SEMESTER II

18BTU203 GENERAL MICROBIOLOGY 4H - 4C

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. BARATHKUMAR, S.
SUBJECT NAME: General Microbiology SUB.CODE:18BTU203
SEMESTER : II CLASS: I B.Sc.

Duration hours	Topics to be covered	Support materials
Unit I - Fundamentals, History, Scope and Evolution of Microbiology		
1	Microbiology - Introduction	**T1 – 1 - 3**
1	History & scope	**T1 – 3 - 36**
1	Classification of microorganisms: Microbial taxonomy	**T1 – 37 – 49**
1	Classification criterias	
1	molecular approaches	
1	Microbial phylogeny	
1	current classification of bacteria	**W1**
1	Recapitalization of unit I / class test	
		Total hours - 08
Unit II - Microbial Diversity		
1	Microbial distribution	**T2 - 2**
1	Characterization of prokaryotes & eukaryotes	**T2- 47 – 70**
1	Morphology and cell structure of Bacteria,	**T1- 73 -98**
1	Morphology and cell structure of Algae, Protozoa	**T1- 365 – 414**
1	Morphology and cell structure of Fungi	**T1- 333 – 364**
1	Unique features of fungi	**T2 – 213 – 233**
1	Recapitalization of unit II / class test	
		Total hours - 07
Unit III - Cultivation and Maintenance of microorganisms		
1	Nutritional categories of micro-organisms	**T2 – 22 – 27**
1	Microbial growth media and its types	**T2 – 27 – 36**
1	Methods of microbes isolation	**T1 – 137**
1	Microbial staining methods	**W2**
1	Purification techniques	**T2 – 20 – 22**
1	Microbial preservation techniques	**W3**
1	Recapitalization of unit III / class test	
		Total hours - 07

Duration hours	Topics to be covered	Support materials
Unit IV - Microbial growth		
1	Microbial Growth curve and Microbial growth kinetics	T3 – 91 – 101 T2 – 183 – 185
1	Batch and continuous culture	T2 – 189 – 195
1	Microbial growth factors and measurement of growth	T2 – 186 – 189
1	Factors affecting growth of bacteria	T3 – 96 – 100
1	Bacterial Reproduction: Transformation, Transduction	T1 – 240 – 253
1	Bacterial Conjugation	
1	Endospores and sporulation in bacteria	T1 – 94 – 96
1	Recapitalization of unit IV / class test	
		Total hours - 08
Unit V - Water and Food Microbiology		
1	Bacterial pollutants of water - coliforms and non coliforms	T1 – 569 - 592
1	Sewage composition and its disposal	T1 – 593 - 617
1	Important microorganism in food Microbiology: Moulds, Yeasts, bacteria	T4 – 13 - 37
1	Food born infections and intoxications	T4 – 455 - 478
1	Food preservation techniques	T4 – 251 - 279
1	Fermented Foods and its types	T4 – 371 - 400
1	Recapitalization of unit IV / class test	
1	Previous Year End semester question paper discussion	
1	Previous Year End semester question paper discussion	
1	Previous Year End semester question paper discussion	
		Total hours - 10

Support materials

T1	Pelczar, M.J., Chan, E.C.S., & Krieg, N.R. (1993). *Microbiology* (5th ed.). McGraw Hill Book Company
T2	Stanier, R.Y., Ingraham, J.L., Wheelis, M.L., & Painter, P.R. (2005). *General Microbiology* (5th ed.). McMillan publishers.
T3	Stuart Hogg (2005). *Essential Microbiology* (1st ed.).John Wiley & Sons Ltd.
T4	Jay, J.M., Loessner, M.J., & Golden, D.A. (2005). *Modern Food Microbiology* (7th ed.). Delhi: India, CBS Publishers and Distributors.
W1	http://www.onlinebiologynotes.com/classification-of-bacteria/
W2	https://www.cliffsnotes.com/study-guides/biology/microbiology/microscopy/staining-techniques
W3	http://www.biologydiscussion.com/micro-biology/preserving-microbial-cultures-top-5-methods/17821

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

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 areusually 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 maybe 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 a vailability of the microscope and the ability to isolate and grow pure cultures of microorganisms.
- Microorganisms are responsible for many of thechanges observed in organic and inorganic matter(e.g., fermentation and the carbon, nitrogen, andsulfur cycles that occur in nature).
- Microorganisms have two fundamentally different types of cells—procaryotic and eucaryotic—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.

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 between1625 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 (figure1.1*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 a45° angle to the specimen plane. This would have provided aform 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 RoyalSociety of London. It is clear from his descriptions that he saw both bacteria and protozoa.

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

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 boilingwater for 3/4 of an hour, no growth took place as long as theflasks remained sealed. He proposed that air carried germs to theculture medium, but also commented that the external air

might berequired for growth of animals already in the medium. The supportersof spontaneous generation maintained that heating the airin 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 ared-hot tube. The flask remained sterile. Subsequently GeorgFriedrich Schroder and Theodor von Dusch allowed air to enter aflask of heat-sterilized medium after it had passed through sterile cottonwool. No growth occurred in the medium even though the air had not been heated. Despite these experiments the French naturalist FelixPouchet 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 airthrough cotton and found that objects resembling plant sporeshad 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.

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 thatdust did indeed carry germs and that if dust was absent, broth remainedsterile even if directly exposed to air. During the course of his studies, Tyndall provided evidence for the existence of exceptionally heat-resistant

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

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.

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 toestablish 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.

