up to a point

- (2) mycoplasmas maintain their cytoplasm at the same osmolarity as the environment using a Na pump

- c) environments with high a_w /low osmotic pressure: water, blood

4. osmotolerant organisms can grow over a wide range of a_w

C. pH

1. measure of the H^+ activity in a solution (Fig. 6-13)

$pH = -\log[H^+] = \log (1/[H^+])$ low pH = acidic

high pH = basic/alkaline

2. Growth of microbes

- a) acidophiles (0-5.5)

- (1) many fungi, few bacteria

- (2) locations: acid thermal springs, gastric juice, acid mine drainage, acid

soils

- b) neutrophiles (5.5-8)
 - (1) most bacteria
 - (2) locations: most foods except for acidic ones, rain water, milk, saliva, blood
- c) alkalophiles (8.5 – 11)
 - (1) locations: alkaline soils and lakes
- 3. EXTERNAL pH ? INTERNAL pH
 - a) Internal pH must be near neutral to avoid harming acid or alkali labile molecules (for *E. coli* internal pH is 7.4-7.8 when grown at pH between 5 and 9)
 - b) Mechanism for maintaining internal pH
 - (1) General
 - (a) plasma membrane is impermeable to H^+
 - (b) Buffering agents in the cytoplasm
 - (2) Neutrophiles

Keep cytoplasm slightly basic by pumping out H^+ in exchange for K^+

 - (3) Acidophiles
 - (a) Decarboxylases take the carboxyl group off acidic amino acids to generate alkaline products to keep the cytoplasm from acidifying
 - (b) up regulate fermentative pathways that generate less acid
 - (c) pump in cations to decrease proton motive force in membrane so H^+ are less likely to flow back into the cell
 - (4) Alkalophiles
 - (a) Pump H^+ into the cell to prevent the cytoplasm from becoming too alkaline
- 4. Microbes themselves change the pH of their environment by producing acidic or

basic waste products

5. Temperature

1 Microbes are poikilothermic – their temperature varies with the environmental temperature

2 Why does temperature matter?

a Enzyme activity

low temp = slow rate of enzyme reactions

high temp = denaturation of enzymes

b Membrane structure

low temp = not fluid enough

high temp = disrupted

3 Cardinal temperatures (Fig. 6-14)

a) minimal

b) optimal

c) maximal

2. Range

a) stenothermal = small temp range

b) eutothermal = large temp range

3. Classes based on growth at a variety of temperatures (Fig. 6-15)

a) Psychrophiles

(1) min = $\leq 0^{\circ}\text{C}$ / opt = $\leq 15^{\circ}\text{C}$ / max = 20°C

(2) location – mostly in deep sea

(3) adaptations

(a) enzymes have evolved to function best at cold temps

(b) membranes have high levels of unsaturated fatty acids that remain fluid in the cold

b) Psychrotrophs or facultative psychrophiles

(1) min = 0°C / opt = 20-30°C / max = 35°C

(2) location – cold, temperate environments that warm up in the summer; food in fridge

c) Mesophiles

(1) min = 15-20°C / opt = 20-45°C / max = 45°C

(2) location – varied as most identified microbes fall in this category; human pathogens

d) Thermophiles

(1) min = 45°C / opt = 55-65°C / max = 80°C

location – hot springs, hot water lines, compost bins, soils

(1) exposed to direct sunlight

(2) adaptations –

(i) heat stable proteins; evolved to function best at high temperatures

(ii) several critical amino acid changes increase stability (as opposed to drastically altered protein)

(iii) increase in salt bridges in the proteins (bridges between negatively charged amino acids with cations)

(iii) densely packed hydrophobic interiors (less unfolding)

(b) membranes have high levels of saturated fatty acids and thus have higher melting temperatures

e) Hyperthermophiles

(1) min = 55°C / opt = 80-113°C / max = ? °C

(2) location - hydrothermal vents on the ocean floor, hot springs

(3) adaptations – as above except that membranes are Archeal

(4) no eukaryotes – Why? internal membranes of organelles must remain porous for transport of large macromolecules and thus would not stand high heat

(5) interests

(a) protein and membrane stability

(b) industrial uses

(c) Taq polymerase for PCR

E. Oxygen concentration

1. Classification based on oxygen use/sensitivity (Fig. 6-16)

a) Obligate aerobes – require atmospheric oxygen for growth

b) Facultative anaerobes – do not require oxygen for growth but grow better in its presence

c) Microaerophiles – require oxygen at levels below that in the atmosphere

d) Aerotolerant anaerobes – can tolerate oxygen but do not use it for growth

e) Obligate anaerobes – killed by oxygen and obviously do not use it for growth

2. Sensitivity to oxygen

a) Inactivation of proteins that are sensitive to O₂ (generally because of sulfhydryl groups or bound cofactors)

b) Inability to detoxify toxic O₂ derivatives produced by interaction with cell components and radiation

3. Cultivation of anaerobes

a) "Anaerobic media" which contains reducing agents such as thioglycollate or cysteine to eliminate any O₂ by conversion to H₂O

b) Elimination of O₂ in container or work area (glove box)

(1) by flushing with nitrogen

- (2) applying vacuum
- (3) GasPak jar (Fig. 6-18): O₂ consuming gas removes O₂
- 4. Cultivation of aerobes
 - a) Vigorous shaking
 - b) Bubbling in sterile air
- 5. Location of organisms depends on the O₂ available and the presence of other organisms that consume or generate O₂
- F. Pressure
 - 1. Most organisms exist at 1 atm
 - 2. In deep sea □ higher pressures
 - a) barotolerant organisms – survive at high pressures but grows better at 1 atm
 - b) moderate barophiles – grow at high pressures but still grow at 1 atm
 - c) extreme (obligate) barophiles – only grows at high pressures
 - 3. Adaptations
 - a) Because water flows into bacteria, hydrostatic force will not crush them
 - b) Enzymes are adapted so that the molecular volume of the activated state of a reaction complex is smaller than the reactants
 - c) Membranes are composed of unsaturated fatty acids that are less likely to gel at high pressures
- G. Radiation
 - 1. Ionizing radiation (radiation that is very short wavelength/high energy)
 - a) Types
 - (1) Gamma
 - (2) X-rays – artificial
 - b) Effects that damage cell components and DNA
 - (1) break H bonds
 - (2) oxidize double bonds

- (3) destroy rings
 - (4) polymerizes some molecules
 - (5) formation of hydroxyl radical
2. UV radiation
- a) Far UV (260 nm) - DNA damage via formation of T-T dimers
 - b) Near UV (325-400nm) – Tryptophan breakdown to toxic products which introduce breaks in DNA
3. visible
- a) interaction of light with certain photosensitizer pigments excites the pigment excited pigment transfers energy to O₂ to generate singlet O₂
□singlet O₂□is a higher energy form of O₂□in which outer shell electrons become highly reactive
 - b) carotenoid pigments absorb energy to convert singlet oxygen back into the unexcited state

The Common Nutritional Requirements

Analysis of microbial cell composition shows that over 95% of cell dry weight is made up of a few major elements: carbon, oxygen hydrogen, nitrogen, sulfur, phosphorous, potassium, Calcium, magnesium and iron. These are called macro elements or macronutrients because they are required by microorganism in relatively large amounts. The first six are components of carbohydrates, proteins ,lipids and nucleic acids. The remaining four macronutrients exist in the cell as cat ions and play a variety of roles. For example potassium (K⁺) is required for activity for a number of enzymes including some of those involved in protein synthesis. Calcium, among other functions, contributes to the heat résistance bacterial endospores. Magnesium serves as cofactor for many enzymes, complexes with ATP, and stabilizes ribosomes and cell membranes. Iron is a part of cytochromes and cofactor for enzymes and electron carrying proteins.

All organisms, including microorganisms, require several micronutrients or trace elements besides macro elements. The micronutrients- manganese, cobalt, zinc, molybdenum, nickel, and copper- are needed by most cells. However, cells require regular media components are often adequate for growth. Therefore it is very difficult to demonstrate a micronutrient requirement. In nature, micronutrients are ubiquitous and probably do not usually limit growth. Micronutrients are normally a part of enzymes and cofactors, and they aid in the catalysis of reactions and maintenance of protein structure. For example zinc is present at the active sites of some enzymes but is also involved in the regulatory and catalytic subunits of *E.coli* aspartate carbamoyltransferase.

Besides the common macroelements and trace elements, microorganisms may have particular requirements that affect the nature of their morphology or environment. Diatoms require silicic acid to construct their beautiful cell walls of silica. Although most bacteria do not require large amounts of sodium, many bacteria growing in saline lakes and oceans depend on the presence of high concentrations of sodium ions.

Finally it must be emphasized that microorganisms require a balanced mixture of nutrients. If an essential nutrient is in short supply, microbial growth will be limited regardless of concentration of other nutrients.

Growth Factors

Microorganisms often grow and reproduce when minerals and sources of energy, carbon, nitrogen, phosphorus and sulfur are supplied. These organisms have the enzymes and pathways necessary to synthesize all cell components required for their well being. Many microorganisms on the other hand, lack one or more essential enzymes. Therefore they cannot manufacture all indispensable constituents but must obtain them or their precursors from the environment. Organic components required because they are essential cell components or precursors of such components and cannot be synthesized by the organism are called growth factors. There are three major classes of growth factors: (1) amino acids (2) purines and pyrimidines, and (3) vitamins.

Amino acids are required for protein synthesis, purines and pyrimidines for nucleic acid synthesis. Vitamins are small organic molecules that usually make up all or part of enzyme

cofactors and only very small amounts sustain growth. Some microorganisms require many vitamins for growth. Other growth factors are also seen heme (from hemoglobin) is required by *Haemophilus influenzae*, and some mycoplasmas need cholesterol.

Knowledge of the specific growth factor requirements of many microorganisms makes possible quantitative growth-response assays for a variety of substances. For example species from the bacterial genera *Lactobacillus* and *Streptococcus* can be used in microbiological assays of mostly vitamins and amino acids. The appropriate bacterium is grown in a series of culture vessels, each containing medium with an excess amount of all required components except the growth factors to be assayed. A different amount of growth factor is added to each vessel. The standard curve is prepared by plotting the growth factor quantity or concentration against the total extent of bacterial growth. Ideally the amount of growth resulting is directly proportional to the quantity of growth factor present : if the growth factor concentration doubles the final extent of bacterial growth doubles.

Factors affecting growth of bacteria

Nutrients

Nutrients such as carbohydrates, fats, proteins, vitamins, minerals and water, required by, man are also needed by microorganisms to grow. Microbes differ in their abilities to use substrates as nutrient sources. Their enzyme systems are made available according to their genetic code. They vary in ability to use nitrogen sources to produce amino acids and, therefore, proteins. Some require amino acids to be supplied by the substrate. When organisms need special materials provided by their environment, we refer to them as fastidious. Difference in the utilization of nutrients and the waste products they produce are important in differentiating between organisms.

Oxygen

Microbes also differ in their needs for free oxygen. Aerobic organisms must grow in the presence of free oxygen and anaerobic organisms must grow in the absence of free oxygen. Facultative organisms can grow with or without oxygen, while microaerophilic organisms grow in the presence of small quantities of oxygen.

Water

Water is necessary for microbes to grow, but microbes cannot grow in pure water. Some water is not available. A measurement of the availability of water is a_w or water activity. The a_w of pure water is 1.0 while that of a saturated salt solution is 0.75. Most spoilage bacteria require a minimum a_w of 0.90. Some bacteria can tolerate an a_w above 0.75 as can some yeasts and most molds. Most yeasts require 0.87 water activity. An a_w of 0.85 or less suppresses the growth of organisms of public health significance.

Temperature

Microorganisms can grow in a wide range of temperatures. Since they depend on water as a solvent for nutrients, frozen water or boiling water inhibits their growth. General terms are applied to organisms based on their growth at different temperatures. Most organisms grow best at or near room and body temperature. These are mesophiles. Those growing above 40°C (105°F) are called thermophiles while those growing below 25°C (75°F) are called psychrotrophs.

Acidity

The nature of a solution based on its acidity or alkalinity is described as pH. The pH scale ranges from 0, strongly acidic, to 14, strongly basic. Neutral solutions are pH 7, the pH of pure water. Most bacteria require near neutral conditions for optimal growth with minimums and maximums between 4 and 9. Many organisms change the pH of their substrate by producing by-products during growth. They can change conditions such that the environment can no longer support their growth. Yeasts and molds are more tolerant of lower pH than the bacteria and may outgrow them under those conditions.

Light & Chemicals

Ultraviolet light and the presence of chemical inhibitors may also affect the growth of organisms. Many treatments such as hydrogen peroxide and chlorine can kill or injure microbes. Under certain conditions those given a sublethal treatment are injured, but can recover.

Bacterial reproduction

Cytological observations and genetic studies indicate something like sexual reproduction, involving the fusion of two different cells and a transfer of hereditary factors occurs in bacteria although infrequently. Genetic recombination occurs in those bacteria that have been carefully studied and presumably occurs in other species as well. One of the most intensively studied species of bacteria, *Escherichia coli* has been shown to have sex-some act as males and transfer genetic information by direct contact with females. This ability to transfer genes is regulated by a fertility factor F^+ which can itself be transferred to a female, thereby converting her into a male.

The usual vegetative bacterial cells are haploid and in sexual reproduction part or the entire chromosome passes from the male cell to the female cell, yielding a cell, i.e., partly or completely diploid. Crossing over then occurs between the female chromosome and the male chromosome or fragment, followed by a process of segregation that yields haploid progeny cells.

Types of sexual reproduction that occurs in bacteria are as follows**1. Bacterial transformation:**

The genetic transfer in bacteria also occurs by transformation, in which the DNA molecule of the donor cell, when liberated by its disintegration, is taken up by another recipient cell and its offspring inherit some characters of the donor cell. When different strains of bacteria are found in a mixed state either in culture or in nature, some of the resultant offspring possess a combination of characters of the parent strains. This phenomenon is known as recombination.

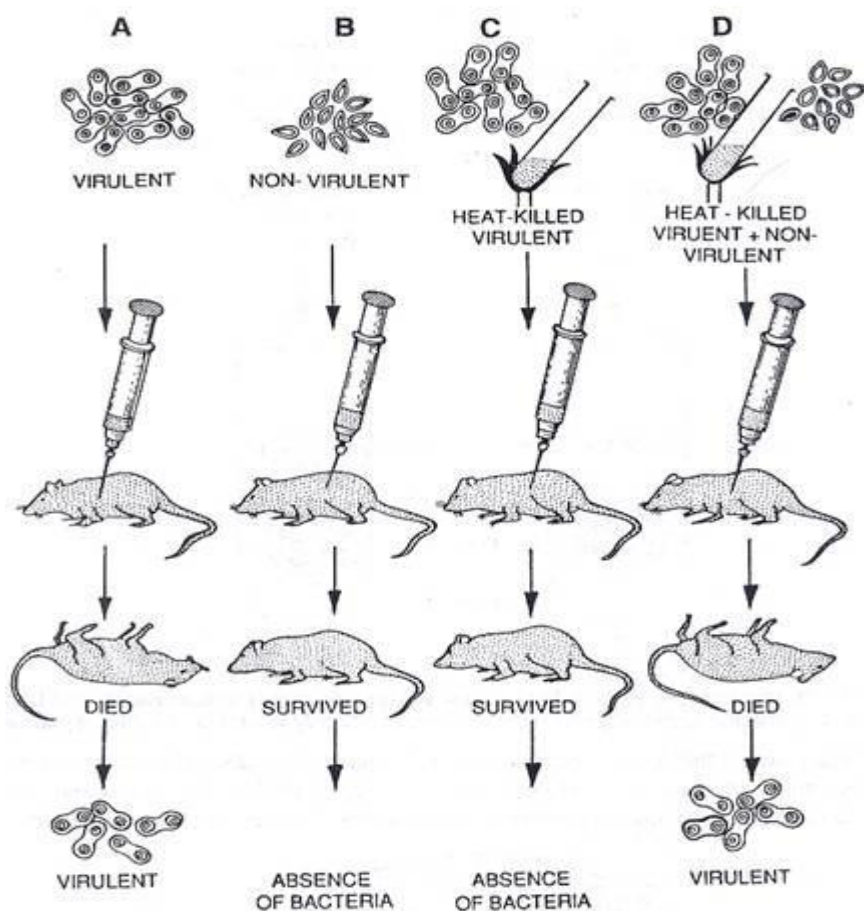
The phenomenon of transformation was first recorded by Griffith (1928). Avery, Macleod and McCarty (1944) demonstrated that the transforming principle being DNA in the sequence of events in bacterial transformation.

The lines of inquiry that led to an understanding of the chemical nature of genetic material arose from a study of the pestilent organism *Diplococcus pneumoniae*. This bacterium causes pneumonia in males. In 1928, Frederick Griffith found that there are two strains of *D. pneumoniae*, one that forms smooth colonies protected by a capsule, and the other one that formed irregular or rough colonies without a capsule when grown on a suitable medium in petri dishes.

When injected into mice (A) only capsulated smooth cells (virulent) produced the disease, but not the non-virulent rough cells (B). On the other hand when the heat killed capsulated (virulent) smooth cells were mixed with non-virulent rough cells (D) and then were injected in the mice the disease was produced. This shows that some factors from the dead capsulated smooth cells, converted the living non-virulent rough cells into living smooth capsulated (virulent) cells.

In 1944, Avery, McCarty and Macleod supported the Griffith's experiment by molecular explanation. They found that the DNA isolated from the heat killed smooth cells, when added to rough cells changed their surface character from rough to smooth, and also made them virulent.

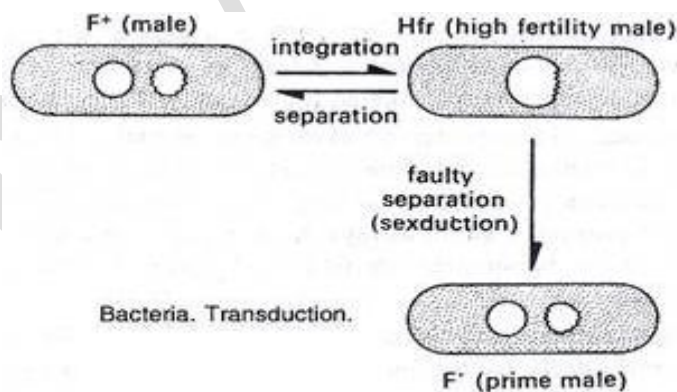
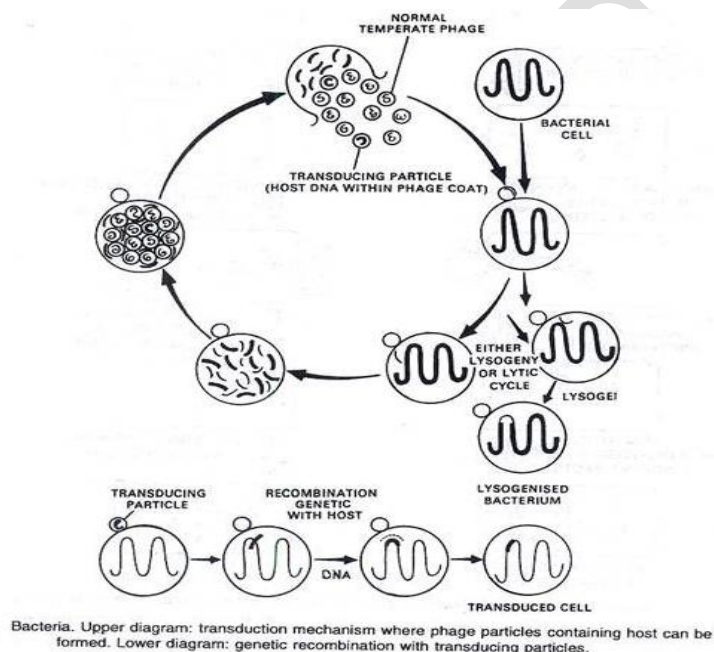
By this experiment, this was shown that DNA was the genetic material responsible for inducing the smooth character of the cells and their property of virulence in mice. Their experiment proved that bacterial transformation involves transfer of a part of DNA from the dead bacterium (i.e., donor) to the living bacterium (i.e., recipient), that expresses the character of dead cell, and so is known as a recombinant.



2. Bacterial transduction:

The genetic transfer in bacteria is achieved by a process known as transduction. Lederberg and Zinder's (1952) experiment in U-tube *Salmonella typhimurium* indicated that bacterial viruses or phages are responsible for the transfer of genetic material from one to the other lysogenic and lytic phages. Thus the host acquires a new genotype. Transduction has been demonstrated in many bacteria.