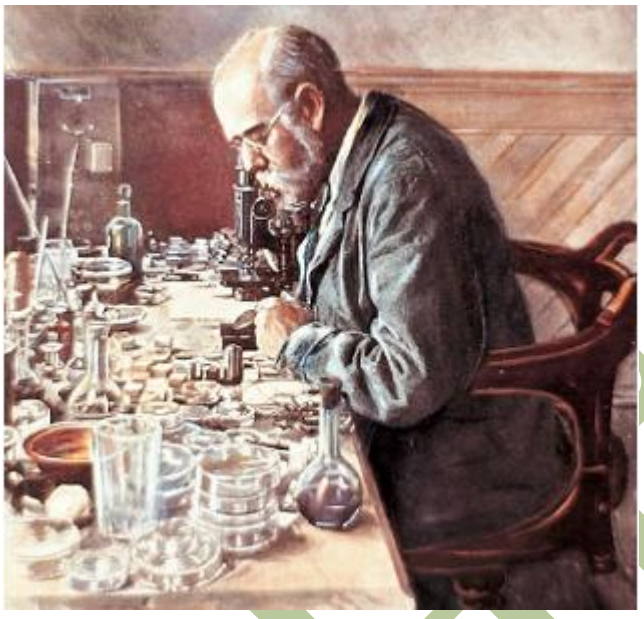
Recognition of the Relationship between Microorganisms and Disease

Although Fracastoro and a few others had suggested that invisibleorganisms produced disease, most believed that disease was due to causes such as supernatural forces, poisonous vaporscalled miasmas, and imbalances between the four humorsthought to be present in the body. The idea that an imbalance betweenthe four humors (blood, phlegm, yellow bile [choler], andblack bile [melancholy]) led to disease had been widely acceptedsince the time of the Greek physician Galen (129–199). Supportfor the germ theory of disease began to accumulate in the earlynineteenth century. Agostino Bassi (1773–1856) first showed amicroorganism could cause disease when he demonstrated in1835 that a silkworm disease was due to a fungal infection. Healso suggested that many diseases were due to microbial infections.In 1845 M. J. Berkeley proved that the great Potato Blightof Ireland was caused by a fungus. Following his successes with

the study of fermentation, Pasteur was asked by the French governmentto investigate the pébrine disease of silkworms that wasdisrupting 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 byhealthy moths.Indirect evidence that microorganisms were agents of humandisease 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 microorganismsin fermentation and putrefaction, developed a system of antisepticsurgery designed to prevent microorganisms from entering wounds. Instruments were heat sterilized, and phenol was used onsurgical dressings and at times sprayed over the surgical area. The

approach was remarkably successful and transformed surgery afterLister published his findings in 1867. It also provided strong indirectevidence 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 causingdisease came from the study of anthrax by theGerman physician Robert Koch (1843–1910).



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).

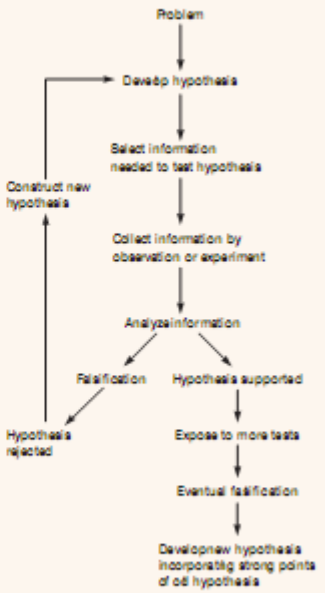
Box 1.1

The Scientific Method

Although biologists employ a variety of approaches in conducting research, microbiologists and other experimentally oriented biologists often use the general approach known as the scientific method. They first gather observations of the process to be studied and then develop a tentative **hypothesis**—an educated guess—to explain the observations (see **Box figure**). This step often is inductive and creative because there is no detailed, automatic technique for generating hypotheses. Next they decide what information is required to test the hypothesis and collect this information through observation or carefully designed experiments. After the information has been collected, they decide whether the hypothesis has been supported or falsified. If it has failed to pass the test, the hypothesis is rejected, and a new explanation or hypothesis is constructed. If the hypothesis passes the test, it is subjected to more severe testing. The procedure often is made more efficient by constructing and testing alternative hypotheses and then refining the hypothesis that survives testing. This general approach is often called the hypothetico-deductive method. One deduces predictions from the currently accepted hypothesis and tests them. In deduction the conclusion about specific cases follows logically from a general premise ("if . . . , then . . ." reasoning). Induction is the opposite. A general conclusion is reached after considering many specific examples. Both types of reasoning are used by scientists.

When carrying out an experiment, it is essential to use a control group as well as an experimental group. The control group is treated precisely the same as the experimental group except that the experimental manipulation is not performed on it. In this way one can be sure that any changes in the experimental group are due to the experimental manipulation rather than to some other factor not taken into account.

If a hypothesis continues to survive testing, it may be accepted as a valid theory. A **theory** is a set of propositions and concepts that provides a reliable, systematic, and rigorous account of an aspect of nature. It is important to note that hypotheses and theories are never absolutely proven. Scientists simply gain more and more confidence in their accuracy as they continue to survive testing, fit with new observations and experiments, and satisfactorily explain the observed phenomena.



The Hypothetico-Deductive Method. This approach is most often used in scientific research.

Koch injected healthymice with material from diseased animals, and the mice becameill. After transferring anthrax by inoculation through a series of 20 mice, he incubated a piece of spleen containing the anthrax bacillusin beef serum. The bacilli grew, reproduced, and producedspores. When the isolated bacilli or spores were injected into mice, anthrax developed. His criteria for proving the causal relationshipbetween a microorganism and a specific disease are known as

Koch's postulates and can be summarized as follows:

1. The microorganism must be present in every case of the disease but absent from healthy organisms.

2. The suspected microorganism must be isolated and grownin a pure culture.
3. The same disease must result when the isolatedmicroorganism 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 and 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:

1. The virulence trait under study should be associated much more with pathogenic strains of the species than with nonpathogenic strains.c
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 infectionand disease process.
5. Antibodies or immune system cells directed against the geneproducts should protect the host.

The molecular approach cannot always be applied because of problemssuch as the lack of an appropriate animal system. It also is difficultto employ the molecular postulates when the pathogen is notwell 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

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 ofpure 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 pathogen 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

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 attenuatedanthrax vaccine in two ways: by treating cultures withpotassium bichromate and by incubating the bacteria at 42 to43°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 spinalcords were removed and dried. During the course of these studies, Joseph Meister, a nine-year-old boy who had been bitten by a rabiddog, 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 Institutewas vaccine production. After the discovery that the diphtheria bacillus produced atoxin, 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 wouldinactivate the toxin and protect against the disease. A tetanus antitoxin was then prepared and both antitoxins were used in the treatment of people. The antitoxin work provided evidence that immunity could result from soluble substances in the blood, now

known to be antibodies(humoral immunity). It became clear that blood cells were also important in immunity (cellular immunity) when Elie Metchnikoff(1845–1916) discovered that some blood leukocytes could engulf disease-causing bacteria. He called these cells phagocytes and the process phagocytosis [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.

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

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

Modern microbiology. Modern microbiology reaches into many fields of human endeavor, including the development of pharmaceutical products, the use of quality-control methods in food and dairy product production, the control of disease-causing microorganisms in consumable waters, and the industrial applications of microorganisms. Microorganisms are used to produce vitamins, amino acids, enzymes, and growth supplements. They manufacture many foods, including fermented dairy products (sour cream, yogurt, and buttermilk), as well as other fermented foods such as pickles, sauerkraut, breads, and alcoholic beverages.

One of the major areas of applied microbiology is **biotechnology.** In this discipline, microorganisms are used as living factories to produce pharmaceuticals that otherwise could not be manufactured. These substances include the human hormone insulin, the antiviral substance

interferon, numerous blood-clotting factors and clot dissolving enzymes, and a number of vaccines. Bacteria can be reengineered to increase plant resistance to insects and frost, and biotechnology will represent a major application of microorganisms in the next century.

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

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 _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 (about0.5 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.

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 classifified? 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.

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	*Bacteria*
Phylum	*Proteobacteria*
Class	*γ- Proteobacteria*
Order	*Enterobacteriales*
Family	*Enterobactericeae*
Genus	*Shigella*
Species	*S.dysentriae*

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.

- 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*

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. Archaebacteria are in one domain, eubacteria in a second, and eukaryotes in the third. Domain Eukarya is subdivided into four kingdoms plants, animals, fungi, protists.

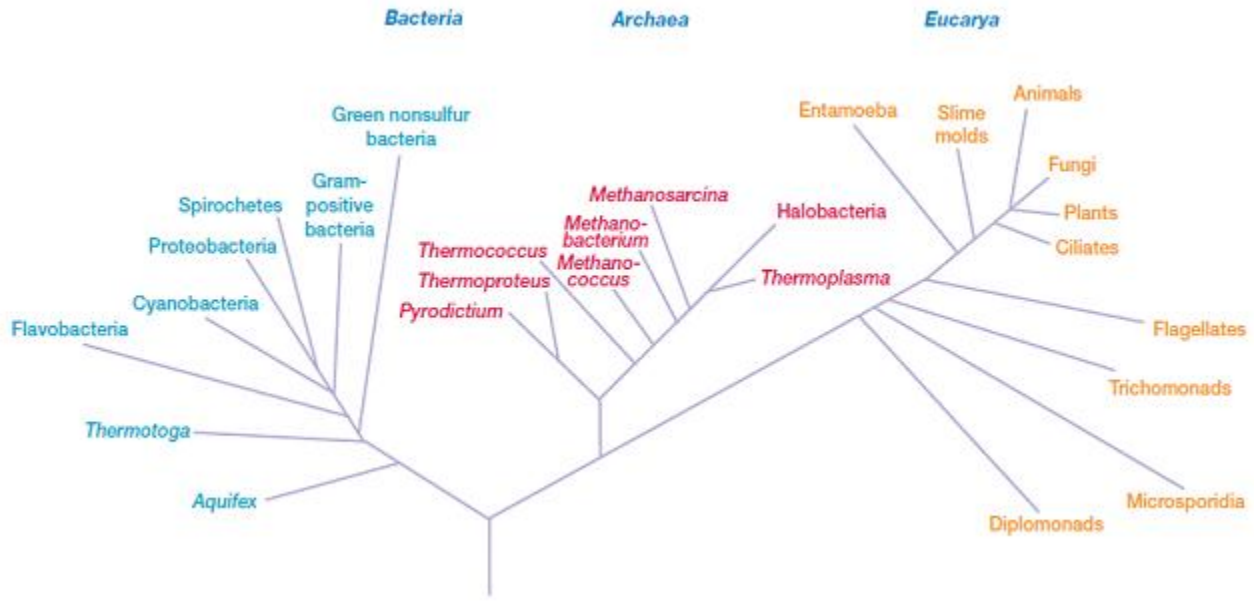


Fig. Three domains based on Woese rRNA sequence analysis

Domain- Archaebacteria

The term *archaebacteria* (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 archaebacteria are grouped (based primarily on the environments in which they live) into three general categories methanogens, extremophiles and non extreme Archaebacteria.

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.

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.

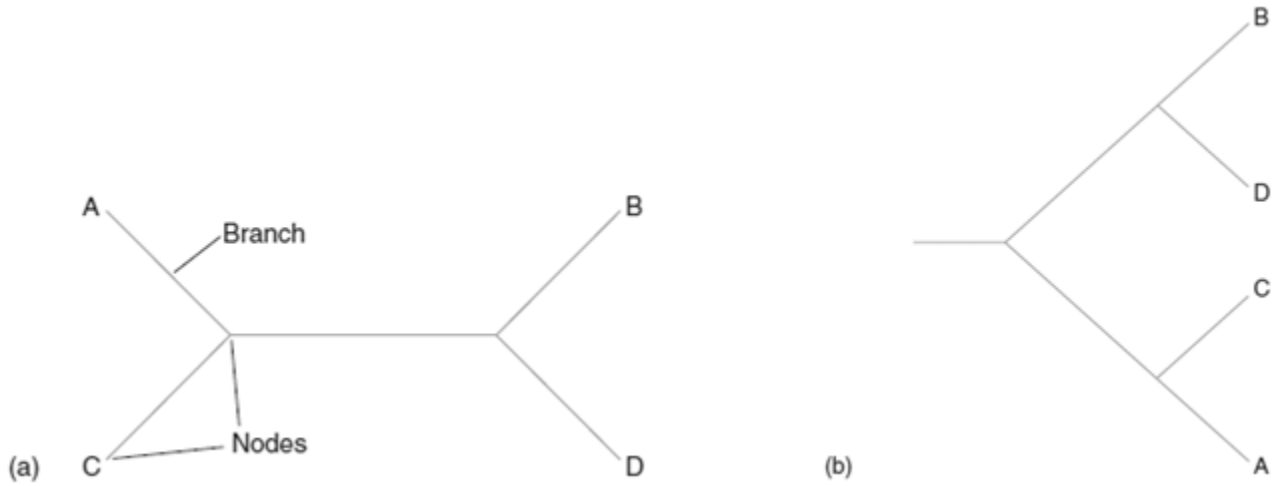


Fig. Phylogenetic tree. a) unrooted tree, b) rooted tree.