In this process, the DNA molecule that carries the hereditary characters of the donor bacterium is being transferred to the recipient cell through the agency of the phage particle. In this process very few closely linked characters can be transferred by each particle. Thus the bacteriophage brings about genetic changes in those bacteria which survive the phage attack.



When a bacterial cell is being infected with a temperate virus either lytic-cycle or

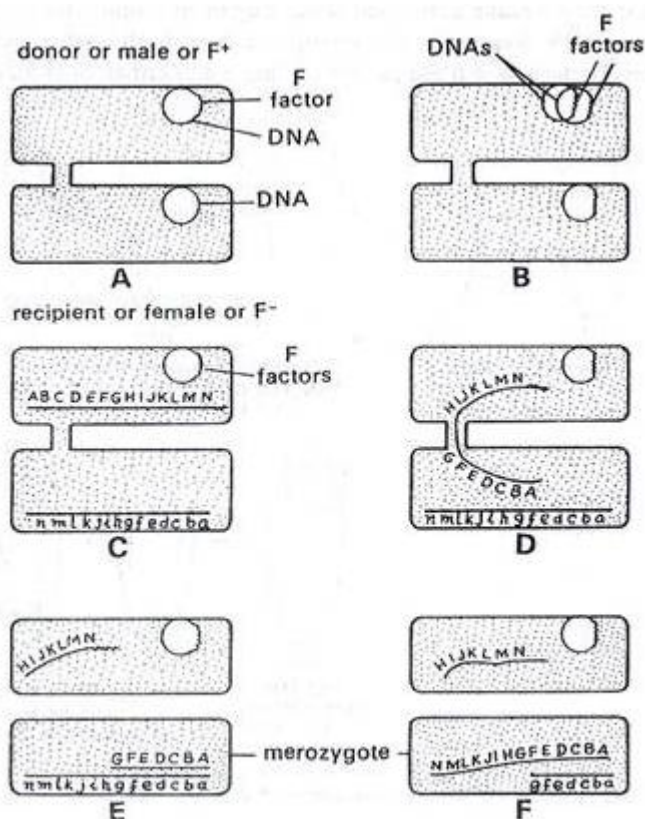
lysogeny starts. Thereafter, host DNA breaks down into small fragments along with the multiplication of virus. Some of these DNA fragments are incorporated with the virus particles becoming transducing one. When bacteria lyse these particles along with normal virus particles are released when this mixture of transducing and normal virus particles is allowed to infect the population of recipient cells, most of the bacteria are infected with normal virus particles and with the result lysogeny or lytic-cycle occurs again. A few bacteria are infected with transducing particles, transduction takes place and the DNA of virus particles undergo genetic recombinations with the bacterial DNA.

3. Bacterial conjugation:

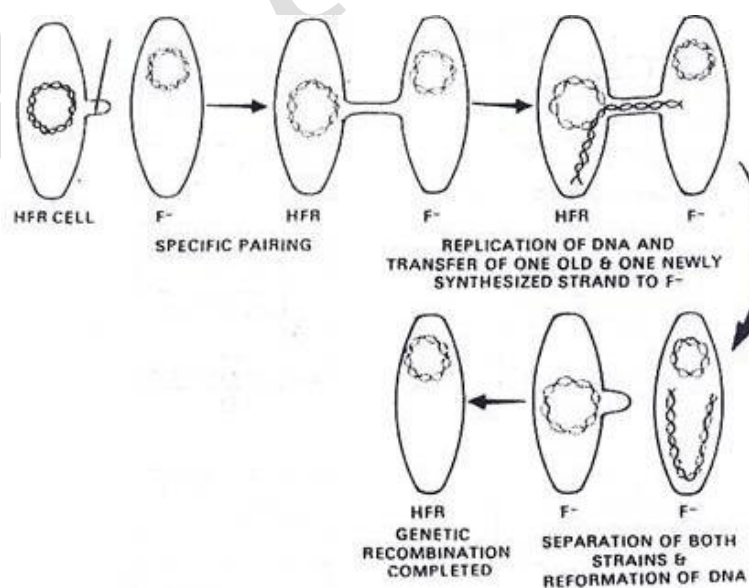
Wollman and Jacob (1956) have described conjugation in which two bacteria lie side by side for as much as half an hour. During this period of time a portion of genetic material is slowly passed from one bacterium which is designated as a male to a recipient designated as a female. This was established that the male material entered the female in a linear series.

The genetic recombination between donor and recipient cells takes place as follows: The Hfr DNA after leaving a part in fragment to recipient cell again reforms in circular manner. In F strain genetic recombination takes place between donor fragment and recipient DNA. Gene transfer is a sequential process and a given Hfr strain always donates genes in a specific order. A single stranded donor DNA (F factor) is integrated in the host chromosome with the help of nuclease enzyme, (see figs. 2.21 and 2.22).

In bacterial conjugation the transfer of genetic material (DNA) takes place by cell to cell contact of donor and recipient cells. During the process of conjugation large portion of the genome is transferred, while in transformation and transduction only small fragment of DNA is transferred. The process of conjugation was discovered by Lederberg and Tatum (1944) in a single strain of *Escherichia coli*. Conjugation has also been demonstrated in *Salmonella*, *Pseudomonas* and *Vibrio*.



Bacteria. Conjugation between sterile male and female of *Escherichia coli*.



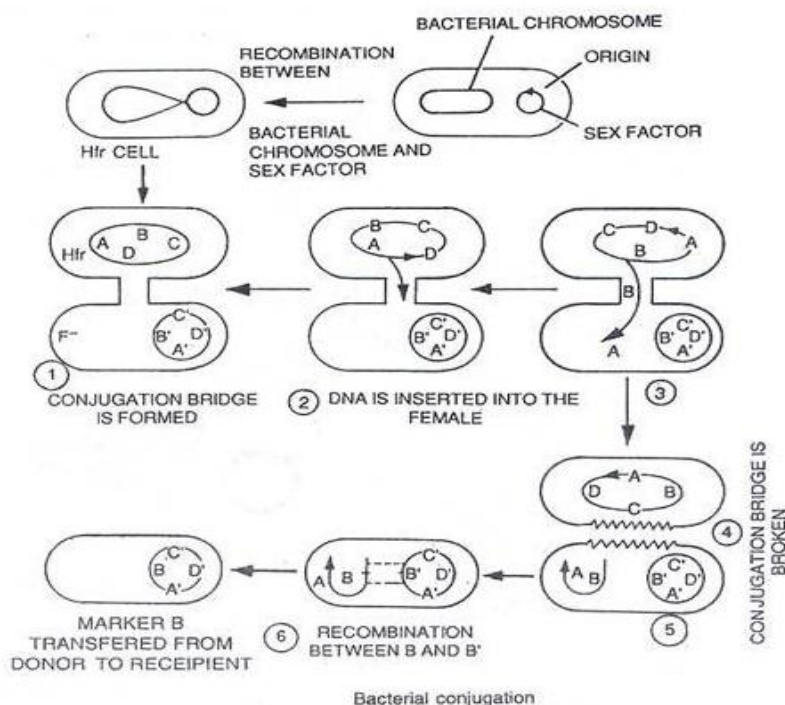
Bacteria. Diagrammatic representation of possible mechanism of conjugation.

In conjugation one way transfer of genetic material takes place from donor to recipient strain. The donor and recipient strains are always determined genetically. Recipient strain is designated as F^- , while donor strains are of two kinds and are designated as F^+ and H fr (high frequency of recombination). If the strain donates only a small portion of its genome it is called F^+ , and if it donates large amount of genome it is called H fr. These F^+ and H fr factors are called episomes.

Strains F^+ and Hfr are characterised by the presence of specific flagellum like structures, the so called sex pilus. The sex pilus is absent in F^+ strains, and is responsible for bacterial mating. Sex pili of F^+ and H fr touch the opposite mating type of cells specifically to transfer the genetic material.

Sex pilus has a hole of $2.5\mu\text{m}$ diameter which is large enough for a DNA molecule to pass through it lengthwise. At the time of pairing DNA of H fr strain (donor) is transferred to F^- strain (recipient) immediately. The circular DNA of H fr cells opens and replicates but during transfer, one strand of DNA is newly synthesized, whereas the other strand is derived from a pre-existing strand of H fr strain. After transfer of DNA both the cells are separated from each other. The H fr DNA after leaving apart its fragment to recipient cell again reforms in circular manner. In F^- strain genetic recombination takes place between donor fragment and recipient DNA. Gene transfer is a sequential process a given H fr strain always donates genes in a specific order. If F^- and H fr strains are allowed to mix in a suspension, different genes in a sequence of time are transferred from the genome of H fr to F^- strain. Genes that enter early, always appear in larger percentage of the recombinations than do genes that enter late, (see figs. 2.22, 2.23 and 2.24).

Conjugation results in a number of recombinants in a suspension of F^+ and H fr cells. These recombinants are variable in their genotypic constitution and so also in their phenotypic expression. These recombinants are entirely new and different from their parents.

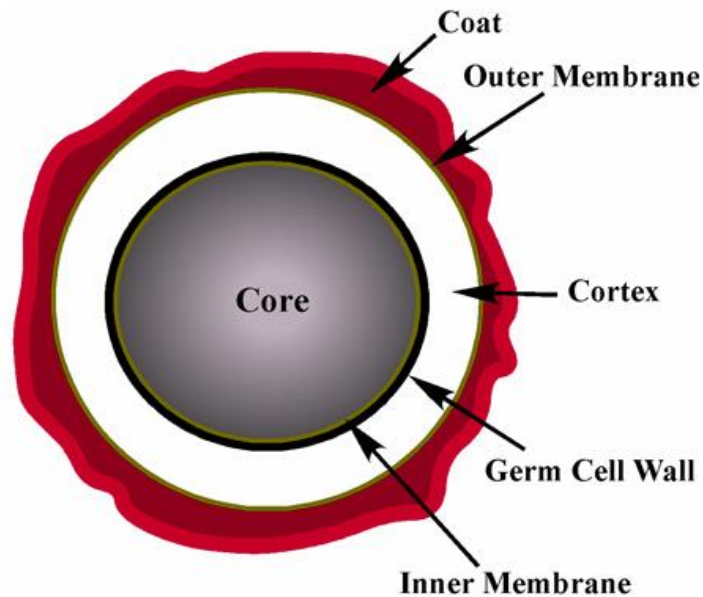


Endospores

Microorganisms sense and adapt to changes in their environment. When favored nutrients are exhausted, some bacteria may become motile to seek out nutrients, or they may produce enzymes to exploit alternative resources. One example of an extreme survival strategy employed by certain low G+C Gram-positive bacteria is the formation of endospores. This complex developmental process is often initiated in response to nutrient deprivation. It allows the bacterium to produce a dormant and highly resistant cell to preserve the cell's genetic material in times of extreme stress.

Endospores can survive environmental assaults that would normally kill the bacterium. These stresses include high temperature, high UV irradiation, desiccation, chemical damage and enzymatic destruction. The extraordinary resistance properties of endospores make them of particular importance because they are not readily killed by many antimicrobial treatments. A variety of different microorganisms form "spores" or "cysts", but the endospores of

low G+C Gram-positive bacteria are by far the most resistant to harsh conditions.



Endospore Structure

The resilience of an endospore can be explained in part by its unique cellular structure. The outer proteinaceous coat surrounding the spore provides much of the chemical and enzymatic resistance. Beneath the coat resides a very thick layer of specialized peptidoglycan called the cortex. Proper cortex formation is needed for dehydration of the spore core, which aids in resistance to high temperature. A germ cell wall resides under the cortex.

This layer of peptidoglycan will become the cell wall of the bacterium after the endospore germinates. The inner membrane, under the germ cell wall, is a major permeability barrier against several potentially damaging chemicals. The center of the endospore, the core, exists in a very dehydrated state and houses the cell's DNA, ribosomes and large amounts of dipicolinic acid.

This endospore-specific chemical can comprise up to 10% of the spore's dry weight and appears to play a role in maintaining spore dormancy. Small acid-soluble proteins (SASPs) are also only found in endospores. These proteins tightly bind and condense the DNA, and are in part responsible for resistance to UV light and DNA-damaging chemicals. Other species-specific

structures and chemicals associated with endospores include stalks, toxin crystals, or an additional outer glycoprotein layer called the exosporium.

Sporulation in bacteria

Endospore Development

The process of forming an endospore is complex. The model organism used to study endospore formation is *Bacillus subtilis*. Endospore development requires several hours to complete. Key morphological changes in the process have been used as markers to define stages of development. As a cell begins the process of forming an endospore, it divides asymmetrically (Stage II).

This results in the creation of two compartments, the larger mother cell and the smaller forespore. These two cells have different developmental fates. Intercellular communication systems coordinate cell-specific gene expression through the sequential activation of specialized sigma factors in each of the cells. Next (Stage III), the peptidoglycan in the septum is degraded and the forespore is engulfed by the mother cell, forming a cell within a cell.

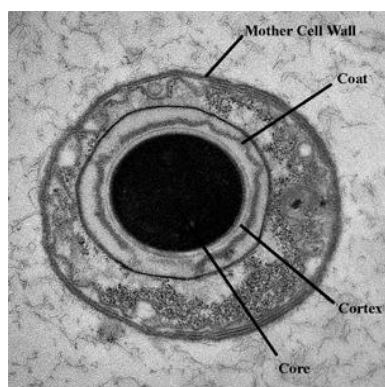
The activities of the mother cell and forespore lead to the synthesis of the endospore-specific compounds, formation of the cortex and deposition of the coat (Stages IV+V). This is followed by the final dehydration and maturation of the endospore (Stages VI+VII). Finally, the mother cell is destroyed in a programmed cell death, and the endospore is released into the environment.

The endospore will remain dormant until it senses the return of more favorable conditions. [A sigma factor is a small protein that directs RNA polymerase to specific sites on

DNA to initiate gene expression.]

Endospores and *Epulopiscium*

Some *Epulopiscium*-like surgeonfish symbionts form mature endospores at night. These spores possess all of the characteristic protective layers seen in *B. subtilis* endospores and also contain large amounts of dipicolinic acid. These are the largest endospores described thus far, with

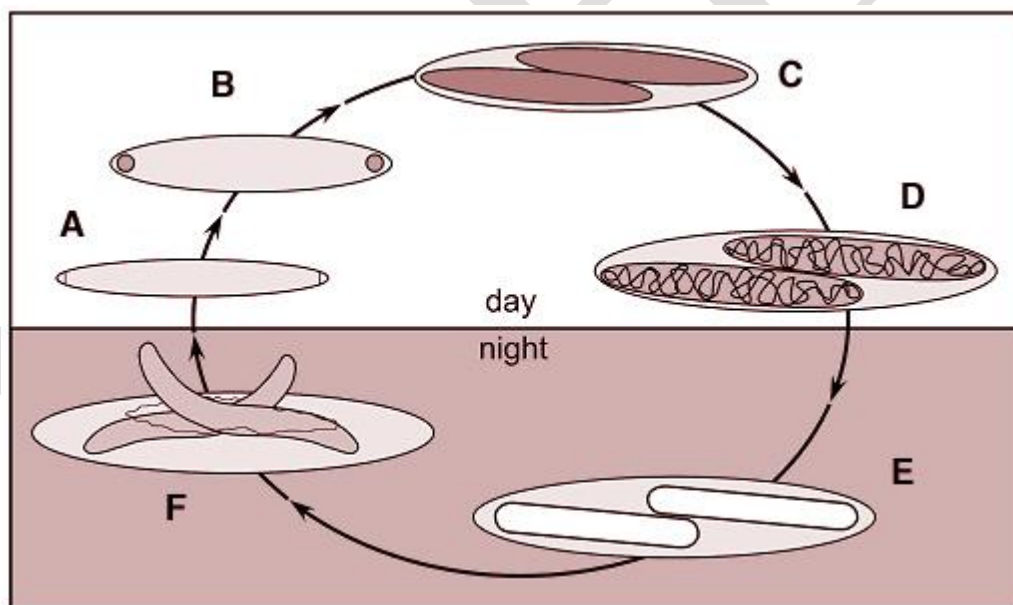


the largest being over 4000 times larger than a *Bacillus subtilis* endospore.

The formation of endospores may help maintain the symbiotic association between these *Epulopiscium*-like symbionts and their surgeonfish hosts. Since endospore formation coincides with periods in which the host surgeonfish is not actively feeding, the cells do not need to compete for the limited nutrients present in the gut at night.

The protective properties of the endospores also allow them to survive passage to new surgeonfish hosts. The fish may also benefit from this relationship because it is able to maintain stable microbial populations that assist in digestion and may receive a nutritional gain from microbial products released during mother cell death and spore germination.

Daily life cycle of endospore-forming *Epulopiscium*-like symbionts.



Possible Questions

1. What is meant by Microbial Growth kinetics
2. Explain Bacterial growth Curve
3. Define Coulter counter
4. Write about Viable cell counting techniques ?
5. Write in detail about Alkalophiles
6. Describe the general characteristics of Psychrotrophs
7. What are the Factors affecting growth of bacteria
8. Write about the General characteristics of mesophiles and thermophiles
9. Explain Bacterial reproduction
10. Discuss in detail about the Bacterial transformation and transduction

UNIT V

SYLLABUS

Water Microbiology Bacterial pollutants of water, coliforms and non-coliforms. Sewage composition and its disposal. Food Microbiology: Important Microorganism in food Microbiology: Important Microorganism in food Microbiology: Moulds, Yeast, bacteria. Major food born infections and intoxications Preservation of various types of foods Fermented foods

Water microbiology is concerned with the microorganisms that live in water, or can be transported from one habitat to another by water.

Water can support the growth of many types of microorganisms. This can be advantageous. For example, the chemical activities of certain strains of yeasts provide us with beer and bread. As well, the growth of some bacteria in contaminated water can help digest the poisons from the water.

For example, bacteria that live in the intestinal tracts of humans and other warm blooded animals, such as *Escherichia coli*, *Salmonella*, *Shigella*, and *Vibrio*, can contaminate water if feces enters the water.

Contamination of drinking water with a type of *Escherichia coli* strain O157:H7 can be fatal. The intestinal tract of warm-blooded animals also contains viruses that can contaminate water and cause disease. Examples include rotavirus, enteroviruses, and coxsackievirus. Another group of microbes of concern in water microbiology are protozoa.

The two protozoa of the most concern are *Giardia* and *Cryptosporidium*. They live normally in the intestinal tract of animals such as beaver and deer.

Giardia and *Cryptosporidium* form dormant and hardy forms called cysts during their life cycles. The cyst forms are resistant to chlorine, which is the most popular form of drinking water disinfection, and can pass through the filters used in many water treatment plants. If ingested in drinking water they can cause debilitating and prolonged diarrhea in humans, and can be life threatening to those people with impaired immune systems.

Many microorganisms are found naturally in fresh and saltwater. These include bacteria, cyanobacteria, protozoa, algae, and tiny animals such as rotifers. These can be important in the food chain that forms the basis of life in the water. For example, the microbes called

cyanobacteria can convert the energy of the sun into the energy it needs to live. The plentiful numbers of these organisms in turn are used as food for other life. The algae that thrive in water is also an important food source for other forms of life.

A variety of microorganisms live in fresh water. The region of a water body near the shoreline (the littoral zone) is well lighted, shallow, and warmer than other regions of the water. Photosynthetic algae and bacteria that use light as energy thrive in this zone. Further away from the shore is the limnetic zone.

Photosynthetic microbes also live here. As the water deepens, temperatures become colder and the oxygen concentration and light in the water decrease. Now, microbes that require oxygen do not thrive. Instead, purple and green sulfur bacteria, which can grow without oxygen, dominate. Finally, at the bottom of fresh waters (the benthic zone), few microbes survive. Bacteria that can survive in the absence of oxygen and sunlight, such as methane producing bacteria, thrive.

Saltwater presents a different environment to microorganisms. The higher salt concentration, higher pH, and lower nutrients, relative to freshwater, are lethal to many microorganisms. But, salt loving (halophilic) bacteria abound near the surface, and some bacteria that also live in freshwater are plentiful (i.e., *Pseudomonas* and *Vibrio*).