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 defied 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.

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) Vibro: -the vibro are the curved, comma shaped bacteria and represented by a single genus. Example: – Vibro 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) **Autotropic 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 bacterio-chlorophyll 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**

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 has no flagella. Example: -Corynebacterium diptherae. ii) **Monotrichous:** - One flagellum is attached to one end of the bacteria cell. Example: - Vibro 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 end of the bacteria cell. Example: - Rhodospirillum rubrum. v) **Peritrichous** : - The flagella are evenly distributed surrounding the entire bacterial cell. Example: -Bacillus.

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 phototrops?
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

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.

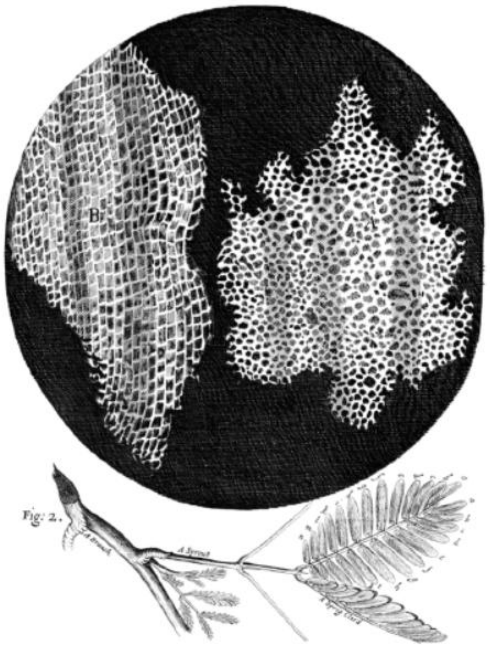


Fig: Drawing of the structure of cork by Robert Hooke that appeared in Micrographia.

"The cell is the fundamental element of organization"

The bservations 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.

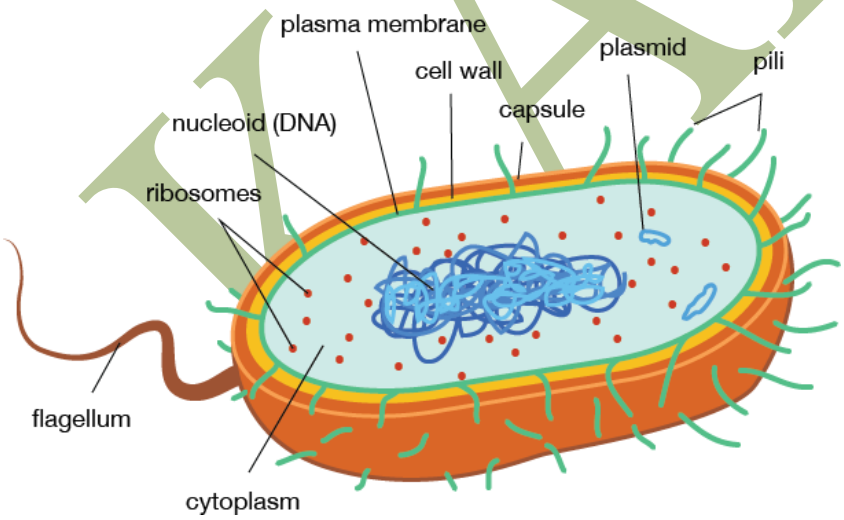


Fig: Schematic diagram of a prokaryotic cell.

Flagella:

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

- 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 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***.

- 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 arebecause 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.

Nucleiod 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.

2. Eukaryotes:

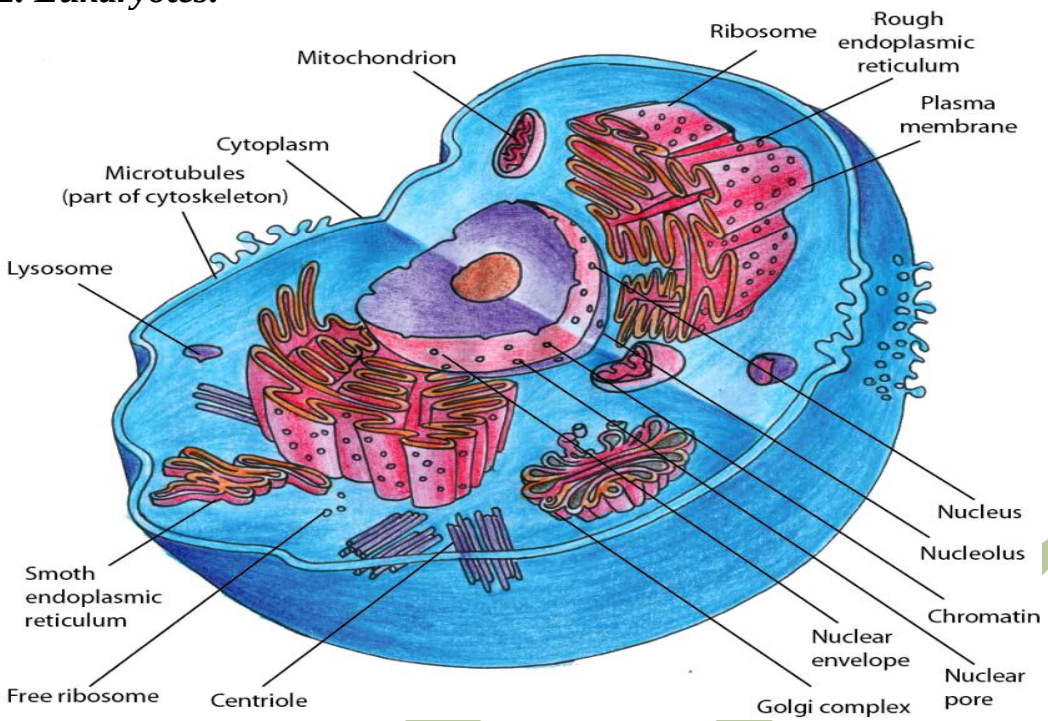


Fig: Eukaryotic cell.

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- 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

	(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 cytosketeton 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

reproduction	fragments only (conjugation)	

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, creats enclosed compartments that are separate from Cytosol.
- As a result, the cell is able to retain specific molecules and cartry out certain reactions in oderly 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.

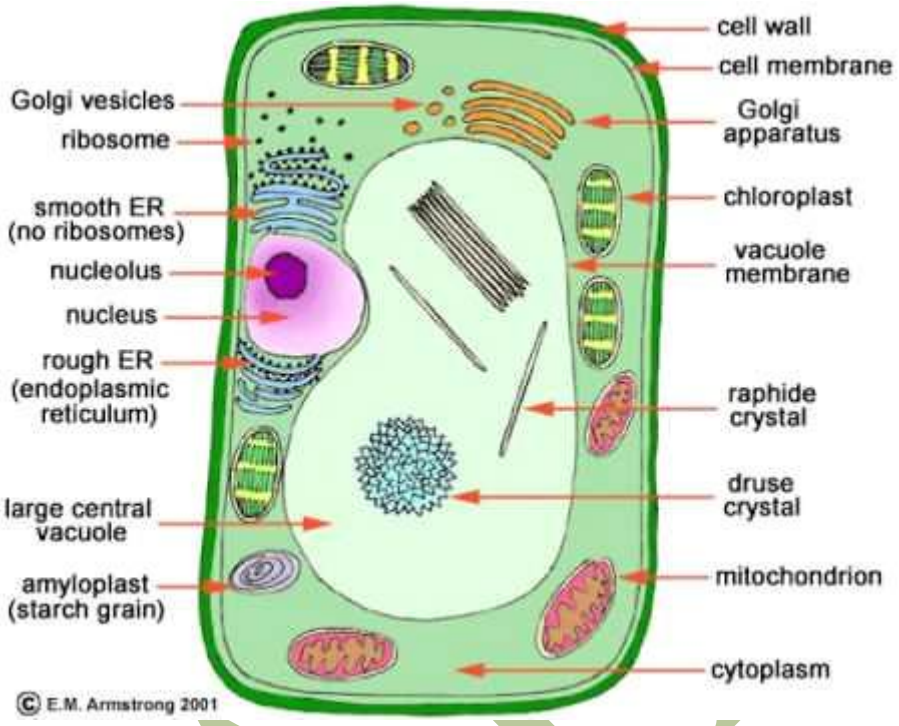


Fig:*Anatomy of Plant Cell.*

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.

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.

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.

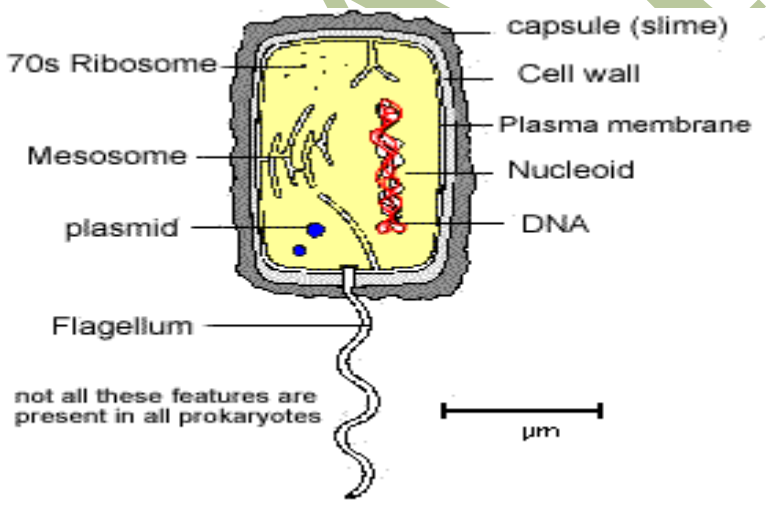
Structure of Bacteria

- Bacteria are unicellular, microscopic, prokarvotic organisms lacking chlorophyll.
- Bacteria were omnipresent. They range in size from 0.5micro 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 tetracocci. 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. *Spirllum*. In addition there are filamentous bacteria and fungus like bacteria. They are multicellular.

- 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 are 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 spiralla 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 cells 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 in stead 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.

- Mesosomes are pocket-like structures formed by the invagination of plasma membrane.
- The general size of a prokaryotic cell is about 1-2 um.
- 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

Structure	Function of bacteria
Cell Wall	• 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	• Controls the entry and exit of substances, pumping some of them in by active transport
Mesosome	• 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	• Contains all the enzymes needed for all metabolic reactions, since there are no organelles
Ribosome's	• 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	• 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

Slime Capsule	• 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:	• 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	• 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%.

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 cholorophyll. 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 posses a true nucleus)
- They are non-green plants.
- The body of fungus is known as thallus.It consist of a single cell as in yeast or it consists of filaments (Mould)
- They donot possesss stems,roots,leaves or vascular system.
- They do not show division of labour.

- 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.
- They 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 20ºC and 30ºC
- Fungi can withstand extremely low temperature as low as 195ºC 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 prostists.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, Puccina etc. Fungi are either unicellular or multicellular forms.The yeast are unicellular.The moulds and mushrooms are multicellular.

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

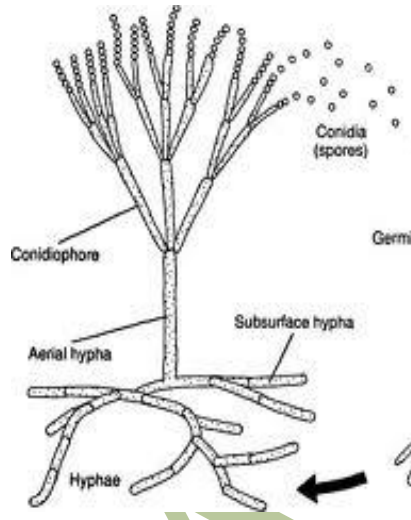
Mycelium It can be divided into vegetarian mycelium which grows into the medium and the aerial mycelium which projects from the surface.