Bacterial pollutants of water :

For human beings, the critical issue when using water is hygiene. More than 4 million people die of illnesses contacted through microorganisms, and most cases are caused by water contaminated by microorganisms. There are many forms of water use in daily life, but the greatest threat to human life occurs when there is direct contact between water and human beings, for example bathing spots where sewage is mixed into the water, office buildings that treat and recycle waste water from toilets for reuse, and water works that use river water as the water supply source. In such cases, microorganisms that affect human health greatly include pathogenic bacteria, pathogenic viruses, pathogenic protozoa, and cyanobacteria (following Table).

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: I B.Sc Biotechnology COURSE NAME: GENERAL MICROBIOLOGY

COURSE CODE: 19BTU203 UNIT: V WATER MICROBIOLOGY BATCH-2019-2022

	Microorganisms	Disease
Bacteria	<i>Salmonella typhi</i>	typhoid
	<i>Salmonella choleraesuis</i>	typhoid, gastroenteritis
	<i>Salmonella enteritidis</i>	typhoid, gastroenteritis
	<i>Shigella</i> sp	dysentery
	<i>Vibrio cholerae</i>	cholera
	<i>Camplobacter jejuni</i>	enteritis
	<i>intestinal pathogenic coliform</i>	gastroenteritis
Virus	<i>Mycobacterium tuberculosis</i>	tuberculosis
	rotavirus	gastroenteritis
	poliovirus	infantile paralysis
	<i>Cryptosporidium</i>	typhoid
Protozoa	<i>Giardia</i>	typhoid
	<i>Entamoeba</i>	dysentery
	<i>Microcystis</i>	liver disorder
Algae	<i>Aphanizomenon</i>	nervous disorder
	<i>Anabaena</i>	nervous disorder
	<i>Cylindrospermopsis</i>	liver disorder

Harmful microorganism in water environment

These pathogenic microorganisms reproduce within the body and infect the body. Cyanobacteria that produce toxic substances, on the other hand, do not reproduce inside the body, but infect the body when more than the tolerable volume of toxic substances that it produces is ingested through contaminated tap water.

Water contamination caused by pathogenic microorganisms and microorganisms that produce toxic substances has become a serious problem. Toxic microorganisms are becoming increasingly common in eutrophic or polluted water, and such problems must be solved. This part explains separately at bacteria, viruses, and pathogenic protozoa that cause contamination, and also discusses the elimination of pathogenic microorganisms during biological treatment of sewage water.

Coliforms and non coliforms

Coliform is a general term that includes multiple genera like *Klebsiella*, *Enterobacter* etc. Coliform bacteria are Gram negative & ferment lactose. Any other genera are, therefore, Non Coliform bacteria, for example *Salmonella* & *Shigella*.

Fecal Coliform (coliform present in the intestines of organisms) are often used as Indicator bacteria, that is, its presence or absence classifies drinking water as unfit or fit for human consumption respectively. *E. coli* is one such indicator bacteria. Its presence in drinking water sample indicates impurity in the water, probably due to interaction with sewage water. Hence, when initial test for *E. coli*/

Total coliform are positive for a drinking water sample, we carry out specific test for other fecal bacteria like *Pseudomonas aeruginosa* which are often pathogenic. Also some strains of *E. coli* may be pathogenic even though most strains are harmless.

Bacteria that are currently targeted by Japanese water quality standards are general bacteria, total coliforms, and fecal coliforms. The standards are determined depending on how the water is used. One of the most widely used of the various water standards in Japan is the number of total coliforms.

The number of general bacteria is regulated by the Waterworks Law water quality standard (following table), while fecal coliforms are regulated only for public recreational waters. This is because in recreational waters, there is a high possibility of water being ingested orally, and so it is important to prevent contamination by pathogenic microorganisms to maintain water quality. It should also be noted that when treated water from sewage works is recycled for use as water for sprinklers or landscaping, where human beings may come in direct contact with the water, the water quality standard requires that no total coliforms be detected.

Standard for bacteria in drinking water, water environment and effluent

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: IB.Sc Biotechnology COURSE NAME: GENERAL MICROBIOLOGY

COURSE CODE: 19BTU203 UNIT: V WATER MICROBIOLOGY BATCH-2019-2022

Standard	Objective bacteria	value
World Health Organization, guideline for drinking water quality	Total coliform or	0 • 100ml-1
	fecal coliform	
Drinking water quality based on waterworks law in Japan	General bacteria	Less than 100 • l-1
	Total coliform	No detection
Environmental standards for lakes and reservoirs in Japan	Total coliform	Less than 1000 MPN • 100ml-1 for drinking water resource and bathing
Effluent standard based on water pollution control law in Japan	Total coliform	3000 • ml-1

As seen from these examples, water quality standards depend on how the water is to be used, but in general when human beings come in direct contact, the water quality is confirmed using fecal coliform numbers. When lakes, reservoirs and ground water are contaminated by pathogenic bacteria, or are not completely sterilized by the water purification treatment, they may become a source of infectious diseases.

Typical water-borne infectious diseases include cholera and dysentery, which took the lives of many from the 19th century to the first half of the 20th century.

Modern water systems and water sterilization efforts have slowed the spread of such water-borne infectious diseases, but there was a major cholera outbreak in 1991 which led to many deaths in Central and South America, and in Africa. The cause is believed to have been poor treatment systems of waterworks and sewage sewer, and deteriorating living conditions.

Vibrio cholerae is the bacteria causing cholera, while *Shigella* causes dysentery. Pathogenic colon *bacillus*, *Campylobacter*, *Clostridium*, *Salmonella*, and *Staphylococcus* are some of the bacteria known to cause water-borne infectious diseases. All of these pathogenic bacteria infect the human intestine, are released into the outside environment with excrement, pass through the treatment process, and are released into rivers, lakes and reservoirs.

Escherichia coli O157 and other fecal coliforms are increasingly causing water-borne infectious diseases in recent years in Japan. O157 is part of the pathogenic colon bacillus which produces verotoxin and causes hemorrhagic colitis, an intense form of diarrhea, hemolytic uraemia syndrome, and thrombotic thrombocytopenic purpura, in cases leading to death. The

prevention of infection by such pathogenic bacteria is of utmost importance. Japanese regulations concerning bacteria levels for drinking water safety are detailed in ministry orders on water quality standards, and require that in 1 ml of test water, the number of colony of general bacteria created is under 100, that no total coliforms are detected, and that residual concentration of free chlorine at the faucet is 0.1 mg • l-1 or more.

Sewage composition

‘ Sewage’ is a collective noun used to represent liquid or solid wastes carried in sewers. It consists of domestic water-borne wastes including human and animal excrete, washing waters and everything that goes down the drains of a town or a city. It also consists of industrial water-borne wastes as well as ground, surface and atmospheric waters which enter the sewerage system.

The amount of sewage produced in our country is of the order of 3.61 million cubic metres/day (about 800 million gallons/day). About 30% of the above amount comes from urban areas. It is estimated that only about 20% of one day sewage production of our country is treated and utilized, and the rest (about 80%) still remains untreated and unutilized.

Composition of Sewage:

The composition of sewage mainly depends upon per capita consumption of water and varies from place to place and season to season.

Microbial Composition

The microbial population per millilitre of sewage may vary from a few lacs to several millions. Various types of microorganisms, viz., micro-fungi, bacteria and protozoa, collectively called ‘sewage fungus’, are known to grow profusely in sewage.

In addition, viruses and many micro-algal genera have also been recorded from sewage. Bacteria occurring in sewage are mainly intestinal and soil inhabiting and their common types are coliforms, *streptococci*, *Clostridia*, *micrococci*, *Proteus*, *Pseudomonas*, and *lactobacilli*.

Disposal of Sewage:

Sewage disposal has become of prime importance now-a-days as it brings undesirable and harmful effects on living beings. Untreated or inadequately treated sewage is generally disposed of into natural water reservoirs without taking its pros and cons into account.

It is so either because we are indifferent to the consequences or because we assume that the water reservoirs are sufficiently large and so located that sewage-dilution prevents hazards.

However, we can no longer rely on disposed-sewage dilution in our natural water reservoirs; the solution of sewage pollution is not its dilution. It is necessary, therefore, that the sewage must be treated before its disposal so that we can, on one hand, save organisms including men from bad effects and, on the other hand, can utilize it to the maximum for our welfare.

Disposal of sewage as such or inadequately treated one, generally leads to following consequences:

- (i) Frequent dissemination of water-borne disease causing microorganisms in large number.
- (ii) Depletion of dissolved oxygen in water leading to anoxic (oxygen-less) condition which may ultimately kill O₂ dependent aquatic life.
- (iii) Creation of offensive odour and debris-accumulation due to which value of property decreases.
- (iv) Increased danger of swimming in water and diminished value of water for other recreational purposes.

Food Microbiology

Important Microorganism in food Microbiology

Molds:

Mold growth on foods, with its fuzzy or cottony appearance, sometimes colored, is familiar to everyone, and usually food with a moldy or "mildewed" food is considered unfit to eat. Special molds are useful in the manufacture of certain foods or ingredients of foods. Thus, some kinds of cheese are mold-ripened, e.g., blue, Roquefort, Camembert, Brie, Gammelost, etc., and molds are used in making Oriental foods, e.g., soy sauce, miso, shoyu, and other discussed later. Molds have been grown as food or feed and are employed to produce products used in foods, such as

amylase for bread making or citric acid used in soft drinks. Some molds do produce various toxic metabolites (mycotoxins).

General characteristics of molds:

The term "mold" is a common one applied to certain multicellular filamentous fungi whose growth on foods usually is readily recognized by its fuzzy or cottony appearance. Colored spores are typical of mature mold of some kinds and give color to part or all of the growth. The thallus, or vegetative body, is characteristic of thallophytes, which lack true roots, stems, and leaves.

Morphological Characteristics:

Hyphae and Mycelium The mold thallus consists of a mass of branching, intertwined filaments called hyphae (singular hypha), and the whole mass of these hyphae is known as the mycelium.

The hyphae may be submerged, or growing within the food, or aerial, or growing into the air above the food.

Molds are divided into two groups: septate, i.e., with cross walls dividing the hypha into cells; and noncoenocytic, septate with the hyphae apparently consisting of cylinders without cross walls. The non-septate hyphae have nuclei scattered throughout their length and are considered multicellular.

Special, mycelial structures or parts aid in the identification of molds. Examples are the rhizoids, or "holdfasts," of *Rhizopus* and *Absidia*, the foot cell in *Aspergillus*, and the dichotomous, or Y-shaped, branching in *Geotrichum*.

Reproductive Parts or Structures.

Molds can grow from a transplanted piece of mycelium. Reproduction of molds is chiefly by means of asexual spores. Some molds also form sexual spores. Such molds are termed "perfect" and are classified as either *Oomycetes* or *Zygomycetes* if nonseptate, or *Asco-mycetes* or *Basidiomycetes* if septate, in contrast to "imperfect" molds, the Fungi Imperfecti (typically septate), which have only asexual spores.

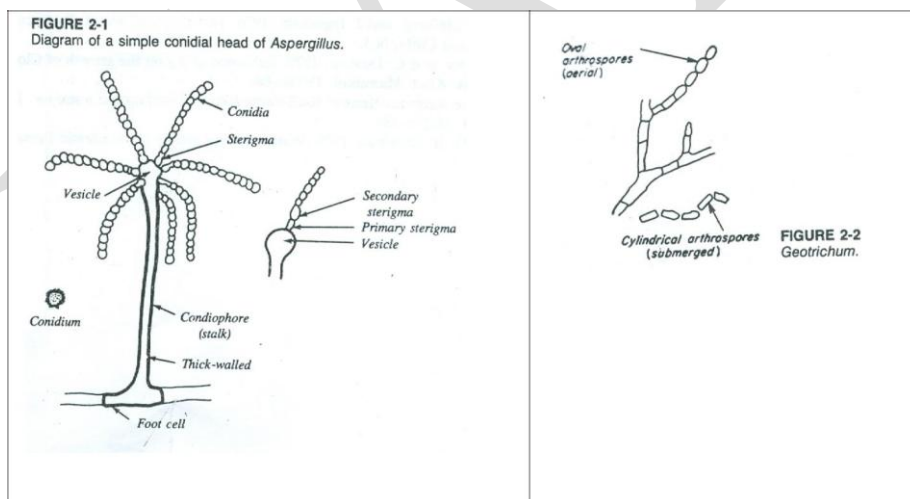
Asexual Spores

The asexual spores of molds are produced in large numbers and are small, light, and resistant to drying. They are readily spread through the air to alight and start new mold thallus where conditions are favorable.

The three principal types of asexual spores are (1) conidia (singular conidium), (2) arthrospores or oidia (singular oidium), and (3) sporangiospores. Conidia are cut off, or bud, from special fertile hyphae called conidiophores and usually are in the open, i.e., not enclosed in any container, in contrast to the sporangiospores, which are in sporangium (plural sporangia), or sac, at the tip of a fertile hypha, the sporangiophore. Arthrospores are formed by fragmentation of a hypha, so that the cells of the hypha become arthrospores.

Examples of these three kinds of spores will be given in the discussion of important genera of molds. A fourth kind of asexual spore, the chlamydospore, is formed by many species of molds when a cell here and there in the mycelium stores up reserve food, swell, and forms a thicker wall than that of surrounding cells.

This chlamydospore, or resting cell, can withstand unfavourable conditions better than ordinary mold mycelium can and later, under favorable conditions, can grow into a new mold



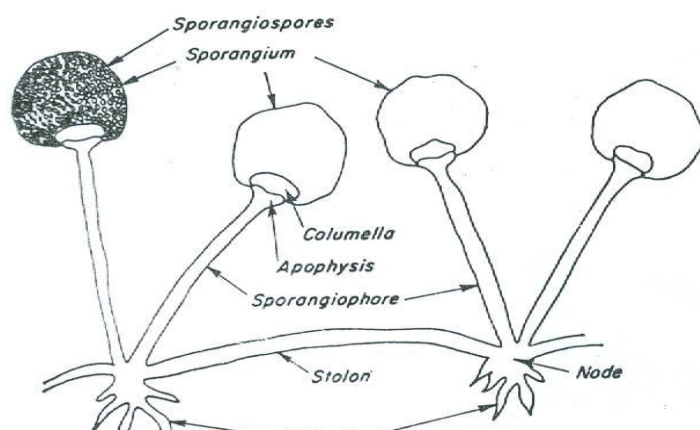


FIGURE 2-3
Rhizopus.

Sexual Spores: The molds which can produce sexual spores are classified on the basis of the manner of formation of these spores and the type produced. The non septate molds (*Phycomycetes*) that produce.

1. Oospores are termed *Oomycetes*. These molds are mostly aquatic; however, included in this group are several important plant pathogens. The oospores are formed by the union of a small male gamete and a large female gamete.

2. Zygospores: *Zygomycetes* form zygospor

es by the union of the tips of two hyphae which often appear similar and which may come from the same mycelium or from different mycelia. Both Oospores and zygospor

es are covered by a tough wall and can survive drying for long periods. **3. Ascospores:** The *Ascomycetes* (septate) form sexual spores known as ascospores, which are formed after the union of two cells from the same mycelium or from two separate mycelia. The ascospores, resulting from cell division after conjugation, are in an ascus, or sac, with usual eight spores per ascus.

4. Basidiospores: The *Basidiomycetes*, which include most mushrooms, plant rusts, smuts, etc., form a fourth type of sexual spore, the basidiospor

Cultural Characteristics

Some molds are loose and fluffy; others are compact. Some look velvety on the upper surface, some dry and powdery, and others wet or gelatinous. Definite zones of growth in the thallus distinguish some molds, e.g., *Aspergillus niger*.

Pigments in the mycelium-red, purple, yellow, brown, gray, black, etc - are characteristic, as are the pigments of masses of asexual spores; green, blue-green, yellow, orange, pink, lavender, brown, gray, black, etc. The appearance of the reverse side of a mold on an agar plate may be striking, like the opalescent blue-black or greenish-black color of the underside of *Cladosporium*.

Physiological characteristics:

The physiological characteristics of molds will be discussed briefly.

Moisture Requirements In general most molds require less available moisture than do most yeasts and bacteria. An approximate limiting total moisture content of a given food for mold growth can be estimated, and therefore it has been claimed that below 14 to 15 percent total moisture in flour or some dried fruits will prevent or greatly delay mold growth.

Temperature Requirements Most molds would be considered mesophilic i.e., able to grow well at ordinary temperatures. The optimal temperature for most molds is around 25 to 30⁰ C, but some grow well at 35 to 37⁰C or above, e.g., *Aspergillus spp.*, and some at still higher temperatures. A number of molds are psychrotrophic; i.e., they grow fairly well at temperatures of refrigeration, and some can grow slowly at temperatures below freezing. Growth has been reported at as low as - 5 to - 10⁰C. A few are thermophilic; i.e., they have a high optimal temperature.

Oxygen and pH Requirements Molds are aerobic; i.e., they require oxygen for growth; this is true at least for the molds growing on foods. Most molds can grow over a wide range of hydrogen-ion concentration (pH 2 to 8.5), but the majority are favored by an acid pH.

Food Requirements Molds in general can utilize many kinds of foods, ranging from simple to complex. Most of the common molds possess a variety of hydrolytic enzymes, and some are grown for their amylases, pectinases, proteinases, and lipases.

Inhibitors Compounds inhibitory to other organisms are produced by some molds, such as *penicillin* from *Penicillium chrysogenum* and clavacin from *Aspergillus clavatus*. Certain chemical compounds are mycostatic, inhibiting the growth of molds (sorbic acid, propionates, and acetates are examples), or are specifically fungicidal, killing molds.

Classification and identification of molds

Molds are plants of the kingdom Myceteae. They have no roots, stems, or leaves and are devoid of chlorophyll. They belong to the Eumycetes, or true fungi, and are subdivided further to subdivisions, classes, orders, families, and genera.

The following criteria are used chiefly for differentiation and identification of molds:

- 1 Hyphae septate or non-septate
- 2 Mycelium clear or dark (smoky)
- 3 Mycelium colored or colorless
- 4 Whether sexual spores are produced and the type: oospores, zygospores, or ascospores
- 6 Characteristics of the spore head
 - a) Sporangia: size, color, shape, and location
 - b) Spore heads bearing conidia: single conidia, chains, budding conidia, or masses; shape and arrangement of sterigmata or phialides; gumming together of conidia
- 7 Appearance of sporangiophores or conidiophores: simple or branched, and if branched the type of branching; size and shape of columella at tip of sporangiophore; whether conidiophores are single or in bundles
- 8 Microscopic appearances of the asexual spores, especially of conidia: shape, size, color; smooth or rough; one-, two-, or many-celled
- 9 Presence of special structures (or spores): stolons, rhizoids, foot cells, apo-physis, chlamydospores, sclerotia, etc.

Molds of Industrial Importance

Mucor: *Mucor* are involved in the spoilage of some foods and the manufacture of others. A widely distributed species is *M. racemosus*; *M. rouxii* is used in the "Amylo" process for the saccharification of starch, and mucors help ripen some cheese, (e.g., Gammelost) and are used in making certain Oriental foods.

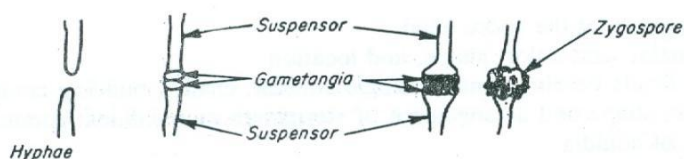


FIGURE 2-5
Zygospore formation in *Mucor*.

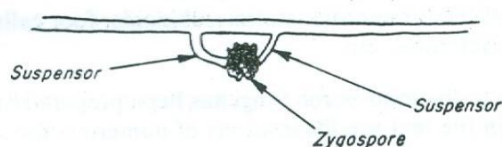


FIGURE 2-6
Zygospore formation in *Zygorrhynchus*.

FIGURE 2-8
Diagram of *Absidia*, showing locations of sporangiophores and rhizoids.

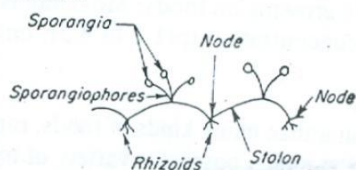


FIGURE 2-9
Thamnidium.

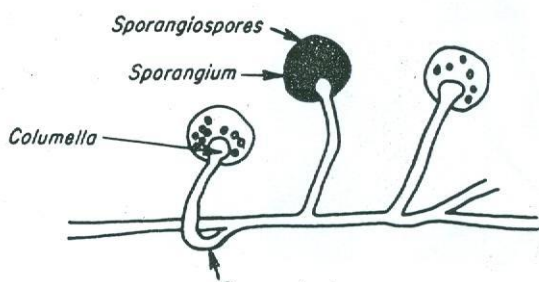
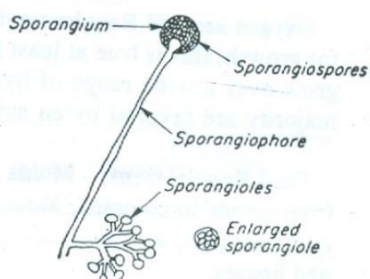


FIGURE 2-4
Mucor.

zygorrhynchus These soil molds are similar to *Mucor* except that the zygo-spore suspensors are markedly unequal in size.

Rhizopus *Rhizopus stolonifer*, the so-called bread mold, is very common and is involved in the spoilage of many foods: berries, fruits, vegetables, bread, etc.

Absidia: Similar to *Rhizopus*, except that sporangia are small and pear-shaped.

Thamnidium: *Thamnidium elegans* is found on meat in chilling storage, causing "whiskers" on the meat.

Aspergillus: The aspergilli are very widespread. Many are involved in the spoilage of foods, and some are useful in the preparation of certain foods. The molds grow well in high concentrations of sugar and salt and hence in many foods of low moisture content. Conidia of this group are some shade of green. *Eurotium*, a name reserved for members having a perfect (sexual) stage.

The *A. niger* group, with *A. niger* as a leading species, is widespread and may be important in foods. The spore-bearing heads are large, tightly packed, and globular and may be black, brownish-black, or purple-brown.

The *A. flavus-oryzae* group includes molds important in the making of some Oriental foods and the production of enzymes. Conidia give various yellow to green shades to the spore heads, and dark sclerotia may be formed.

Penicillium: This is another genus that is widespread in occurrence and important in foods. The genus is divided into groups and subgroups, and there are numerous species. The genus is divided into large groups on the basis of the branching of the spore-bearing heads, or penicilli (little brushes). These heads, or verticillata, are a whorl or cluster of three or more elements: sterigmata, metulae (subbranches), and branches.

P. expansum, the blue-green-spored mold, causes soft rots of fruits. Other important species are *P. digitatum*, with olive, or yellowish-green conidia, causing a soft rot of citrus fruits; *P. italicum*, called the "blue contact mold" with blue green conidia, also rotting citrus fruit; *P. camemberti*, with grayish conidia, useful in the ripening of Camembert cheese; and *P. roqueforti*, with bluish-green conidia, aiding in the ripening of blue cheeses, e.g., *Roquefort*.

Trichothecium The common species, *T. roseum* (Figure 2-14), is a pink mold which grows on wood, paper, fruits such as apples and peaches, and vegetables such as cucumbers and cantaloupes.

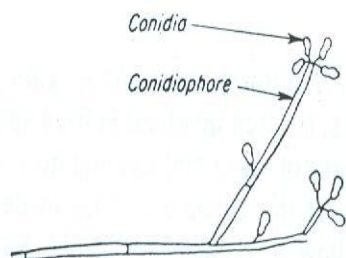


FIGURE 2-16
Sporotrichum.

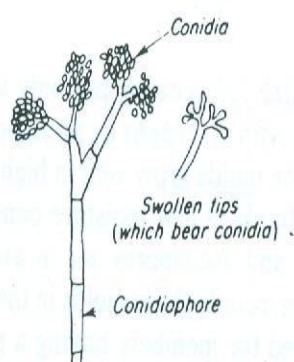


FIGURE 2-17
Botrytis cinerea.

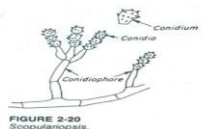


FIGURE 2-20
Scopulariopsis.

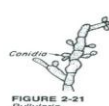


FIGURE 2-21
Pullularia.

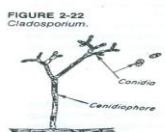


FIGURE 2-22
Cladosporium.

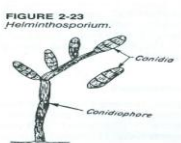


FIGURE 2-23
Helminthosporium.

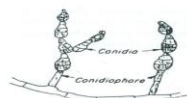


FIGURE 2-24
Alternaria.



FIGURE 2-25
Stemphylium.

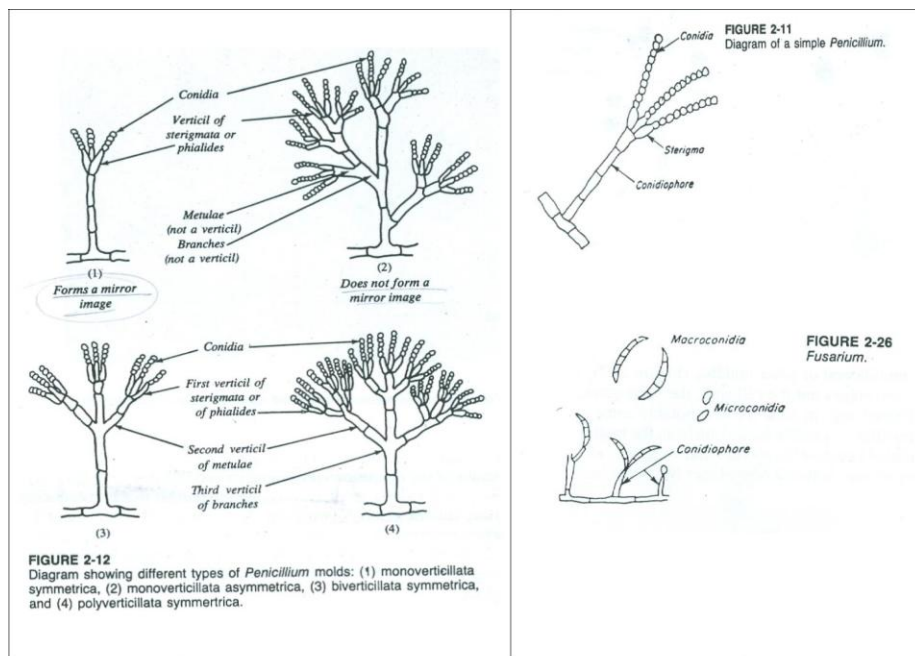


FIGURE 2-14
Trichothecium.

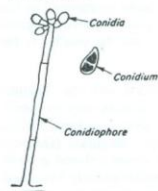


FIGURE 2-15
Neurospora (Monilia).

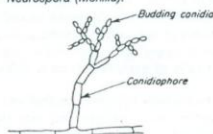


FIGURE 2-18
Cephalosporium.

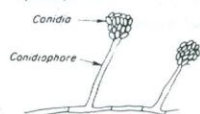
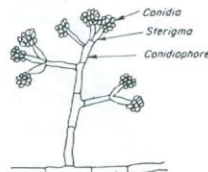


FIGURE 2-19
Trichoderma.



Sporotrichum Among the saprophytic species is *S. carnis*, found growing on chilled meats, where it causes "white spot."

Botrytis One species important in foods is *B. cinerea*. It causes a disease of grapes but may grow saprophytically on many foods.

Cephalosporium: *Cephalosporium acremonium* is a common species.

Trichoderma *T. viride* is a common species. The mature mold plant is bright green because the balls of green conidia are glued together, and tufts of white hyphae (sterile) stick up well above the conidiophores.

Scopulariopsis *S. brevicaulis* is common species. Colonies are brownish and cottony.

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Pullularia: Ovate, hyaline conidia (blastospores or buds from preexisting cells) borne as lateral buds on all parts of the mycelium. Colonies are pale and slimy and yeastlike when young, becoming mycelial and dark and leathery in age. *P. pullulans* is a common species.

Cladosporium *C. herbarum* is a leading species. These dark molds cause "black spot" on a number of foods, on cellar walls, etc. Colonies of *C. herbarum* are restricted in growth and are thick, velvety, and olive - to gray-green; the reverse side of the plant is a striking opalescent blue-black to greenish-black.

Helminthosporium Species of this genus are for the most part plant pathogens but may grow saprophytically on vegetable materials

Alternaria Molds of this genus are common causes of the spoilage of foods. *A. citri* (rotting citrus fruits), *A. tenuis*, and *A. brassicae* common species.

Stemphylium: This, too, is a common genus, The conidia are dark and multicellular but have, fewer cross - walls than those of *Alternaria* and are rounded at both ends.

Fusarium Molds of this genus often grow on foods. The species are very difficult to identify, and the appearance of growth is variable.

Endomyces Yeast like fungi, forming mycelium and arthrospores. Some species rot fruits.

Monascus Colonies of *M. purpureus* are thin and spreading and reddish or purple in color. Found on dairy products and on Chinese red rice (ang -khak).

Sclerotinia Some species cause rots of vegetables and fruits, where they are present in the conidial stage. The lemon-shaped conidia are in chains, with a "plug" separating conidia.

Yeasts and Yeast like fungi – General characteristics of yeasts, classification and identification of yeasts, yeasts of industrial importance Yeasts and yeast like fungi

Like mold, the term "yeast" is commonly used but hard to define. It refers to those fungi which are generally not filamentous but unicellular and ovoid or spheroid and which reproduce by budding or fission.

Yeasts may be useful or harmful in foods. Yeast fermentations are involved in the manufacture of foods such as bread, beer, wines, vinegar, and surface ripened cheese, and yeasts are grown for enzymes and for food. Yeasts are undesirable when they cause spoilage of sauerkraut, fruit juices, syrups; molasses, honey, jellies, meats, wine, beer, and other foods.

General characteristics of yeasts

Yeasts are classified chiefly on their morphological characteristics, although their physiological ones are more important to the food microbiologist.

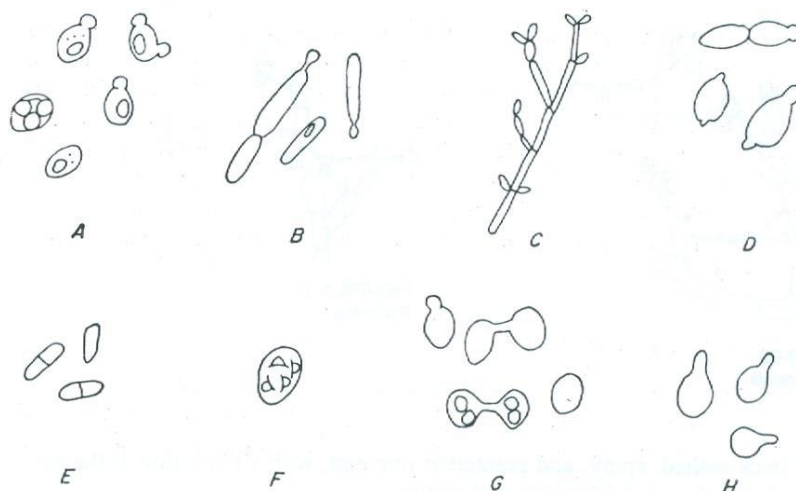
Morphological Characteristics

The morphological characteristics of yeasts are determined by microscopic examination.

Form and Structure The form of yeasts may be spherical to ovoid, lemon shaped, pear-shaped, cylindrical, triangular, or even elongated into a false or true mycelium. They also differ in size. Visible parts of the structure are the cell wall, cytoplasm, water vacuoles, fat globules, and granules, which may be metachromatic, albuminous, or Starchy. Special staining is necessary to demonstrate the nucleus.

Reproduction

Most yeasts reproduce asexually by multilateral or polar budding, a process in which some of the protoplasm bulges out the cell wall; the bulge grows in size and finally walls off as a new yeast cell. In some yeasts, notably some of the film yeasts, the bud appears to grow from a tube like projection from the mother cell. Replicated nuclear material is divided between the mother and daughter cells. A few species of yeasts reproduce by fission, and one reproduces by a combination of fission and budding.

**FIGURE 2-27**

Yeasts of different shapes: (A) *Saccharomyces cerevisiae*, with budding cells and one ascus with four ascospores, (B) *Candida* yeast, with elongated cells, (C) *Candida*, showing pseudomycelium, (D) apiculate (lemon-shaped) yeast, (E) *Schizosaccharomyces*, multiplying by fission, (F) *Hansenula*, with ascospores shaped like derby hats, (G) *Zygosaccharomyces*, showing conjugation with ascus and four ascospores, (H) flask-shaped yeasts.

Cultural Characteristics

Growth as a film on the surface of liquid media suggests an oxidative or film yeast, and production of a carotenoid pigment indicates the genus *Rhodotorula*. The appearance of the growth is important when it causes colored spots on foods. It is difficult to tell yeast colonies from bacterial ones on agar plates; the only certain way is by means of microscopic examination of the organisms. Most young yeast colonies are moist and somewhat slimy but may appear mealy; most colonies are whitish, but some are cream-colored or pink. Some colonies change little with age, but others become dry and wrinkled.

Yeasts are oxidative, fermentative, or both. The oxidative yeasts may grow as a film, pellicle, or scum on the surface of a liquid and then are termed film yeasts. Fermentative yeasts usually grow throughout the liquid and produce carbon dioxide.

Physiological Characteristics

Most common yeasts grow best with a plentiful supply of available moisture. But since many yeasts grow in the presence of greater concentrations of solutes (such as sugar or salt) than most bacteria.

Most yeast requires more moisture than molds, however. on the basis of water activity or a_w , yeasts may be classified as ordinary if they do not grow in high concentrations of solutes, i.e., in a low a_w , and as osmophilic if they do.

Lower limits of a_w for ordinary yeasts range from 0.88 to 0.94.

Osmophilic yeasts have been found growing slowly in media with an a_w as low as 0.62 to 0.65 in syrups, although some osmophilic yeasts are stopped at about 0.78 in both salt brine and sugar syrup.

The a_w values will vary with the nutritive properties of the substrate, pH, temperature, availability of oxygen, and presence or absence of inhibitory substances.

The range of temperature for growth of most yeasts is 25 to 30⁰C and the maximum about 35 to 47⁰ C. Some kinds can grow at 0⁰ C or less. The growth of most yeasts is favored by an acid reaction in the vicinity of pH 4 to 4.5, and they will not grow well in an alkaline medium unless adapted to it. Yeasts grow best under aerobic conditions, but the fermentative types can grow anaerobically, although slowly.

In general, sugars are the best source of energy for yeasts, although oxidative yeasts, e.g., the film yeasts, oxidize organic acids and alcohol. Carbon dioxide produced by bread yeasts accomplishes the leavening of bread, and alcohol made by the fermentative yeasts is the main product in the manufacture of wines, beer, industrial alcohol, and other products. The yeasts also aid in the production of flavors or "bouquet" in wines.

Nitrogenous foods utilized vary from simple compounds such as ammonia and urea to amino acids and polypeptides. In addition, yeasts require accessory growth factors.

Yeasts may change in their physiological characteristics, especially the true, or ascospore-forming, yeasts, which have a sexual method of reproduction. These yeasts can be bred for certain characteristics or may mutate to new forms. Most yeasts can be adapted to conditions which previously would not support good growth. Illustrative of different characteristics within a species is the large number of strains of *Saccharomyces cerevisiae* suited to different uses, e.g., bread strains, beer strains, wine strains, and high-alcohol-producing strains or varieties.

Classification and identification of yeasts

The true yeasts are in the subdivision *Ascomycotina*, and the false, or asporogenous, yeasts are in the subdivision *Fungi Imperfecti* or *Deuteromycotina*. Certain yeasts are actually represented in two different genera based on whether they reproduce sexually.

The principal bases for the identification and classification of genera of yeasts are as follows:

- 1 Whether ascospores are formed.
- 2 If they are spore-forming:
 - a) The method of production of ascospores:
 - (1) Produced without conjugation of yeast, cells (parthenogenetically). Spore formation may be followed by
 - (a) Conjugation of ascospores. (b) Conjugation of small daughter cells.
 - (2) Produced after isogamic conjugation (conjugating cells appear similar).
 - (3) Produced by heterogamic conjugation (conjugating cells differ in appearance).
 - b) Appearance of ascospores: shape, size, and color. Most spores are spheroidal or ovoid, but some have odd shapes, e.g., most species of *Hansenula*, which look like derby hats
 - c) The usual number of ascospores per ascus: one, two, four, or eight.
- 3 Appearance of vegetative cells: shape, size, color, inclusions.
- 4 Method of asexual reproduction:
 - a. Budding. b. Fission. c. Combined budding and fission. d. Arthrospores (oidia).
5. Production of a mycelium, pseudo mycelium, or no mycelium.
6. Growth as a film over surface of a liquid (film yeasts) or growth throughout medium.
- 7 Color of macroscopic growth.
- 8 Physiological characteristics (used primarily to differentiate species or strains within a species):
 - a. Nitrogen and carbon sources. b. Vitamin requirements. c. Oxidative or fermentative: film yeasts are oxidative; other yeasts may be fermentative or fermentative and oxidative.
 - d. Lipolysis; urease activity, acid production, or formation of starch like compounds.

Yeasts of industrial importance

Most yeasts used industrially are in the genus *Saccharomyces*. The term "wild yeast" is applied to any yeast other than the one being used or encouraged. Thus yeast employed in one process could be a wild yeast in another. Most of the troublesome wild yeasts are asporogenous, or false, yeasts.

Genus *Schizosaccharomyces* These yeasts, which reproduce asexually by fission and form four or eight ascospores per ascus after isogamic conjugation, have been found in tropical fruits, molasses, soil, honey, and elsewhere. A common species is *S. pombe*.

Genus *Saccharomyces* Cells of these yeasts may be round, ovate, or elongated and may form a pseudo-mycelium. Reproduction is by multipolar budding or by ascospore formation. The ascospores, one to four per ascus, are usually round or ovate. The leading species, *S. cerevisiae*, is employed in many food industries, with special strains used for the leavening of bread, as top yeasts for ale, for wines, and for the production of alcohol, glycerol, and invertase.

Top yeasts are very active fermenters and grow rapidly at 20⁰ C. The clumping of the cells and the rapid evolution of CO₂ sweep the cells to the surface, hence the term top yeast.

Bottom yeast do not clump, grow more slowly, and are best fermenters at lower temperatures (10 to 15⁰ C). The absence of clumping and the slower growth and evolution of CO₂ permit the yeast to settle to the bottom, hence the term bottom yeast. These characteristics of brewers' yeast are observations and do not explain why some yeast clump, or flocculate.

S. cerevisiae var. *ellipsoideus* is a high-alcohol-yielding variety used to produce industrial alcohol, wines, and distilled liquors. *S. uvarum*, a bottom yeast, is used in making beer. *S. fragilis* and *S. Lactis*, because of their ability to ferment lactose, may be important in milk or milk products. *S. rouxii* and *S. mellis* are osmophilic.

Many of the *Saccharomyces* have been reclassified. For example, *S. uvarum* is now considered a variant of *S. cerevisiae*, *S. fragilis* is now *Kluyveromyces marxianus*, and *S. lactis* is now *K. marxianus* var. *lactis*.

S. rouxii, *S. mellis*, and *S. nussbaumeri* are now *Zygosaccharomyces rouxii*. *Debaryomyces hansenii* is now *D. hansenii*.

Genus *Kluyveromyces*. These yeasts reproduce by multilateral budding, and ascospores are liberated upon maturity.

Genus *Zygosaccharomyces*. These yeasts are notable for their ability to grow in high concentrations of sugar (hence they are termed osmophilic) and are involved in the spoilage of honey, sirups, and molasses and in the fermentation of soy sauce and some wines. *Zygosaccharomyces nussbaumeri* grows in honey.

Genus *Pichia* These oval to cylindrical yeasts may form pseudomycelia.

Ascospores are round or hat-shaped, and there are one to four per ascus. A pellicle is formed on liquids; e.g., *P. membranaefaciens* grows a pellicle on beers or wines.

Genus *Hansenula* These yeasts resemble *Pichia* in appearance but are usually more fermentative, although some species form pellicles. Ascospores are hat- or Saturn-shaped. **Genus *Debaryomyces*** These round or oval yeasts form pellicles on meat brines. Ascospores have a warty surface. *D. kloeckeri* grows on cheese and sausage.

Genus *Hanseniaspora* These lemon-shaped (apiculate) yeasts grow in fruit juices. *Nadsonia* yeasts are large and lemon-shaped.

False Yeasts (Fungi Imperfect)

Genus *Torulopsis* These round to oval fermentative yeasts with multilateral budding cause trouble in breweries and spoil various foods. *T. sphaerica* ferments lactose and may spoil milk products. Other species can spoil sweetened condensed milk, fruit-juice concentrates, and acid foods.