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.

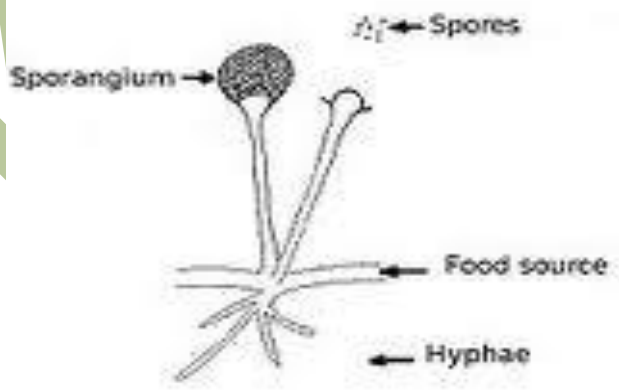
The mycelium without septa is called aseptate mycelium.The mycelium with septum is called a septate mycelium.

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.

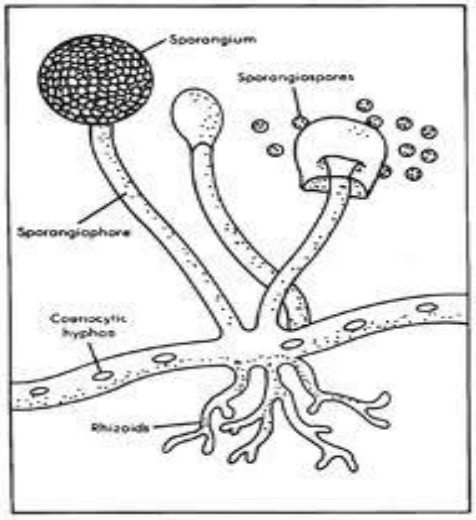
The nutrition in fungus is heterotrophic. They live as saprophytes or parasites or symbionts. Reproduction occurs by asexual and sexual methods.



Aspergillus species



Fungus



Asexual fruiting structure

Economic importance of fungi

Medicines: Antibiotics are obtained from fungi.

- Penicillum is obtained from *Penicillum 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 produce by fungi Eg.*Aspergillus flavus* produce digestion.

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

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*, athlets 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

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

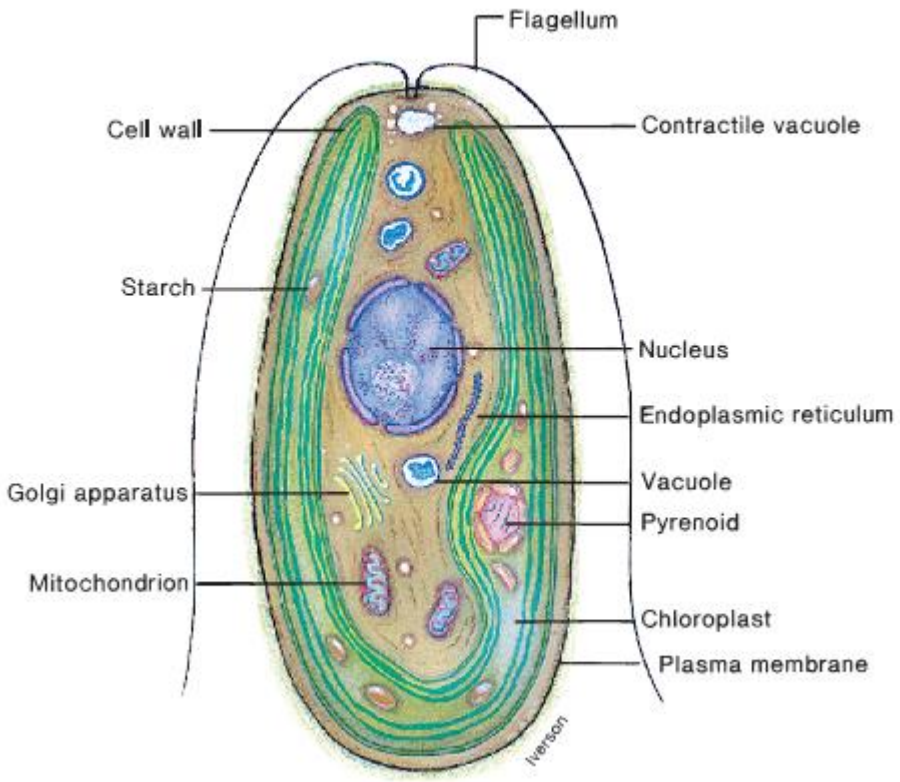
According to the five-kingdom system of Whittaker, the algae belong to seven divisions distributed between two different kingdoms. This classical classification is based on cellular, not organismal, properties. Some more important properties include: (1) cell wall (if present) chemistry and morphology; (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
Chrysophyta (yellow-green and golden-brown algae; diatoms)	*Protista* (single cell or colonial; eucaryotic)
Euglenophyta (photosynthetic euglenoid flagellates)	*Protista*
Pyrrhophyta (dinoflagellates)	*Protista*
Charophyta (stoneworts)	*Protista*
Chlorophyta (green algae)	*Protista*
Phaeophyta (brown algae)	*Plantae* (multicellular; eucaryotic)
Rhodophyta (red algae)	*Plantae*