Genus *Candida* These yeasts form pseudohyphae or true hyphae, with abundant budding cells or blastospores, and may form chlamydospores. Many form films and can spoil foods high in acid and salt. *C. utilis* is grown for food and feed. *C. krusei* has been grown with dairy starter cultures to maintain the activity and increase the longevity of the lactic acid bacteria. Lipolytic *C. lipolytica* can spoil butter and oleomargarine.

Genus *Brettanomyces* These ogive - or arch-shaped yeasts produce high amounts of acid and are involved in the late fermentation of Belgian lambic beer and English beers. They also are found in French wines. *B. bruxellansis* and *B. lambicus* are typical species.

Genus *Kloeckera* These are imperfect apiculate or lemon-shaped yeasts. *K. apiculata* is common on fruits and flowers and in the soil.

Genus *Trichosporon* These yeasts bud and form arthrospores. They grow best at low temperatures and are found in breweries and on chilled beef. *T. pullulans* is a common species.

Genus *Rhodotorula* These red, pink, or yellow yeasts may cause discolorations on foods, e.g., colored spots on meats or pink areas in sauerkraut.

Apiculate or lemon-shaped yeasts, in *Saccharomyces*, *Hanseniaspora*, *Nadsonia*, and *Kloeckera*, are considered objectionable in wine fermentations because they give off-flavors, low yields of alcohol, and highly volatile acid.

Osmophilic yeasts (*Saccharomyces rouxii* and *S. mellis*) grow well in an environment of high osmotic pressure, i.e., in high concentrations of sugars, salts, or other solutes, causing spoilage of dry fruits, concentrated fruit juices, honey, maple sirup, and other high-sugar solutions.

Salt-tolerant yeasts grow in curing brines, salted meats and fish, soy sauce, miso paste, and tamari sauce. The most salt-tolerant of the film yeasts are species of *Debaryomyces*, which grow on curing brines and on meats and cucumbers in them, as does *Saccharomyces rouxii*, which can grow as a film on brine. Yeasts in various other genera (*Torulopsis*, *Brettanomyces*, and others) also grow in brines. Yeasts grow in soy sauce with its high content of salt (about 18 percent). *Saccharomyces rouxii* is considered of great importance in the production of alcohol and flavor, but species of *Torulopsis*, *Pichia*, *Candida*, and *Trichosporon* also may grow. Film-forming *S. rouxii* and *Pichia* are sometimes involved in the spoilage of soy sauce. Similar yeasts are involved in miso production, but kinds will vary as the salt concentration is varied between 7 and 20 percent.

Bacteria – Morphological characteristics important in Food Bacteriology. Cultural and Physiological characteristics important in food bacteriology. Genera of bacteria important in Food Bacteriology groups of bacteria important in food bacteriology Bacteria

Morphological characteristics important in food bacteriology

One of the first steps in the identification of bacteria in a food is microscopic examination to ascertain the shape, size, aggregation, structure, and staining reactions of the bacteria present. The following characteristics may be of special significance.

Encapsulation

The presence of capsules or slime may account for sliminess or ropiness of a food. In addition, capsules serve to increase the resistance of bacteria to adverse conditions, such as heat or chemicals. To the organism they may serve as a source of reserved nutrients. Most capsules are polysaccharides of dextrin, dextran, or levan.

Formation of Endospores

Bacteria of the genera *Bacillus*, *Clostridium*, *Desulfotomaculum*, *Sporolactobacillus* (rods), and *Sporosarcina* (cocci) share the ability to form endospores. *Bacillus* - aerobic and some facultative anaerobic and *Clostridium* - anaerobic.

Endospores are formed at an intracellular site, are very refractile, and are resistant to heat, ultraviolet light, and desiccation.

Formation of Cell Aggregates

It is characteristic of some bacteria to form long chains and of others to clump under certain conditions. It is more difficult to kill all bacteria in intertwined chains or sizable clumps than to destroy separate cells.

Bacterial growth in and on foods often is extensive. Pigmented bacteria cause discolorations on the surfaces of foods; films may cover the surfaces of liquids; growth may make surfaces slimy; or growth throughout the liquids may result in undesirable cloudiness or sediment.

Physiological characteristics important in food bacteriology

These changes include hydrolysis of complex carbohydrates to simple ones; hydrolysis of proteins to polypeptides, amino acids, and ammonia or amines; and hydrolysis of fats to glycerol and fatty acids. O-R reactions, which are utilized by the bacteria to obtain energy from foods

(carbohydrates, other carbon compounds, simple nitrogen-carbon compounds, etc.), yield such products as organic acids, alcohols, aldehydes, ketones, and gases.

Genera of bacteria important in food bacteriology

The classification given in Bergey's Manual of Systematic Bacteriology, vols. I and II, 1984 and 1986, will be followed.

Genus *Acetobacter* These bacteria oxidize ethyl alcohol to acetic acid. They are rod-shaped and motile and are found on fruits, vegetables, souring fruits, and alcoholic beverages. They are a definite spoilage problem in alcoholic beverages.

Genus *Aeromonas* These are gram-negative rods with an optimum temperature for growth of 22 to 28 C. They are facultative anaerobes and can be psychrophilic. They are frequently isolated from aquatic environments. *A. hydrophila* can be a human pathogen; it is also pathogenic to fish, frogs, and other mammals.

Genus *Alcaligenes* As the name suggests, an alkaline reaction usually is produced in the medium of growth. *A. viscolactis*. causes ropiness in milk, and *A. metalcaligenes* gives a slimy growth on cottage cheese. These organisms come from manure, feeds, soil, water, and dust. This genus also contains organisms which were formerly classified in the genus *Achromobacter*.

Genus *Alteromonas* Several former species of *Pseudomonas* are now classified as *Alteromonas*. They are marine organisms that are potentially important in sea foods.

Genus *Arthrobacter* A predominant soil organism, it is inert in most foods. However, some species can grow at 5⁰ C and would be considered psychrotrophs.

Genus *Bacillus* The endospores of species of this aerobic to facultative genus usually do not swell the rods in which they are formed. Different species may be mesophilic or thermophilic, actively proteolytic, moderately proteolytic, or non proteolytic, gas-forming or not, and lipolytic or not. In general the spores of the mesophiles, e.g., *B. subtilis*, are less heat-resistant than spores of the thermophiles. Spores of the obligate thermophiles, e.g., *B. stearothermophilus*, are more

resistant than those of facultative thermophiles, e.g., *B. coagulans*. The actively proteolytic species usually may also sweet-curdle milk; *B. cereus* is such a species. The two chief acid- and gas-forming species, *B. polymyxa* and *B. macerans*, sometimes are termed "aerobacilli." Many of the mesophiles can form acid from glucose or other sugar but usually form only a small amount that often is neutralized by ammonia produced from the nitrogenous food. The thermophilic flat sour bacteria that spoil canned vegetables can produce considerable amounts of lactic acid from sugar, and such a culture, e.g., *B. coagulans*, may be employed for the manufacture of lactic acid. The soil is an important source of *Bacillus* species.

Genus *Brevibacterium* *B. linens* is related to *Arthrobacter globiformis* and may be synonymous. *B. linens* may be important in the surface smear of certain cheeses, e.g., brick or Limburger, where the culture produces an orange red pigmentation and helps ripening.

Genus *Brochotrix* These are gram-positive rods which can form long filamentous like chains that may fold into knotted masses. The optimum temperature for growth is 20 to 25⁰C, but growth can occur over a temperature range of 0 to 45⁰C depending on the strain. They can spoil a wide variety of meats and meat products when they are stored aerobically or vacuum packed and held refrigerated. *B. thermosphacta* is the only species listed.

Genus *Campylobacter* These bacteria were originally classified in the genus vibrio. Several strains of *C. fetus subsp. jejuni* have been associated with gastroenteritis in humans.

Genus *Clostridium* The endospores of species of this genus of anaerobic to microaerophilic bacteria usually swell the end or middle of the rods in which they are formed. Different species may be mesophilic or thermophilic and proteolytic or non-proteolytic. *Clostridium thermosaccharolyticum* is an example of a saccharolytic obligate thermophile; this organism causes gaseous spoilage of canned vegetables. Putrefaction of foods often is caused by mesophilic, proteolytic species, such as *C. lentoputrescens* and *C. putrefaciens*. The violent disruption of the curd in milk by *C. perfringens* or similar species results in a "stormy fermentation" of milk, and the lactate fermenting *C. butyricum* is a cause of late gas in cured cheese. The soil is the primary source of *Clostridium* spp., although they also may come from bad silage, feeds, and manure.

Genus *Corynebacterium* The diphtheria organism, *C. diptheriae*, may be transported by foods. *C. bovis*, with the slender, barred, or clubbed rods characteristic of the genus, is commensal on the cow's udder, can be found in aseptically drawn milk, and may be a cause of bovine mastitis.

Genus *Dessulfotomaculum* A gram, negative rod which swells when an endospore forms. They are common inhabitants of the soil, fresh water, and the rumen. Sulfur compounds can serve as the terminal electron acceptor in respiration and thereby be reduced to hydrogen sulfide. *Clostridium nigrificans*, which is responsible for sulfide stinker spoilage in canned foods, is now called *D. nigrificans*.

Genus *Enterobacter* Some were formerly classified as *Aerobacter*. They are widely distributed in nature; a member of the coliform group.

Genus *Erwinia* The species of this genus are plant pathogens that cause necrosis, galls, wilts, or soft rots in plants and therefore damage the plants and vegetable and fruit products from them. *E. carotovora* is associated with the market disease called "bacterial soft rot." *E. carotovora subsp. carotovora* causes rotting in a large number of plants. *E. carotovora subsp. atroseptica* produces a black rot in potatoes. *E. carotovora subsp. betavascularum* causes soft rot in sugar beets.

Genus *Escherichia* Found in feces, a predominant gram-negative rod isolated from the intestinal tract of warm-blooded animals and widely distributed in nature. One of the "coliform group," the genus is divided into many biotypes and serotypes, some of which can be pathogenic to humans.

Genus *Flavobacterium* The yellow to orange-pigmented species of this genus may cause discolorations on the surface of meats and be involved in the spoilage of shellfish, poultry, eggs, butter, and milk. Some of the organisms are psychrotrophic and have been found growing on thawing vegetables.

Genus *Gluconobacter* (Formerly *Acetomonas*). Species can oxidize ethanol to acetic acid. *G. oxydans* causes ropiness in beer following viscous growth in beer or wort.

Genus *Halobacterium* Bacteria of this genus, e.g., *H. salinarium*, are obligate halophiles and are usually chromogenic. They may grow and cause discolorations on foods high in salt, such as salted fish.

Genus *Klebsiella* Many are capsulated. Commonly associated with the respiratory and intestinal tracts of humans. *K. pneumoniae* is the causative organism for a bacterial pneumonia in humans.

Family Lactobacillaceae

Genus *Lactobacillus* The lactobacilli are rods, usually long and slender, that form chains in most species. They are microaerophilic, (some strict anaerobes are known), are catalase-negative and gram-positive, and ferment sugars to yield lactic acid as the main product.

Homofermentation: They ferment sugar chiefly to lactic acid if they are homo-fermentative, with small amounts of acetic acid, carbon dioxide, and trace products. The homo-fermentative lactobacilli with optimal temperatures of 37 C or above include *L. bulgaricus**, *L. helveticus*, *L. lactis**, *L. acidophilus*, *L. thermophilus*. The homo-fermentative lactobacilli with lower optimal temperatures include *L. casei*, *L. plantarum*, and *L. leichmannii**

Heterofermentation: If they are hetero-fermentative, they produce appreciable amounts of volatile products, including alcohol, in addition to lactic acid. *L. delbrueckii*, *L. fermentum* is the chief example of a hetero-fermentative lactobacillus growing well at higher temperatures. Hetero-fermentative species grow at lower temperatures are *L. brevis*, *L. buchneri*, *L. pastorianus**, *L. hilgardii*, and *L. trichodes**.

All the above species except *L. delbrueckii*, *L. leichmannii*, *L. hilgardii*, *L. trichodes**, and some strains of *L. brevis* ferment lactose with the production of lactic acid and therefore may be of importance in the dairy industries. Chief sources of the lacto-bacilli are plant surfaces, manure, and dairy products.

Characteristics that make the lactobacilli important in foods are

- (1) their ability to ferment sugars with the production of considerable amounts of lactic acid, making it possible to use them in the production of fermented plant and dairy products or the manufacture of industrial lactic acid but resulting in the deterioration of some products, e.g., wine or beer,
- (2) Production of gas and other volatile products by hetero fermentative species, sometimes with damage to the quality of food, as with *L. fermentum* growing in Swiss cheese or *L. hilgardii* or *L. trichodes** in wines.

(3) Their inability to synthesize most of the vitamins they require, making them unable to grow well in foods poor in vitamins but useful in assays for the vitamin content of foods.

(4) The heat resistance, or thermotolerant properties, of most of the high-temperature lactobacilli, enabling them to survive pasteurization or other heating processes, such as that given the curd in the manufacture of Swiss and similar cheeses.

Species of *Lactobacillus* different from the ones already mentioned have been found growing in refrigerated meats, but only a few names for these lactobacilli have been suggested, e.g., *L. viridescens* for one causing greening of sausage and *L. salinmandus** for one growing in sausage. These lactobacilli are exceptional because of their ability to grow at low temperatures.

Genus *Leuconostoc* This genus, called *Betacoccus* by Orla-Jensen, contains the heterofermentative lactic streptococci, which ferment sugar to lactic acid plus considerable amounts of acetic acid, ethyl alcohol, and carbon dioxide. The ability of *L. dextranicum** and *L. cremoris** to ferment citric acid of milk and produce the desirable flavoring substance diacetyl and to stimulate the lactic streptococci. This led to their inclusion as "lactic starter" for buttermilk, butter, and cheese. The habitat of this genus is the surface of plants.

Some of the characteristics of *Leuconostoc* species that make them important in foods are

- (1) Production of diacetyl and other flavoring products,
- (2) Tolerance of salt concentrations, e.g., in sauerkraut and dill-pickle fermentations, permitting *L. mesenteroides* to carry on the first part of the lactic fermentation,
- (3) Ability to initiate fermentation in vegetable products more rapidly than other lactics or other competing bacteria and to produce enough acid to inhibit nonlactics,
- (4) Tolerance of high sugar concentrations (up to 55 to 60 percent for *L. mesenteroides*), permitting the organism to grow in sirups, liquid cake and ice-cream mixes, etc.,
- (5) production of considerable amounts of carbon dioxide gas from sugars, leading to undesirable "openness" in some cheeses, spoilage of foods high in sugars (sirups, mixes, etc.), and leavening in some breads,

(6) Heavy slime production in media containing sucrose. This is a desirable characteristic for the production of dextran but a hazard in materials high in sucrose, as in the production of sucrose from sugarcane or beets.

Genus *Listeria* These bacteria are gram-positive, non-spore-forming rods with tumbling motility. *L. monocytogenes* can cause food-borne disease outbreaks.

Genus *Microbacterium* Bacteria of this genus are important because of their resistance to adverse conditions and their use in production of vitamins. They are small, nonmotile, gram-positive, asporogenous, catalase-positive, aerobic, homo-fermentative, lactic acid-producing rods, which sometimes produce palisade arrangements. *M. tacticum* is the species usually encountered. Microbacteria are very resistant to heat for non-spore-forming bacteria, surviving pasteurization of milk readily and even temperatures of 80 to 85⁰ C for 10 min. They therefore are among the thermodurics.

Genus *Micrococcus* Most of the species prominent in foods are gram-positive, aerobic, and catalase-positive. The following characteristics make various groups of micrococci important in foods:

- (1) Some species can utilize ammonium salts or other simple nitrogenous compounds as a sole source of nitrogen,
- (2) Most species can ferment sugars with the production of moderate amounts of acid,
- (3) Some are acid-proteolytic (*M. freudenreichii**),
- (4) some are very salt tolerant and hence able to grow at relatively low levels of available moisture; these grow in meat-curing brines, brine tanks, etc.,
- (5) Many are thermoduric, i.e., survives the pasteurization treatment given market milk (*M. varians*),
- (6) Some are pigmented and discolor the surfaces of foods on which they grow; *M. luteus* is yellow, for example, and *M. roseus* is pink, and
- (7) Some of the micrococci can grow fairly well at temperatures around 10 C or below.

Micrococci are widespread in nature but have been isolated most often from dust and water. They often are found on inadequately cleaned and sanitized food utensils and equipment.

Genus *Mycobacterium* The tubercle bacilli that cause tuberculosis, *M. tuberculosis*, has been spread by foods, especially raw milk from infected cows.

Genus *Pediococcus* The cocci occur singly, in pairs or short chains, or in tetrads (division in two planes) and are gram-positive, catalase-negative, and microaerophilic. They are homo-fermentative. The characteristics that make the organism important in foods have been mentioned: salt tolerance, acid production, and temperature range, especially the ability to grow at cool temperatures. *Pediococcus* have been found growing during the fermentation of brined vegetables and have been found responsible for the spoilage of alcoholic beverages, e.g., beer, where their production of diacetyl is undesirable. *P. damnosus* can spoil beer. *P. cerevisiae* has been used as starter culture in fermented sausages.

Genus *Photobacterium* The genus includes coccobacilli and occasional rods which can be luminescent. They are not widespread; however, *P. phosphoreum* has been known to cause phosphorescence of meats and fish.

Genus *Propionibacterium* Members of this genus may be found in foods. In Swiss cheese certain species (e.g., *Propionibacterium freudenreichii*) ferment the lactates to produce the gas that helps form the holes or eyes, and also contribute to the flavor. Pigmented propionic bacteria can cause color defects in cheese.

Genus *Proteus* Bacteria of this genus have been involved in the spoilage of meats, seafood, and eggs. The presence of these bacteria in large numbers in non refrigerated foods has made them suspect as a cause of food poisoning.

Genus *Pseudomonas* A number of species of *Pseudomonas* can cause food spoilage. These bacteria are gram-negative, usually motile, asporogenous rods.

Characteristics of some of the *Pseudomonas* species that make them important in foods are

- (1) Their ability to utilize a large variety of non carbohydrate carbon compounds for energy and their inability to use most carbohydrates.
- (2) Their ability to produce a variety of products that affect flavor deleteriously, (3) their ability to use simple nitrogenous foods.
- (4) Their ability to synthesize their own growth factors or vitamins.

- (5) The proteolytic and lipolytic activity of *I*, some species.
- (6) Their aerobic tendencies, enabling them to grow rapidly and produce oxidized products and slime at the surfaces of foods, where heavy contamination is most likely.
- (7) Their ability to grow well at low (refrigeration) temperatures.
- (8) Pigment production by some species, e.g., the greenish fluorescence by pyoverdinin of *Pseudomonas fluorescens* and white, cream-colored, reddish, brown, or even black (*P. nigrificiens**) colors of other species.
- (9) Their resistance to many disinfectants and sanitizers used in the food industry.

On the other hand, the pseudomonads are limited by a fairly high a_w (0.97 to 0.98), are readily killed by heat, grow poorly if oxygen is not readily available, are not especially resistant to drying, and grow poorly or not at all above 43 °C.

Genus *Salmonella* Species of these enteric pathogens may grow in foods and cause food infections.

Genus *Serratia* Many species produce a pink or magenta pigment and may cause red discolorations on the surface of foods. *S. marcescens* is the most common species.

Genus *Shigella* Species of *Shigella*, causing bacillary dysenteries, may be transported by foods.

Genus *Sporolactobacillus* *Lactobacillus inulinus* has been classified as *S. inulinus* because of its ability to form endospores. It resembles *Lactobacillus* in many characteristics.

Genus *Sporosarcina* A gram-positive coccus that forms endospores. *S. ureae* and *S. halophila* are the two species listed.

Genus *Staphylococcus* The gram-positive staphylococci grow singly, in pairs, in tetrads, or in irregular, grapelike clusters. The most important species, *S. aureus*, usually gives yellow to orange growth, although it may be white on occasion. Many of the beta-hemolytic, coagulase-positive strains are pathogenic, and some produce an enterotoxin which causes food poisoning.

Genus *Streptococcus* The cocci in this genus are characteristically in pairs, in short chains, or in long chains, depending on the species and the conditions of growth, and all are homofermentative. The streptococci important in foods are divided into four groups: the pyogenic, viridans, lactic, and, enterococcus groups.

The pyogenic (pus-producing) group pathogenic streptococci, of which *S. agalactiae*, a cause of mastitis in cows, and *S. pyogenes*, a cause of human septic sore throat, scarlet fever, and other diseases are representatives that have been found in raw milk. The pyogenic streptococci cannot grow at 10 or 45⁰C.

The viridans groups includes *S. thermophilus*, a coccus important in cheeses making at high temperatures and yogurt, and *S. bovis* is like *S. thermophilus* and it is thermoduric and therefore counted in the plating of pasteurized milk. These species can grow at 45⁰C but not at 10⁰C.

The lactic group contains the important dairy bacteria, *S. lactis* and *S. cremoris**, grow at 10⁰C but not at 45⁰C. These bacteria are used as starters for cheese, cultured buttermilk, and some types of butter, along with *Leuconostoc spp.*, and *S. lactis* often is concerned in the souring of raw milk.

The enterococcus group consists of *S. faecalis* and *S. faecium*, and some related subspecies. *S. faecalis* is usually the more heat-resistant and comes more from human sources, whereas *S. faecium* has been reported to be more from plant sources. *S. faecalis* subsp. *liquefaciens** is an acid proteolytic variety of *S. faecalis*, and *S. faecalis* subsp. *zymogenes** is a beta-hemolytic variety. Bacteria of this group can grow at both 10 and 45⁰C.

The enterococci have several characteristics in common that make them unusual streptococci:

- (1) They are thermoduric, readily surviving the pasteurization treatment of milk or even more heating.
- (2) They tolerate 6.5 percent and more of salt.
- (3) They can grow at the alkaline pH of 9.6.
- (4) They can grow over a wide range of temperatures, some multiplying at as low as 5 to 8⁰C and most of them at as high as 48 to 50⁰C.

The term "fecal streptococci" is often used in the food industry to describe those enterococci which are used as indicator organisms.

Genus *Streptomyces* Members of this genus can cause undesirable flavors and appearance when growing on foods; musty or earthy odors and tastes from these organisms may be absorbed by nearby foods when growth of the *Streptomyces* is near at hand.

Genus *Vibrio* Bacteria in this genus are widely distributed in fresh and salt water, in soil, and in the alimentary canal of humans and animals. Some are moderately halophilic. Some species are pathogenic to humans.

Genus *Yersinia* Can be found in the soil. *Y. pestis* is the causative-organism of plague in humans and in rats and other rodents. Some strains of *Y. enterocolitica* are also pathogenic and causative agents of food-borne disease outbreaks.

Groups of Bacteria important in food Bacteriology

1. Lactic acid – forming bacteria or lactics:

These bacteria ferment sugars to lactic acid. This may be desirable in making products such as sauerkraut and cheese. But undesirable in terms of spoilage of wines because they usually form acid rapidly.

Ex: *Leuconostoc*, *Lacto bacillus*, *Streptococcus* and *Pediococcus*.

2. Acetic acid forming bacteria or acetics:

Most of the acetic acid belong to two genera *Acetobacter* and *Gluconobacter*. Both oxidize ethyl alcohol to acetic acid, but *Acetobacter* is capable of oxidizing acetic acid further to CO₂. Characteristics that make acetic acid bacteria important are

1. Their ability to oxidize ethanol to acetic acid.
2. Their strong oxidising power, result in oxidation of desired product like acetic acid, by desirable sps or undesirable sps under favourable conditions.
3. Excessive sliminess of some species

Ex: *Acetobacter acetic sub sp. suboxydans*. This bacteria clog vinegar generators.

3. Butyric acid forming bacteria or butyrics:

Most bacteria of this group are spore forming anaerobes of the genus *Clostridium*.

4. Propionic acid – forming bacteria or propionics: Ex: Propionic bacterium

5. Proteolytic bacteria:

They produce extracellular proteinases proteolytic bacteria may be aerobic, facultative, spore forming, anaerobic and spore forming.

Bacillus cereus – Aerobic, spore forming

Pseudomonas fluorescens – Non spore forming and aerobic to facultative.

Clostridium sporogenes – Spore forming and anaerobic other examples are *clostridium*, *bacillus*, *proteus*. Acid proteolytic bacteria carry on an acid fermentation and proteolysis simultaneously.

Ex: *Streptococcus faecalis var. liquefaciens*

Micrococcus caseolyticus are acid proteolytic.

Some bacteria are putrefactive i.e., they decompose proteins anaerobically to produce foul smelling compounds such as H₂S, mercaptans, amines, indole and fatty acids.

Ex: *Clostridium*, *Proteus*, *Pseudomonas*.

4. Lipolytic Bacteria:

This bacteria produce lipases which catalyze the hydrolysis of fats to fatty acids and glycerol. Many of the aerobic, actively proteolytic bacteria also are lipolytic.

Pseudomonas fluorescens – Strongly lipolytic

Pseudomonas, *Alcaligenes*, *Staphylococcus*, *Serratia* and *Micrococcus* are genera that contain lipolytic bacteria.

5. Saccharolytic bacteria:

These bacteria hydrolyze disaccharides or polysaccharides to simpler sugars. Amylolytic bacteria possess amylase to bring about the hydrolysis of starch outside the cell. Amylolytic bacteria are *Bacillus subtilis* and *Clostridium butyricum*.

6. Pectinolytic Bacteria:

Pectins are complex carbohydrates that are responsible for cell wall rigidity in vegetables and fruits pectic substances derived from citrus fruits can be used in commercial products as gelling agents.

Ex: *Erwinia*, *Bacillus*, *Clostridium*, *Achromobacter*, *Aeromonas*, *Arthrobacter*, *Flavobacterium*.

7. Thermophilic Bacteria or Thermophiles:

Optimum temperature required for these bacteria 45°C - 55°C. *Bacillus stearothermophilus* – thermophilic flat sour spoilage of low acid canned foods.

8. Thermotolerant Bacteria:

Thermotolerant bacteria are usually defined as those which can survive a heat treatment such as pasteurization. Ex: *Bacillus* sps, Micrococci, Enterococci can survive pasteurization of

liquid eggs. Fungi like *Byssoschlamys fulva*, *Aspergillus* and *Penicillium* are thermotolerant. Some thermotolerant bacteria like *Bacillus* and enterococci can also be psychrotrophic.

9. Psychrotrophic Bacteria or psychrotrophs:

These bacteria are able to grow at commercial refrigeration temperatures. Unlike psychrophiles, psychrotrophs do not have their optimal temperature for growth at refrigeration temperature and their optimum between 25°C and 30°C.

Ex: *Pseudomonas*, *Flavobacterium*, *Achromobacter* and *Alcaligenes*, *Micrococcus*, *Lactobacillus* etc.

10. Halophilic Bacteria or Halophiles:

Halophilic Bacteria require certain minimal concentrations of dissolved sodium chloride for growth.

Ex: *Pseudomonas*, *Moraxella*, *Acinetobacter*, *Flavobacterium*, *Vibrio* spp which grow best in media with 0.5 – 3.0 percent salt. These micro organisms are isolated from fish shell fish. These are slightly halophilic.

Moderate halophiles are grown in the media.

Containing 3.0 – 15% salt salted fish, brined fish, brined meats and some salted vegetables.

Extreme halophiles grow in the heavily brined foods 15 – 30% salt.

EX: *Halobacterium*, *Halococcus*.

Other bacteria are salt tolerant i.e., halotolerant bacteria can grow with or without salt. Usually they are capable of growing in foods containing 5.0% salt or more. Ex: *Bacillus*, *Micrococcus*, *Corynebacterium*, *Streptococcus* and *Clostridium* spp., *Sarcina*, *pediococcus*, *Alcaligenes*.

11. Osmophilic or Saccharophilic Bacteria:

Osmophilic bacteria are those which grow in high concentrations of sugar. Ex: *Leuconostoc*.

12. Pigmented Bacteria:

Colors produced by pigmented bacteria growing on or in foods.

Flavobacterium – Yellow to orange; *Serratia* – Red; *Halobacterium* – Pink

13. Slime or rope forming bacteria:

Alcaligenes viscolactis, *Enterobacter aerogenes* & *Klebsiella oxytoca* causes ropiness of milk and *Leuconostoc* spp., producing slime in sucrose solutions and slimy surface growth of various bacteria occurring on foods.

Streptococcus & *Lactobacillus* make milk slimy or ropy. *Micrococcus* makes curing solutions for meats ropy. *Lactobacillus plantarum* and *Lactobacilli* may cause ropiness in various fruit, vegetable and grain products e.g. in cider, sauerkraut and beer.

14. Gas forming Bacteria:

Many kinds of bacteria produce small amounts of gas and yield it slowly.

Ex: *Leuconostoc*, *Lactobacillus* (heterofermentative), Propionic bacterium, *Escherichia*, *Enterobacter*, *Proteus*, *Bacillus* and *Clostridium*.

Leuconostoc, *Lactobacillus*, *Propionibacterium*, produces only CO₂.

Other genera produce both CO₂ and H₂.

15. Coliform and Fecal coliform group:

Coliforms are short rods that are defined as aerobic and facultative anaerobic, gram negative, non spore forming bacteria. Ex: *Escherichia coli*, *Enterobacter aerogenes*.

Fecal coliform group includes coliforms capable of growing at 44 - 45°C.

Geotrichum candidum is the machinery mold and as an indicator of plant sanitation and contaminated equipment. Some of the characteristics that make the coliform bacteria important in food spoilage are

1. Their ability to grow well in a variety of substrates and synthesise most of the necessary vitamins.
2. Their ability of the group to grow well over a fairly wide range of temperatures from below 10°C to about 46°C.
3. Their ability to produce considerable amounts of acid and gas from sugars.
4. Their ability to cause off – flavours often described as unclean or barny.
5. Their ability of *E. aerogenes* to cause sliminess or ropiness of foods.

Food borne infection and intoxications

Bacillus Cereus – Foodborne Intoxication

Found

Widely distributed in nature; can be isolated from meats, milk, vegetables, and fish.

Transmission

Bacteria produce a toxin that causes illness. Vomiting-type outbreaks have usually been associated with rice products and other starchy foods such as potatoes, pasta, and cheese products. Sauces, puddings, soups, casseroles, pastries, and salads have also been implicated in outbreaks.

Symptoms

Food poisoning is characterized by nausea and vomiting 0.5 to six hours after the ingestion of a contaminated food product. In more severe cases, abdominal cramps and diarrhea might occur with symptoms lasting up to 24 hours.

Campylobacter jejuni – Foodborne Infection

Found

Widely distributed in nature; can be isolated from meats, milk, vegetables, and fish.

Transmission

Bacteria produce a toxin that causes illness. Vomiting-type outbreaks have usually been associated with rice products and other starchy foods such as potatoes, pasta, and cheese products. Sauces, puddings, soups, casseroles, pastries, and salads have also been implicated in outbreaks.

Symptoms

Food poisoning is characterized by nausea and vomiting 0.5 to six hours after the ingestion of a contaminated food product. In more severe cases, abdominal cramps and diarrhea might occur with symptoms lasting up to 24 hours.

Clostridium botulinum – Foodborne Intoxication

Found

Widely distributed in nature; soil and water on plants and intestinal tracts of animals and fish.

Grows only in little or no oxygen.

Transmission

Bacteria produce a toxin that causes illness. Improperly canned foods, garlic in oil, vacuum-packed and tightly wrapped food.

Symptoms

Toxins affect the nervous system. Symptoms usually appear in 18 to 36 hours, but can sometimes appear as few as four hours or as many as eight days after eating. Double vision, droopy eyelids, trouble speaking and swallowing, and difficulty breathing may occur. Can be fatal in three to 10 days if not treated.

Clostridium perfringens

Found

Soil, dust, sewage, and intestinal tracts of animals and humans. Grows only in little or no oxygen.

Transmission

Called "the cafeteria germ" because many outbreaks result from food left for long periods in steam tables or at room temperature. Bacteria destroyed by cooking, but some toxin-producing spores may survive.

Symptoms

Diarrhea and gas pains may appear eight to 24 hours after eating; usually last about 1 day, but less severe symptoms may persist for one to two weeks.

Escherichia coli 0157:H7 – Foodborne Infection

Found

Intestinal tracts of some mammals, raw milk, unchlorinated water; one of several strains of *E. coli* that can cause human illness.

Transmission

Contaminated water, raw milk, raw or rare ground beef, unpasteurized apple juice or cider, uncooked fruits and vegetables, person-to-person.

Symptoms

Diarrhea or bloody diarrhea, abdominal cramps, nausea, and malaise; can begin two to five days after food is eaten, lasting about eight days. Some, especially the very young, have developed Hemolytic Uremic Syndrome (HUS) that causes acute kidney failure. A similar illness, thrombotic thrombocytopenic purpura (TTP), may occur in older adults.

Hepatitis A virus – Foodborne Infection

Found

Hepatitis A is widely distributed throughout the world, occurring in both epidemic and sporadic cases. Hepatitis A is primarily transmitted person to person by the fecal-oral route, but common source transmission does occur.

Transmission

Hepatitis A virus is excreted in feces of infected people and can produce clinical disease when a susceptible individual consumes contaminated water or foods. Cold cuts and sandwiches, fruits, fruit juices, milk and milk products, vegetables, salads, shellfish, and iced drinks all can be considered vehicles for the transmission of Hepatitis A.

Symptoms

Hepatitis A is usually a mild illness characterized by sudden onset of fever, malaise, nausea, anorexia, and abdominal discomfort, followed by several days of jaundice.

Listeria monocytogenes – Foodborne Infection

Found

Some studies suggest that 1% to 10% of humans may be intestinal carriers of *Listeria Monocytogenes*. It has been found in at least 37 mammalian species, both domestic and feral, as well as at least 17 species of birds and possibly some species of fish and shellfish. It can be isolated from soil, silage, and other environmental sources

Transmission

Raw milks, pasteurized fluid milk, cheeses, ice cream, raw vegetables, fermented raw-meat sausages, raw and cooked poultry, raw meats, and raw and smoked fish.

Symptoms

Some studies suggest that 1% to 10% of humans may be intestinal carriers of *Listeria monocytogenes*. It has been found in at least 37 mammalian species, both domestic and feral, as well as at least 17 species of birds, and possibly some species of fish and shellfish. It can be isolated from soil, silage, and other environmental sources.

Listeria monocytogenes

Found

Intestinal tracts of humans and animals, milk, soil, leaf vegetables, and processed foods; can grow slowly at refrigerator temperatures.

Transmission

Soft cheese, raw milk, improperly processed ice cream, raw leafy vegetables, meat, and poultry. Illness caused by bacteria that do not produce toxin.

Symptoms

Fever, chills, headache, backache, sometimes abdominal pain and diarrhea; 12 hours to three weeks; may later develop more serious illness in at-risk patients (meningitis or spontaneous abortion in pregnant women); sometimes just fatigue.

Norwalk virus group – Foodborne Infection

Found

The virus has been identified in clams and oysters by radio immunoassay. It is typically shed in the feces of humans.

Transmission

Norwalk gastroenteritis is transmitted by the fecal-oral route via contaminated water and foods. Shellfish and salad ingredients are the foods most often implicated in Norwalk outbreaks. Ingestion of raw or insufficiently steamed clams and oysters poses a high risk for infection with Norwalk virus.

Salmonella (over 2,300 types)

Found

Intestinal tract and feces of animals; *Salmonella enteritidis* in raw eggs.

Transmission

Raw or undercooked eggs, poultry, and meat; raw milk and dairy products; seafood and food handlers.

Symptoms

Stomach pain, diarrhea, nausea, chills, fever, and headache usually appear eight to 72 hours after eating; may last one to two days.

Shigella (over 30 types)

Found

Human intestinal tract; rarely found in other animals.

Transmission

Person-to-person by fecal-oral route; fecal contamination of food and water. Most outbreaks result from food, especially salads, prepared and handled by workers using poor personal hygiene techniques.

Symptoms

Disease referred to as "shigellosis" or bacillary dysentery. Diarrhea containing blood and mucus, fever, abdominal cramps, chills, and vomiting; 12 to 50 hours from ingestion of bacteria; can last a few days to two weeks.

Staphylococcus aureus – Foodborne Intoxication

Found

On the skin, infected cuts, pimples, noses, and throats.

Transmission

From people to food through improper food handling. Multiply rapidly at room temperature to produce a toxin that causes illness.

Symptoms

Severe nausea, abdominal cramps, vomiting, and diarrhea can occur one to six hours after eating; recovery within two to three days—longer if severe dehydration occurs.

Vibrio parahaemolyticus and other marine *Vibrio spp.*-Food-Borne Infection

Found

Both pathogenic and non-pathogenic forms of the organism can be isolated from marine and estuarine environments and from fish and shellfish dwelling in these environments.

Transmission

Infections with this organism have been associated with the consumption of raw, improperly cooked, or cooked, recontaminated fish and shellfish.

Symptoms

Diarrhea, abdominal cramps, nausea, vomiting, headache, fever, and chills may be associated with infections caused by this organism.

Here is a brief description of the food preservation methods detailed in the book The Home Preserving Bible. Canning, freezing, and drying food are the most common methods for preserving foods at home today. However, there are many other methods, and some are easier and less expensive. Listed below is an overview of 10 methods for preserving foods, including today's popular methods, as well as other old-fashioned and ancient techniques that are worth re-visiting.

1. **Canning** is the process of heating the product at a specified temperature for a specific length of time (pasteurizing), and then vacuum sealing the pasteurized food in special glass jars designed for this purpose. It can be used with most foods, including fruits, vegetables, meats, seafood, and some prepared foods. Canning requires the purchase of reusable canning jars and rings, one-time use sealing lids, and some practice to learn the necessary and detailed steps.
2. **Freezing** is the process of chilling foods to at least 0°F. It can be used with all foods, including fruits, vegetables, meats, seafood, grains, nuts, dairy, eggs, and prepared foods. True freezing is not possible in the freezer compartment of your refrigerator where the temperature is typically much warmer, between 10°F to 32°F. Freezing is easy to do, if you can afford to buy and operate the relatively expensive appliance. For more information, read: A short primer on freezing food.
3. **Drying** is the process of dehydrating foods until there is not enough moisture to support microbial activity. It can be used with most foods, including fruits, vegetables, meats, seafood, grains, legumes, and nuts. There are several different techniques, some are relatively easy to do and require no special equipment. For more information, read: An Introduction to the Drying Food Preservation Method.

4. **Fermenting** is the process of encouraging the growth of “good bugs” to inhibit the “bad bugs” that can spoil food. It can be used with many types of foods, including fruits, vegetables, meats, seafood, grains, legumes, dairy, and eggs to produce a wide range of products such as wine (from grapes), sauerkraut (cabbage), cured sausage (meat), and yogurt (milk). Many fermented products can be produced without any special equipment. The method for each type of product is relatively easy, but requires attention to detail. For information on some of these methods, read: more articles about Fermenting Foods on this website.
5. **Pickling** is the process of soaking food in a solution containing salt, acid, or alcohol. It can be used with most foods, including fruits, vegetables, meats, seafood, legumes, and eggs. Most methods require no special equipment. However, pickled foods can be unsafe if prepared carelessly or stored at room temperature. Pickling is often combined with another method, such as fermenting, canning, or just refrigerating. Here are some simple techniques to get you started:
6. **Dry salting** is either a fermenting or pickling technique used for meat, fish, and vegetables. A low salt concentration (2½% to 5% weight of the salt per weight of the food), promotes fermentation, while a high salt concentration (20% to 25% salt), prevents microbial growth and preserves the food in a more or less fresh, although salty state. Many people familiar with the technique consider salted vegetables such as green beans to be far superior in taste and texture than canned or frozen beans. This old-fashioned method was promoted in the early twentieth century as an alternative to canning, in order to conserve glass, tin, and fuel in time of war. Here is a recipe for salted cauliflower that you can adapt to other vegetables: Salted cauliflower, peas, or green beans in brine without fermenting.
7. **Curing** is similar to pickling, and uses salt, acid, and/or nitrites. It is used for meat and fish. Simple, modern curing methods often reduce the amount of salt and nitrites, which may require that you refrigerate or freeze the final product. Shelf-stable products require the use of adequate amounts of nitrites and a complex drying process using special equipment and exacting technique. Some curing methods also employ a secondary

process such as fermenting, smoking, or sealing. For more information, read: All about brining and curing corned beef and game meat.