Ultrastructure of the Algal Cell

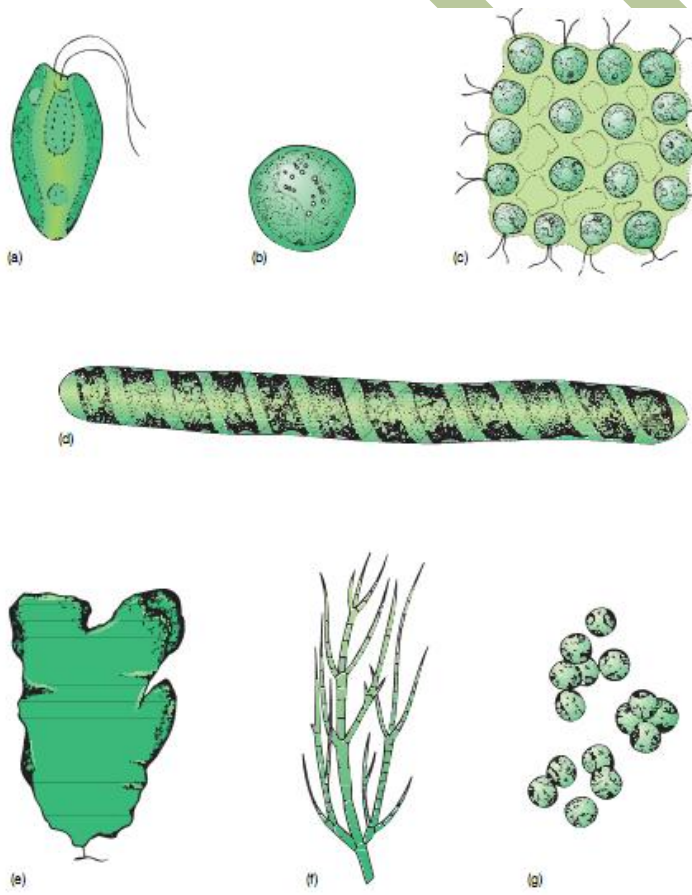
The eucaryotic algal cell is surrounded by a thin, rigid cell wall. Some algae have an outer matrix lying outside the cell wall. This usually is flexible and gelatinous, similar to bacterial capsules. When present, the flagella are the locomotor organelles. The nucleus has a typical nuclear envelope 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.



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.

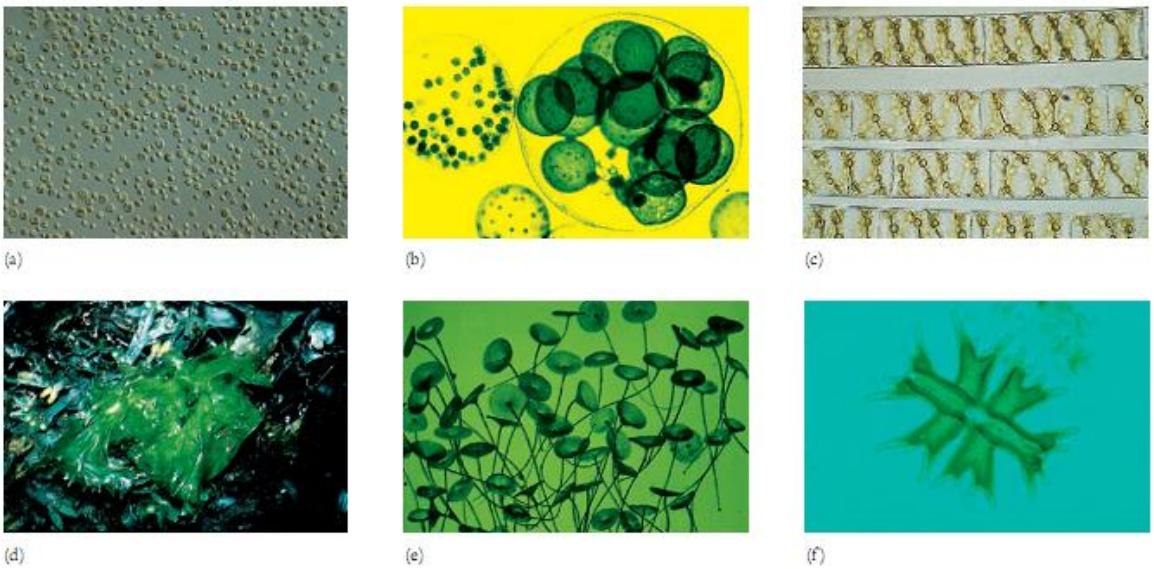


Diagrammatic Algal Bodies: (a) unicellular, motile, Cryptomonas; (b) unicellular, nonmotile, almellopsis; (c) colonial, Gonium; (d) filamentous, Spirotaenia; (e) bladelike kelp, Monostroma; (f) leafy tubular axis, branched tufts or plumes, Stigeoclonium; (g) unicellular, nonmotile, Chrysocapsa.

Characteristics of the Algal Divisions

Chlorophyta (Green Algae)

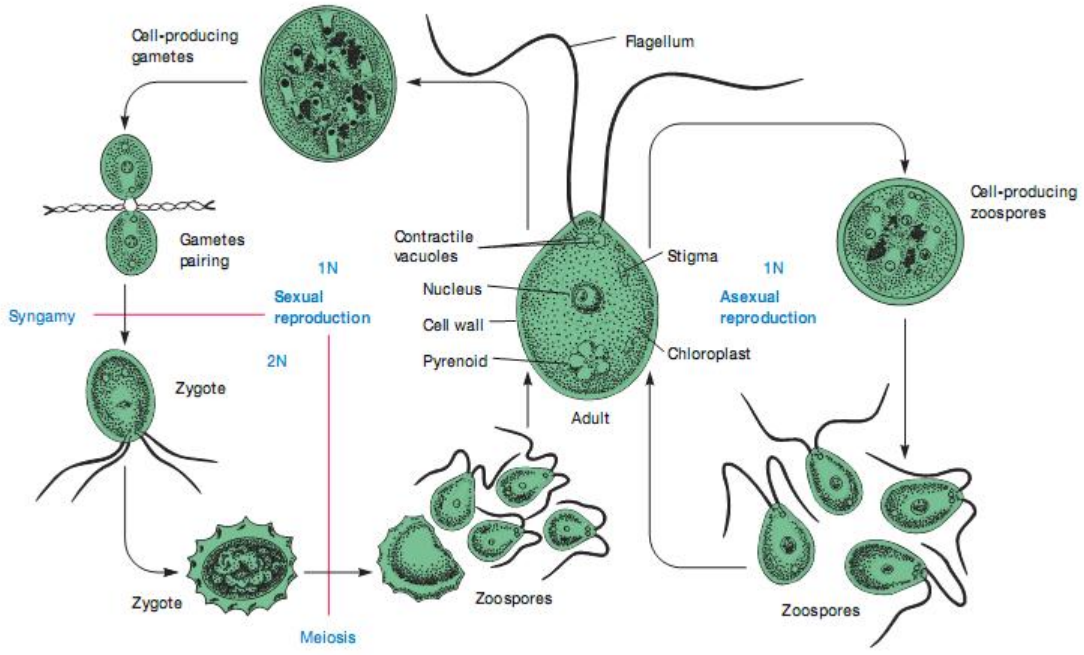
The Chlorophyta or green algae [Greek chloros, green] are an extremely varied division. They grow in fresh and salt water, in soil, on other organisms, and within other organisms. The Chlorophyta have chlorophylls a and b along with specific carotenoids, and they store carbohydrates as starch. Many have cell walls of cellulose. They exhibit a wide diversity of body forms, ranging from unicellular to colonial, filamentous, membranous or sheetlike, and tubular types.