8. **Smoking** is a complementary process to curing that improves flavor and appearance, and can also act as a drying agent. Smoking in the home environment contributes more flavor and appearance benefits than food preservation. However, smoked meats are less likely to turn rancid or grow mold than unsmoked meats.
9. **Sealing** is a process of covering food to keep out air, which *delays* (but does not stop) the activity of spoilage organisms. It is used primarily as a complementary process to other methods such as drying or freezing. Both fat sealing and vacuum sealing methods are relatively easy. Vacuum sealing a relatively inexpensive small appliance.
10. **Cellaring** is the process of storing foods in a temperature-, humidity-, and light-controlled environment. It can be used with many foods, especially vegetables, grains, and nuts, as well as fermented foods and dry-cured meats. There are many different methods for cellaring food, all of which are relatively easy to do. Some require simple, inexpensive equipment you may already own. No matter where you live, whether in an apartment or on a farm, you can use the concept of cellaring to some degree.

Fermented foods

Fermented foods

Fermentation in food processing is the conversion of carbohydrates to alcohols and carbon dioxide or organic acids using yeasts, bacteria, or a combination thereof, under anaerobic conditions. Fermented things can be the kinds of food that people refer to as “acquired tastes.” But some of the most common things we eat and drink are fermented. The words aged and cured

should be your first clue. Beneficial microorganisms beat out the kind that act on and eat up the carbohydrates in the food. The results are interesting flavors, textures, and smells.

Types and changes during fermentation

1. Coffee

Wild yeasts and bacteria from the air eat the slimy layer, called mucilage, still covering the beans after picking. The fermentation deepens the flavor and body of the beans.

2. Cheeses

The bacteria are added to give cream or milk a sour flavor. After the curds and whey are separated and the cheese is formed into a solid shape, it's inoculated with specific kinds of mold to make specific kinds of cheese (like blue cheese) and fermented (aged) again.

3. Yogurt, Sour Cream and Buttermilk

Milk or cream is exposed to souring bacteria, either by inoculation or through the air.

4. Chocolate

After cocoa beans are picked, the pulp surrounding them ferments, darkening the beans beneath and mellowing their flavor.

5. Wine

Yeast is added to the crushed grapes or naturally occurring yeasts already on the grape skins are allowed to thrive next they convert the juice's sugar to alcohol.

6. Beer

Yeast is added to grains that have been heated, soaked, and strained (leaving a sweet, grainy liquid), which converts the sugars to alcohol. Some beers, like Belgian lambics, use naturally occurring bacteria and yeasts from the air.

7. Charcuterie

Meat is heavily salted, sometimes with curing salts containing nitrates, then hung in a cool, well-ventilated place to age. The salt and nitrates discourage harmful bacteria (like those that cause botulism) and encourage beneficial ones. Dry aged beef is also mildly fermented, having hung in the open air (but without salt).

8. Vanilla

Young beans are soaked, dried, and exposed to the air for several months to cure, whereby their rich flavor develops.

9. Vinegar

A starter bacterial culture called a mother is introduced to alcohol (beer and wine are most common), which converts it to acetic acid.

10. Bread

Yeast is introduced to flour and ferments the carbohydrates, leaving behind carbon dioxide, which leavens the bread. Sourdough bread also contains a souring bacterium present in the starter.

11. Pickles, Sauerkraut, and Kimchee

Fresh vegetables are mixed with salt, packed into airtight containers, and aged. Bacteria naturally present on the vegetables' skins help create a kind of vinegar, transforming the vegetables. Not to be confused with vinegar pickles.

12. Fish Sauce

Fish is salted, aged, and pressed, and then the rotting fish juice is mixed with spices to create this staple Southeast Asian condiment.

13. Fermented Fish

Trout or salmon is packed in salt and left for anywhere from 24 hours to a couple of months, so the outside seems cooked but the inside remains moist and deliciously raw.

14. Ginger Beer

Though most commercially available ginger beer is just soda pop with air forced into it, traditionally the drink was naturally carbonated by allowing ginger, sugar, and water to ferment. Try making CHOW's Ginger Beer.

15. Miso

A mold called *Aspergillus oryzae* is mixed with rice, barley or soybeans and then aged in wooden casks for a few months to make this Japanese flavoring paste. Tamari and soy sauce or shoyu are made the same way, but in the case of shoyu wheat is also used.

16. Tempeh

Originally created in Indonesia, this bean cake is made by cooking soybeans and inoculating them with *Rhizopus* mold. The white mold binds the beans together.

17. Natto

Slimy soybean product from Japan that is cooked beans are fermented with the bacterium *Bacillus subtilis natto* for a day, and then aged under refrigeration for a few days more.

18. Marmite

A savory brown spread from Britain often eaten on bread, made from brewer's yeast that is the byproduct of brewing beer.

19 Rumpstuf

A traditional German food, fruit is marinated with sugar and rum in an earthenware crock.

20. Century Egg

A duck egg is treated with quicklime, salt, and tea and then aged, oftentimes after being coated in clay-rich mud and rice husks for months. The egg's yolk turns gelatinous and greenish, and the white becomes amber-colored. The egg is often served cut up over porridge.

Possible Questions

1. What is meant by Water Microbiology?
2. Explain Photosynthetic Microbes
3. Define Pollutants

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: IB.Sc Biotechnology COURSE NAME: GENERAL MICROBIOLOGY

COURSE CODE: 19BTU203 UNIT: V WATER MICROBIOLOGY BATCH-2019-2022

4. Write about Coliforms and non coliforms?
5. Write in detail about Sewage and its disposal
6. Describe the general characteristics of Molds
7. List about the industrial importance of Molds
8. Write about the General characteristics of yeasts
9. Explain the industrial importance of yeasts
10. Discuss in detail about the Genera of bacteria important in food bacteriology

	Opt 1	Opt 2	Opt 3	Opt 4	Answer
Microbiology is a branch of science that deals with _____?	virus	plants	microbes	bacteria	microbes
who is the father of microbiology ?	Muller	Antony van leeuwenhoek	Louis pasteur	Robert koch	Antony van leeuwenhoek
Microbes are _____?	prokaryotes	multicellular	plants	unicellular	prokaryotes
Nuclear material with out a nuclear membrane is called _____?	nucleoid	nucleus	cytoplasm	nucleolus	nucleoid
Who discovered the concept of pasteurization?	Muller	Leeuvanhoeck	Robert koch	Louis pasteur	Louis pasteur
Who discovered bacilli?	Robert koch	Muller	Pasteur	Weigert	Robert koch
The formation of life from non living substances is called _____?	generations	genetics	theory of sapantaneous generation	symbiosis	theory of spontaneous generation
Louis Pasteur defined fermentaion as _____?	life	life with air	life without air	conversion of organic compounds	life without air
Germ plasm theory states that infectious disease are caused by _____?	bacteria	microbes	fungus	virus	microbes
The process of heating liquid s to moderate temperature for a definite time which delays the bacterial growth are known as _____?	pasteurization	sterilization	germination	fermentation	pasteurization
The flow of chemicals between living and non living things is called _____?	chemical cycle	bioloogical cycle	biochemicla cycle	biogeochemical	biogeochemical
_____ are the foods produced by the growth of microorganism?	cheese	yougurt	Mushrooms	pizza	cheese
_____ are used to absorb or adsorb heavy metals?	baker's yeast	bacilli	virus	bacteria	baker's yeast
Extra chromosomal DNA of bacteria are known as _____?	capsule	plasmid	cell wall	nucleus	plasmid
The study of bacteria is known as _____?	bacteriology	microbiology	virology	phycology	bacteriology
The study of algae is called as _____?	bacteriology	microbiology	phycology	virology	physology
Two bacteria are joined together and a portion of DNA of donor is	conjugation	transformation	transduction	gene transfo	conjugation

transferred to the recipient. This process is known as ____?				transformation	
Bacteria are _____	Gram positive	Gram negative	non motile	unicellular	Gram positive
Bacteria possess one or more long sinuous unbranched appendages called _____	capsule	flagella	nucleoid	plasmid	flagella
_____ is the mode of bacterial reproduction	transformation	transduction	conjugation	binary fission	binary fission
_____ are otherwise called as blue green algae	Cyanobacteria	Pseudomonas	Streptococcus	Bacillus	Cyanobacteria
Bright field microscope is also known as _____	Phase contrast microscope	Electron microscope	Light microscope	Fluorescent	Light microscope
The magnification of objective lens in bright field microscope is _____	45x	20x	15x	18x	45x
The magnification of ocular lens in bright field microscope is _____	5x	20x	45x	100x - 40x	5x
The efficiency of light microscope lies on its _____	magnification power	resolving power	virtual image	lens system	resolving power
The resolving power of a microscope is a function of the wavelength of light used and _____ of the lens system	magnification power	virtual image	numerical aperture	fine adjustment	numerical aperture
Dark field microscope is most useful for observing bacteria less than _____ μm	0.2	0.5	0.4	0.1	0.2
In a dark field microscope organisms appear as bright spots against _____ background	bright	dark	black	white	black
Certain chemical substances absorb light and after some time emit the light of longer wavelength and lesser energy. This phenomenon is known as _____	illumination	fluorescence	irradiation	absorption	fluorescence
In fluorescent microscope _____ is used to illuminate the specimen	radiation	UV light	fluorescent	probes	UV light
_____ microscope is used mainly to observe living unstained cells	fluorescence	light	phase contrast	electron	phase contrast
_____ devised a method of converting phase changes to produce differences in light intensity	Frederick Zernike	Robert Koch	Pasteur	Muller	Frederick Zernike
In _____ Frederick Zernike devised a method of converting phase changes to produce differences in light intensity	1928's	1923's	1930's	1940's	1930's
Electron microscopes are _____ types	3	2	4	5	2

With electron microscope it is possible to resolve objectives as small as _____ Å	20	30	40	10	10
The resolving power of the electron microscope is _____ times greater than that of the light microscope	10	100	1000	5	100
Electron microscope produces useful magnification upto _____	x 400,000	x 300,000	x 200,000	x 100,000	x 400,000
In SEM the image is created as the specimen is scanned with a beam of _____	UV	fluorescent	electron	illumination	electron
The TEM employs an electron beam generated by _____	a tungsten filament	lamp	UV	Fluorescent	a tungsten filament
The electron microscope uses electron beams and _____ to produce image	light waves	magnetic fields	glass lenses	illuminator	magnetic fields
In SEM the scattered electrons are collected by electronic devices and displayed on _____	a cathode ray tube	objective lens	magnetic field	image plan	a cathode ray tube
In SEM the specimens are coated with a thin layer of _____	gold	palladium	silver	gold or palladium	gold or palladium
In Phase contrast microscope , in living unstained condition of most microorganism are _____ in liquid medium which they are suspended	transparent	similar to medium	visible	not visible	transparent
The principle of Phase contrast microscope based on the fact that light passing through one material and into another material of a slightly different _____	refractive index	depth	color	in image	refractive index
Which is the technique used in Light microscope?	Wet mount technique	refractive	mercury	uv	wet mount technique
In fluorescent microscope _____ lamp is used as light source	tungsten	mercury	diffracted light	uv	mercury
In fluorescent microscope, the blue light is reflected downwards to the specimen by a _____ mirror	dichroic	emission	blue light	white light	dichroic
In fluorescence microscope the barrier filter allows _____ light to pass to the eye however it blocks	blue	green	white	yellow	green
Several different kind of microscope are available with magnification range from _____	100x - 400,000x	100x - 4000x	10x - 400,000x	100x - 40x	100x - 400,000x
The Phase contrast microscope	angular	annular	mirror	lens	annular

contains an _____ aperture in the condenser that forms a hollow cone of light rays				system	
In Phase contrast microscope light rays that pass through the specimen are _____ and passed outside the phase plate	reflected	deflected	refractive	reflective	deflected
In Phase contrast microscope the resulting darkening of the image increases the contrast seen by the _____	naked eye	through lens	human eye	objective lens	human eye
The Bright field microscope or light microscope is also called as	student compound microscope	compound microscope	phase contrast	dark field	student compound microscope
The examination of stained smear preparation is often called as _____ mount technique	dry	wet	liquid	solid	dry
An acid dye is one which has _____ charge in staining basic cell components	negative	positive	neutral	negative and positive	negative
An basic dye is one which has _____ charge in staining acidic cell components	negative	positive	neutral	negative and positive	positive
_____ are the dyes used for simple staining	methylene blue	nitrogen	iodine	Kovac's reagent	methylene blue
Who developed staining procedure?	Christain Gram	Muller	Robert Koch	Leeu van hoeck	Christain Gram
In _____ Christain Gram developed staining procedure	1882	1883	1884	1886	1884
_____ is used in negative staining	methylene blue	crystal violet	nigrosin	eosin	nigrosin
Unit II					
Microorganisms require _____ elements in large quantities	8	11	10	15	Richard Petri
Nutrients are transported in microorganisms by	passive diffusion	active diffusion	direct diffusion	indirect diffusion	drying
How many mechanisms are present for nutrient transport in microorganism	4	2	3	6	enrichment media
Pure culture technique is	pour plate	incubation	diffusion	serial dilution	physical method
Microorganisms are grown	culture				living

on _____ in laboratories	media				cells
Exact composition of complex media is	unknown	known	partially known	little known	freeze drying
Exact composition of defined media is	unknown	known	partially known	little known	below 100 C
The addition of which will change chemically defined media to complex media	glucose	latose	biotin	yeast extract	refriger ation
Which of the following is a macronutrient	carbon	manganese	zinc	cobalt	cryopres ervation
Which of the following is a micronutrient	carbon	manganese	magnesium	nitrogen	glycerol
The technique used to avoid all microorganisms is accomplished by	sterilization	disinfection	incineration	boiling	ice crystals
Seperation of single bacterial colony is called as	isolation	pure culturing	seperation	All of the above	20-30 years
Media which distinguish one type of bacteria from other is known as	complex media	defined media	differential media	enriched media	vaccum
A _____ permit growth of one group of bacteria	selective media	enriched media	complex media	differtial media	slow freezing
Which one is a selective media	potato dextrose	LB agar	Mannitol salt agar	eosin methylene blue	staining
Example of differential media is	potato dextrose	LB agar	Mac conkey's agar	mannito salt agar	iodine
Enrichment media is used to isolate bacteria from	sputum	soil	water	air	differen tial
Urea media is used to check the presence of enteric bacteria producing _____	amylase	cellulase	urease	lactase	counter stain
Autoclaving is an example of _____ sterilization	moist heat	dry heat	red heat	all of these	crystal violet
Temperature in autoclaving must be	118	121	100	115	malachite green
Sterilization of heat sensitive material is achieved by	dry heat	moist heat	red heat	radiation	fixation
Temperature required for dry heat sterilization is	160	140	150	130	basic dye
_____ is used mainly for surface treatments	UV light	infra red	visible light	ionizing radiation	eosin
Which radiation is used to sterilise complex matrices such as food stuff	UV light	infra red	visible light	ionizing radiation	1884

For sterilizing heat sensitive liquid and gases _____ technique is used	filtration	radiation	autoclave	hot air oven	pink
In depth filter, columns are packed with	glass wool	straw	membrane filter	Hepa filter	gram's postive
Filter pores required to eliminate bacteria is	enrichment media	0.5 μm	0.2 μm	0.4 μm	mycobacteria
Filter pores required to eliminate virus is	0.01-0.1 μm	0.1-0.2 μm	0.001-0.2 μm	0.3-0.5 μm	log phase
A bacterial colony represents	pure culture	complex culture	mixed culture	all of these	depletion of nutrition
Nichrome wire loop is used in	spread plate	streak plate	pour plate	all of these	lag phase
Petri dish was invented by	richard petri	robert koch	louis pasteur	anton van leun hook	richard petri
Which of the following method of sterilization have no effects on spore	drying	hot air oven	autoclave	radiation	drying
Blood agar medium is	enrichment media	enriched media	selective media	differential media	enrichment media
Infrared radiation is a method of sterilization by	dry heat	moist heat	chemical method	physical method	physical method
Viruses can be cultivated on	lab media	living cells	broth	agar	living cells
Lyophilization means	sterilization	freeze drying	burning to ashes	exposure to formation	freeze drying
Temperature required for pasteurization is	above 100 C	below 100 C	100 C	above 50 C	below 100 C
Bacteria can be stored successfully for weeks by	refrigeration	lyophilization	pasteurization	filtration	refrigeration
Technique used for long storage of bacterial culture is	cryopreservation	lyophilization	refrigeration	pasteurization	cryopreservation
Stabilizing agent used for cryopreservation is	glycerol	glucose	acetone	ethanol	glycerol
Glycerol/DMSO is used in cryopreservation to prevent formation of _____	ice crystals	toxins	spores	rupture	ice crystals
Cultures can be stored for _____ by cryopreservation	10-20 months	1-2 years	indefinite	20-30 years	20-30 years
In lyophilization culture is dehydrated by	vacuum	heating	thawing	all of these	vacuum

Low temperature in lyophilization is achieved by	slow freezing	instant freezing	liquid nitrogen	dry ice	slow freezing
Which technique is used to visualize microbes in bacteria	smearing	spreading	staining	dyeing	staining
Mordant used in gram staining is	crystal violet	saffranine	iodine	ethanol	iodine
Grams staining is a _____ staining technique	simple	differentia l	complex	multipl e	different ial
Saffranine used in gram's staining is a	primary stain	counter stain	mordant	solubli zer	counter stain
Primary stain used in grams stainig is	crystal violet	saffranine	iodine	ethanol	crystal violet
Stain used for staining spores is	crystal violet	malachite green	iodine	saffran ine	malachit e green
____ is the process by which the internal and external structures of cells and microorganisms are preserved and fixed in position	staining	fixation	removal	all of these	fixation
Methylene blue is an example of	acidic dye	basic dye	neutral dye	all of these	basic dye
An example of acidic dye is	methylene blue	saffranine	crystal violet	eosin	eosin
Grams staining was developed in the year	1884	1984	1887	1987	1884
Gram negative bacterial are visualized as _____ colored after grams stainig	violet	blue	yellow	pink	pink
In gram's staining violet colored bacteria are	gram's positive	gram's negative	gram's neutral	norma	gram's postive
Acid fast staining is performed to differentiate _____	viruses	fungi	acetobacter	mycob acteria	mycobacteria
Maximum growth of bacteria occurs in	lag phase	log phase	stationary phase	death phase	log phase
Bacteria reaches to death phase due to	depletion of nutrients	lack of water	temperature	humidi ty	depletio n of nutritio n
Phase in which cells adjust to new enviornment is	log phase	declining phase	lag phase	station ary phase	lag phase
Unit III					
A culture of bacteria produces 5 generation in 2 hours what is the generation time for this bacterium under those condition?	15 minutes	24 minutes	30 minutes	1hr	24 minutes
Normal doubling time of the bacteria is _____?	30mins-1hr	1-2hr	3hr	15min utes	30minut es-1hr
Microorganisms is dependent on the _____?	substrate	antibiotics	medium	water conten t	substrat e

The microorganisms can be grown in _____?	Batch bioreactor	temperature	fermentation	antibiotics	Batch bioreactor
Thermophiles can be isolated by using _____?	High temperature	low temperature	Acidic pH	Aerosol dilution	High temperature
Acidophiles can be isolated from _____?	High temperature	low temperature	Acidic pH	Aerosol dilution	Acidic pH
Normal doubling time of the yeast _____?	0.30mins-1hr	1-2hrs	3-6hrs	48hrs	1-2hrs
Batch fermentation is regarded as a _____?	open system	continuous system	closed system	fed batch	closed system
In batch fermentation how many phases are used for growth condition?	5phases	3-4phases	7phases	6phases	6phases
The most active growth of microorganisms and multiplication occur during _____ phase?	lag phase	stationary phase	deceleration phase	log phase	log phase
The microbial growth may either slow down or completely stop in _____ phase?	lag phase	log phase	stationary phase	death phase	Stationary phase
Fed -batch fermentation used for the production of _____?	recombinant proteins	recombinant antibiotics	recombinant lipids	recombinant food	recombinant proteins
Semi-continuous fermentation used the production of _____?	Alcohol	beer	wine	juice	Alcohol
The removal of the culture medium continuously and replacement of this with a fresh sterile medium in a bioreactor is called?	Semi-continuous	continuous system	Fed- batch reactor	batch reactor	Continuous culture system
Which of the following is used to grow bacterial culture continuously?	Chemostat	Hemostat	Petroff-Hausser chamber		
Which of the following procedures uses a photocell to measure absorbance of a culture to regulate the flow of culture media?	Chemostat	Hemostat	Petroff-Hausser chamber	Trubidostat	Trubidostat
Molecules that induced proliferation are _____?	Growth factor	receptor	cyclins	nuclear lamins	Growth factors
Activity of CDKs is mediated by _____?	Receptors	cyclins	growth factor	checkpoints	Cyclins
Extra cellular signals are transduced in part by _____?	Growth factor	receptor	cyclins	checkpoints	receptor
Cancer cells can proliferate ,in part,by escaping _____?	Autocrine stimulation	metastasis	angiogenesis	clonal replication	metastasis

Cancer cells can become independent of external growth factors by way of _____?	clonal replication	metastasis	autocrine stimulation	apoptosis	Autocrine stimulation
Proteins that phosphorylate transcription factors are _____?	Tumor suppressor genes	oncogenes	protooncogenes	cyclin-dependent kinases	cyclin-dependent kinases
The human papilloma virus E6 and E7 genes exemplify _____?	CDK inhibitors	tumor suppressor genes	oncogenes	protooncogenes	oncogenes
After first generation, the cell number will be _____	2^1	2^3	$N_t = N_0 \times 2^2$	0	2^1
Aerobic bacteria require _____ for their growth?	CO ₂	#VALUE!	O ₂	Carbon	O ₂
The presence of oxygen is toxic to _____ type of bacteria?	Aerobic bacteria	Facultative bacteria	Autotrophic bacteria	Anaerobic bacteria	Anaerobic bacteria
_____ type of bacteria can able to grow in both aerobic and anaerobic condition?	Aerobic bacteria	Facultative bacteria	Heterotrophic bacteria	all of the above	Facultative bacteria
Based on their nutrition, the bacteria are categorized as _____?	Autotrophic	symbiosis	anaerobic bacteria	facultative bacteria	Autotrophic
_____ type of bacteria are capable of synthesizing their own food from inorganic substances?	Autotrophic	Heterotrophic	Sporophytes	Parasites	Autotrophic
Heterotrophic bacteria are useful for the production of _____?	Alcohol	carbonic acid	wine	acetic acid	alcohol
which type of bacteria are capable of synthesizing their own food from hosts?	Aerobic bacteria	anaerobic bacteria	parasites	autotrophic bacteria	parasites
_____ is a good example of non pathogenic bacteria?	bacillus	clostridium	<i>E.coli</i>	vibrio	<i>E.coli</i>
fungi are a group of _____ organisms?	eukaryotic, heterotrophic	autotrophic	aerobic	Anaerobic bacteria	eukaryotic, heterotrophic
_____ microbes cannot synthesis their own food from the inorganic compounds?	bacteria	virus	fungi	yeast	fungi
<i>Aspergillus niger</i> is used for production of _____?	Penicillin	Gluconic acid	Citric acid	Enzymes	Citric acid
_____ is the unable to live in a cell-free environment?	Fungi	Bacteria	Yeast	Virus	Virus
An organism is completely dependant on atmospheric O ₂ for	Acidophile	Facultative anaerobe	osmotolerant	obligate	obligate aerobe

growth ,this organism is a _____?				aerobe	
An organism has an optimal growth rate when the hydrogen ion concentration is very high ,this organism is	Neutrophile	Acidophile	alkalophile	osmotolerant	Acidophile
The term facultative anaerobe refers to an organism that _____?	Does not use oxygen but tolerate it	It is killed by oxygen	Prefers to grow without oxygen	Can grow with or without oxygen	Can grow with or without oxygen
Some bacteria contain an extrachromosomal circular DNA is called _____	cosmid	plasmid	vector	Capsule	plasmid
Adenovirus is a _____?	Plant	Animal	Insect	plankton	Animal
Microbes classified based on their energy sources is _____?	Autotrophs, heterotrophs	Lithotrophs, organotrophs	Saprophytic, Parasitic	Phototrophs, Chemotrophs	Phototrophs, Chemotrophs
_____ is not required for all bacteria?	Carbon	Nitrogen	Sodium	calcium	Sodium
Phosphorus is an essential component of _____?	Nucleotide	nutrition	Nucleic acids	carbon bond	nucleotide
Bacterial growth is influenced by which factor _____?	Temperature	humidity	soil	bond	temperature
Binary fission refers to the division of _____	Parent cell into 2 daughter cells	parent cell into 1 daughter cells	2 parent cell into 4 daughter cells	2 parent cell into 8 daughter cells	Parent cell into 2 daughter cells
_____ method is used to count the total number of both live and dead cells.	Smear count method	Membrane filter count	Direct microscopic count	Electronic count method	Direct microscopic count
The oxidation number of an atom in elemental form is _____?	3	2	0	1	0
Luminol is used in a preliminary test for _____?	Saliva	blood	Milk	Clay	Blood
Which type of metal takes the longest to degrade?	Steel	Tin	Iron	Aluminum	Aluminum
_____ reaction releases light energy at room temperature?	All redox	Double replacement	Chemiluminescent redox	Decomposition	Chemiluminescent redox
The sum of the oxidation numbers in all compounds are equal to _____?	1	2	0	4	0
The vitamin C in lemons is a	Antioxidant	CHO	Oxidizing agent	Corrosive	Antioxidant

good_____	t			ve compo und	ant
_____reduce iron ore to iron in a blast furnace?	Calcium carbonate	Calcium	Oxygen	Carbon monoxide	Calcium carbonate
Sodium_____when it result with sulfur to form sodium sulfide	Gains 1 Electron	Gains 2 Electrons	Is oxidized	Is reduced	Is oxidized
Can oxidation and reduction occur without one another?	They are interdependent	They are not interdependent	They are dependent	they are neutral	They are interdependent
Bleach removes stain from clothing by_____colored molecules.	Oxidizing	Protecting	Reducing	Dissolving	Oxidizing
What is the oxidation number of oxygen as a free element?	2 ⁺	2 ⁻	0	-1	0
The microbial growth may either slow down or completely stop in _____phase?	lag phase	log phase	stationary phase	death phase	death phase
Microbes classified based on their energy sources is_____?	Autotrophs, heterotrophs	Lithotrophs, organotrophs	Saprophytic, Parasitic	Phototrophs, Chemotrophs	Autotrophs, heterotrophs
unit IV					
Fungi are non-vascular plants without _____	chlorophyll	cell wall	plastids	chromophores	chlorophylls
Study of fungi is known as	bacteriology	mycology	fycolgy	virology	mycology
Mode of nutrition of fungi is	organotrophic	heterotrophic	autotrophic	lithotrophic	heterotrophic
Fungi reproduces by the means of	spores	binary fission	propagation	all of these	spores
pH of medium for fungus is	acidic	basic	neutral	any of these	acidic
Mushrooms are a type of	algae	fungus	bacteria	virus	fungus
Penicillin is obtained from	algae	fungus	bacteria	plants	fungus
What should be the temperature of compost when spawning is done ?	15 to 20°C	22 to 25°C	30 to 35°C	Not certain	22 to 25°C
What should be the pH of casing soil ?	4	7	9	any	7
Mushroom is a _____	Parasite b)	Saprophyte	Autotroph	Symbiont.	Saprophyte
A mature fruiting body of mushroom has following parts	stipe	leaves	stem	stalk	stipe
Mushrooms contain _____ amount of protein	100%	10%	negligible	20 to 40%	20 to 40%
Wild mushrooms grow during -	Rainy	Summer	Any time	In the	Rainy

	season			month of October.	season
Which of the following statement is true	Normal soil is suitable for growth of mushrooms.	Mushrooms can be cultivated on all types of soil.	Mushrooms cannot be cultivated in south india as temperature is more		
What is the period between two 'flushes' of mushroom crop?	3 to 4 days	one to two months	7 to 10 days	Not certain	7 to 10 days
In casing soil can be replaced by	Cow dung + clay-loam soil	Compost + clay-loam soil	Lime + Peat	All of the above.	Compost + clay-loam soil
Mushroom cultivation was started first in	England	France	Germany	China	France
the optimum temperature needed for <i>Agaricus bisporus</i> is	100-150C	250- 300C	350- 400C	400- 500C	250- 300C
For oyster mushroom cultivation beds are prepared in _____	Plastic bags	Trays	Pots	Bucket.	Plastic bags
The mushroom spawn preparation needs addition of calcium carbonate because it -----	Maintains the pH	nutrition	turbidity	water content	maintains the PH
The method of mushroom harvesting is	Machine lifting	Cutting with scissors	Hand picking	Lifting with trowels	Hand picking
Secondary mycelium of mushroom produces umbrella like structure called as	Primary mycelium	Tertiary mycelium	Pileus	Gills.	Pileus
Mushroom are used in the preparation of	Soups	wine	bread	rice	soups
Mycotrophy is the symbiosis of a fungus with	bacteria	algae	bryophytes	other fungi	other fungi
What is the fruit body of <i>Penicillium</i> called	perithecium	cleistothecium	apothecium	stroma	cleistothecium
Tikka disease of groundnut is caused by	<i>Aspergillus</i>	<i>Puccinia</i>	<i>Cercospora</i>	<i>Fusarium</i>	<i>Cercospora</i>
In the fruit body of <i>Agaricus</i> basidia are produced on the:	gills	pileus	stipe	rhizomorph	gills
Penicillin was extracted by:	Flemming	Huxley	Lamarck	Brown	Flemming
The structure in which the ascospores are formed in	basidium	sporangium	ascus	gametangium	ascus
The mycelium of <i>Erysiphe</i> is	aseptate	septate	flagella	multiseptate	septate
The spermatization takes place in	<i>Neurospora</i>	<i>Penicillium</i>	<i>Peziza</i>	<i>Erysiphe</i>	<i>Neurospora</i>

	a	m		e	ora
The sexual reproduction lacks in	Ascomyces	Basidiomycetes	Phycomycetes	Deuteromycetes	Deuteromycetes
Mycorrhiza is a term to indicate	fungus association with stem	bacteria association with root	fungi association with root	study of fungi	fungi association with root
After fusion of sexual gametes in Mucor the resultant structure is known as	oospore	cleistothecium	zygospore	zygote	zygospore
Which of the following diseases is caused by a fungus ?	small-pox	tuberculosis	cancer	black rust of wheat	black rust of wheat
Same haploid structures of Rhizopus include the	mycelia, sporangia and spores	hyphae, zygote and sporangia	mycelia, zygospore and spores		
Fungal hyphae penetrate hard cell wall of their host with the help of	enzymes	hormones	sharp tips	haustoria	haustoria
Candida albicans cause infections	Surface	Subcutaneous	Cutaneous	Systemic	cutaneous
Trichophyton is classified under	Dermatophyte	Deep mycosis	Superficial mycosis	Opportunistic mycosis	Dermatophyte
All Candida strains are sensitive to	Neomycin	Nystatin	Amphotericin	Clotrimazole	Nystatin
Cell wall in bacteria is	present	absent	present in few		present
Less amount of peptidoglycan is present in	Grams positive	Gram's negative	Gram's neutral	Gram cells	Gram's negative
In a bacteria all the enzymes are present in	plastid	nucleoid	cytoplasm	golgi apparatus	cytoplasm
DNA in a bacteria is always	linear	circular	open	spiral	circular
Bacteria which obtain their energy from sunlight are	phototrophs	lithotrophs	chemotrophs	organo trophs	phototrophs
Lithotrophs obtain their energy by metabolizing	organic compound	inorganic compound	carbohydrates	sunlight	inorganic compound
Nitrifying bacteria are	phototrophs	lithotrophs	chemotrophs	organo trophs	lithotrophs
Study of fungi is known as	bacteriology	mycology	phycology	virology	mycology
Mode of nutrition of fungi is	organotrophic	heterotrophic	autotrophic	lithotrophic	heterotrophic
Fungi reproduces by the means of	spores	binary fission	propagation	all of these	spores

Viruses range in size from	1-100nm	25-300nm	10-100µm	1-10µm	25-300nm
The structural component found in all viruses is	envelope	capsid	spikes	tail fibres	capsid
The common polyhedral capsid shape of virus is	pentagon	circular	icosahedron	sphere	icosahedron
Unit V					
Sickness or ailment caused in animal and humans are called_____	viral diseases	Bacterial diseases	Fungal diseases	Microbial diseases	Microbial diseases
How many types of microbes?	2 types	3 types	4 types	5 types	4 types
protozoa also known as _____	Bacteria	Protoctista	Fungus	Virus	Protoctista
One of the following is a bacterial diseases	Chickungunya	Bird flu	Hepatitis	typhoid	Chickungunya
The organ infected by Hepatitis 'B' virus is	Heart	Kidney	Stomach	Liver	Liver
The enzyme that helps to convert RNA into DNA in HIV virus is_____	Anylase	Invertase	Pepsin	Reverse transcriptase	Invertase
"Reduced platelet count in blood" is one of the symptoms of the diseases is _____	Dengue	Chickungunya	Bird flu	Syphilis	Dengue
TB caused by the_____	Viral infection	Bacterial infection	algal infection	fungal infection	Bacterial infection
Tuberculosis usually affected in the_____	Heart	Kidney	Lungs	Liver	Lungs
S.typhi growing in the _____	Heart	Kidney	Intestine	Lungs	Intestine
How many types of Tuberculosis infection?	Only one	Two	Three	Four	Two
Live vaccine are used for specific prophylaxis of _____	Diphtheria	Hepatitis D	Tuberculosis	Botulism	Tuberculosis
Live vaccine for specific prophylaxis against tuberculosis is called_____	DTP	HLA	MMR	BCG	BCG
Salmonella typhi	Gram positive cocci arranged single	Gram positive rod arranged in chains	Gram negative rod arranged singly		
Typhoid is also known as_____	Pneumonic fever	Hepatic fever	vesical fever	Enteric fever	Enteric fever
Skin test with specific allergens is used for diagnosis of_____	Tuberculosis	Enteric fever	Staphylococcal infection	Cholera	Tuberculosis
what is the pathogen that causes typhoid fever?	S.pneumoniae	S.typhi	C.perfringens	P.aeruginosa	S.typhi
which phage typing is widely used	Dysentery	Typhoid	Botulism	Camph	Typhoid

for epidemiological analysis?		fever		typhoid fever	fever
AIDS is diagnosed by:	Slide agglutination	Latex agglutination	ELISA	CFT	ELISA
Doubling time for Mycobacterium tuberculosis is ____	14-16 seconds	14-16 days	14 -16 minutes	14 -16 hours	14 - 16 hours
which is used to treatment of drug ____	Azidothymidine	Ribavirin	Pencillin G	Acyclovir	Azidothymidine
choose the microorganism which possesses cord-factor:	Mycoplasma pneumoniae	Mycobacterium tuberculosis	Streptococcus pneumoniae	Neisseria meningitidis	Mycobacterium tuberculosis
AIDS is diagnosed by:	Bacteriology method	Biological method	Serological method	Microscope	Serological method
Chemotherapy is treatment of ____	Lungs	Heart	Cancer	Fever	Cancer
Main property of cancer has ____	killing the cells	replication the cell	growing the cell	doubling the cell	killing the cell
Chemotherapy using drugs that convert cytotoxic activity only upon light exposure is called ____	Phototherapy	Chemoradiotherapy	Targeted therapy	Radiation therapy	Phototherapy
Hiv is a ____	Immune virus	Retrovirus	Pathogenic bacteria	fungi	Retrovirus
HIV affected immunosystem as ____	CD4+ T Cell	neutrophil	memory cell	plasma cell	CD4+T cell
The bacteria then perforate through the wall and are phagocytosed by ____	Macrophage	dendritic cell	white cell	RBC cell	Macrophage
Chemotherapy term was coined by ____	Mendel	Edward Jenner	Paul Ehrlich	John	Paul Ehrlich
The first modern Chemotherapeutic agent was	Arsenic	Arsphenamine	Lethen	Protein	Arsphenamine
Any treatment of diseases with drug called as ____	Pharmacodynamics	Pharmacotherapy	Pharmacokinetics	Pharmacology	Pharmacotherapy
protozoan is a ____	single cell nuclei	single cell microorganism	nucleus	plasmid	single cell microorganism
The classic symptom malaria is ____	Paroxysm	Paroxysm	Parenteral	Internal	Paroxysm
Severe malaria is usually caused by ____	P.ovale	P.vivax	P.falciparum	P.malariae	P.Falciparum
symptoms of Falciparum symptoms arise ____ after infection	9-25 days	7-14 days	1-10 days	9-30 days	9-30 days
How many types of malaria caused by humans?	3	2	4	5	5

Antibacterial are used against	Bacteria	Fungi	Protozoa	seaweed	Bacteria
Microbial agents that kill microbes are called	Microbicidal	Antimicrobial	Antibacterial	Antifungal	Microbicidal
Antifungal are used against	Bacteria	Fungi	Protozoa	Seaweed	Fungi
use of antimicrobial medicines to treat infection is known as	Photochemotherapy	Pharmacotherapy	antimicrobial chemotherapy	Chemoradiotherapy	Antimicrobial chemotherapy
The main classes of antimicrobial agents are	Microbes	Disinfectant	Contamination	Media	Disinfectant
HIV as affected to ____	Acquired immune deficiency syndrome	Innate immune deficiency syndrome	Iron deficiency	Calcium deficiency	Acquired immune deficiency syndrome
which immunity level as lost from the affecting of HIV	acquired immunity	cell mediate immunity	innate immunity	host immunity	Cell mediate immunity
AIDS syndrome caused by a virus is called ____	Immuno virus	Retrovirus	HIV virus	Bacteriophage	HIV virus
Typhoid can caused affected by ____	heart attack	Kidney failure	lens failure	Brain tumour	Kidney failure
Which of the following types of interferon is used in the treatment of hepatitis C virus:	Interferon -alpha	Interferon -beta	Interferon -delta	Interferon -gamma	Interferon -alpha
Which one of the following drugs produces hemorrhagic cystitis:	Nitrosurea	Cisplatinum	Cyclophosphamide	Bleomycin	Cyclophosphamide
All of the following are cell cycle non specific anticancer agents except ____	Nitrosurea	Cisplatinum	Cyclophosphamide	Bleomycin	Bleomycin
Which one of the following antibiotics is effective in treatment of typhoid fever ____	Pencillin G	Kefaclor	Demicocycline	Ceprofloxacin	Ceprofloxacin
Which one of the following antibiotics is bacteriostatic	Erythromycin	Gentamycin	Cefradin	Ampicillin	Cefradin
Bone marrow depression is a sideeffect of all of the following drug except ____	Chloramphenicol	Cyclosporin	Interferon A	Pencillin G	Pencillin G
Cotrimoxazole is a combination of ____	Sulfadiazine+trimethoprim	Sulfamethoxazole+trimethoprim	Sulfamethoxazole+dapsone	Sulfadiazine+pyrimethamine	Sulfamethoxazole+trimethoprim
Fanconi syndrome is a side effect	Refampicin	Neomycin	Demicocycline	Clinda	Demicoc

of which one of the following therapeutic agent_____	n			mycin	ycline
which drugs passes through blood brain barrier?	Cephalexin	Cefactor	Ceftriaxone	Micillinam	Micillinam
Which is the longest duration of action_____	Oxytetracycline	Demicocycline	Minocycline	Chlortetracycline	Minocycline
Second generation of cephalosporin is _____	Cephalexine	Cefoperzone	Cefotaxime	Cefoxline	Cefoxline
More active against gram-ve bacilli is _____	Dicloxacillin	Erythromycin	Cephalexine	Micillinam	Micillinam
All of the following chemotherapeutic agents are effective in treatment of anerobic infection except:	Metronidazole	Imipenium	vancomycin	Cefoxitin	Imipenium
Which of the following is NOT an intrinsic factor in food spoilage?	pH	Moisture content	Temperature	Available nutrients	Temperature
There is an increased sensitivity to aflatoxins in individuals previously infected with	measles	hepatitis B	mumps	chickenpox	hepatitis B
Despite efforts to eliminate spoilage organisms during canning, sometimes canned foods are spoiled. This may be due to	UV treatment	spoilage before canning	heat sterilization	Chemical treatment	spoilage before canning
The effectiveness of many chemical preservatives depends primarily on the food	pH	temperature.	water content	acidity	pH
Sodium nitrite is responsible for	increasing rate of spoilage.	protecting against botulism	maintenance of red color in meat	reducing rate of spoilage.	reducing rate of spoilage.
The main reservoir of <i>Staphylococcus aureus</i> is	human buccal	human nasal cavity	human red blood cells.	human feces.	human nasal cavity
Most spoilage bacteria grow at	Acidic pH	Basic	Alkaline pH	Neutral pH	Neutral pH
Which of the following refers to the addition of microorganisms to the diet in order to provide health benefits beyond basic nutritive value?	Prebiotics	Probiotics	Antibiotics	Adjuvants	Probiotics
Which type of fermentation is used to produce yogurt?	Yeast-lactic fermentations	Mesophilic	Therapeutic	Thermophilic	Thermophilic