Chlorophyta (Green Algae); Light Micrographs. (a) Chlorella, a unicellular nonmotile green alga (160). (b) Volvox, a typical green algal colony (450). (c) Spirogyra, a filamentous green alga (100). Four filaments are shown. Note the ribbonlike, spiral chloroplasts within each

filament. (d) Ulva, commonly called sea lettuce, has a leafy appearance. (e) Acetabularia, the mermaid's wine goblet. (f) Micrasterias, a large desmid (150).

Chlamydomonas is a representative unicellular green alga (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.

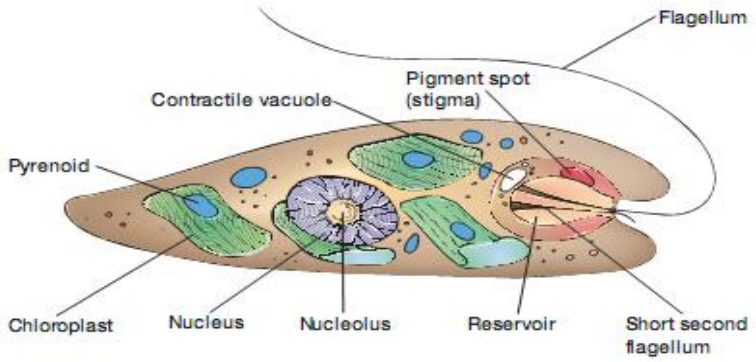
Charophyta (Stoneworts/Brittleworts)

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.



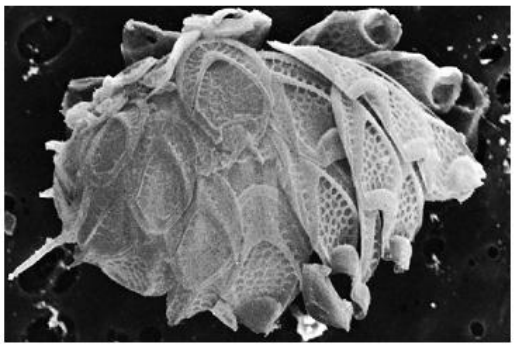
Euglena. A Diagram Illustrating the Principal Structures Found in This Euglenoid. Notice that a short second flagellum does not emerge from the anterior invagination. In some euglenoids both flagella are emergent.

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

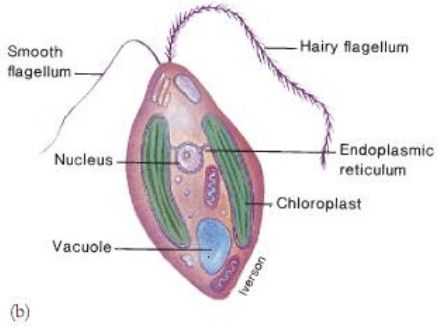
The division Chrysophyta is quite diversified with respect to pigment composition, cell wall, and type of flagellated cells. In molecular classification schemes, these algae are associated with the stramenopiles and have mitochondria with tubular cristae. The division is divided into three major classes: 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 -1,3 linked glucose residues).

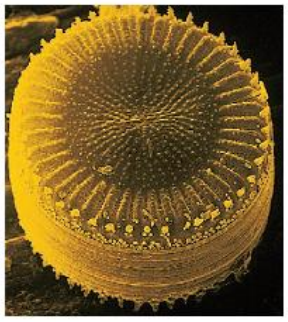
Some Chrysophyta lack cell walls; others have intricately patterned coverings external to the plasma membrane, such as scales (figure 26.6a), walls, and plates. Diatoms have a distinctive two-piece wall of silica, called a frustule. Two anteriorly attached flagella of unequal length are common among 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.

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 carboncontaining 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.



(a)





(c)

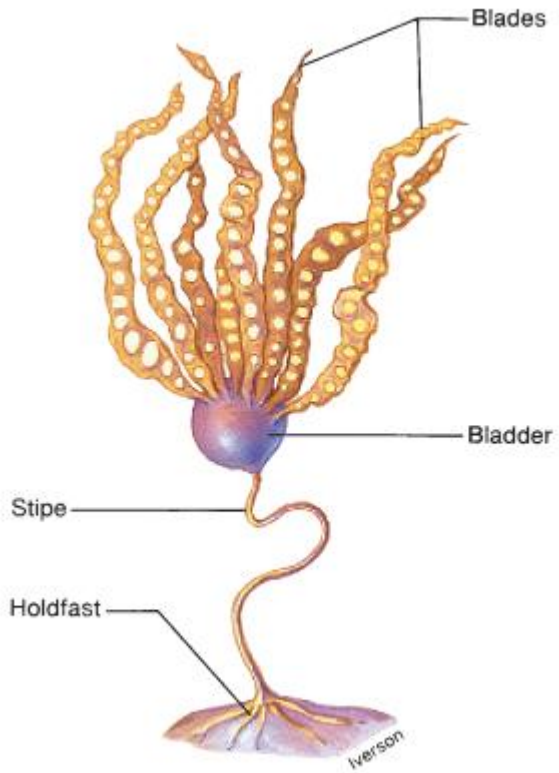
(d)

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

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.

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).

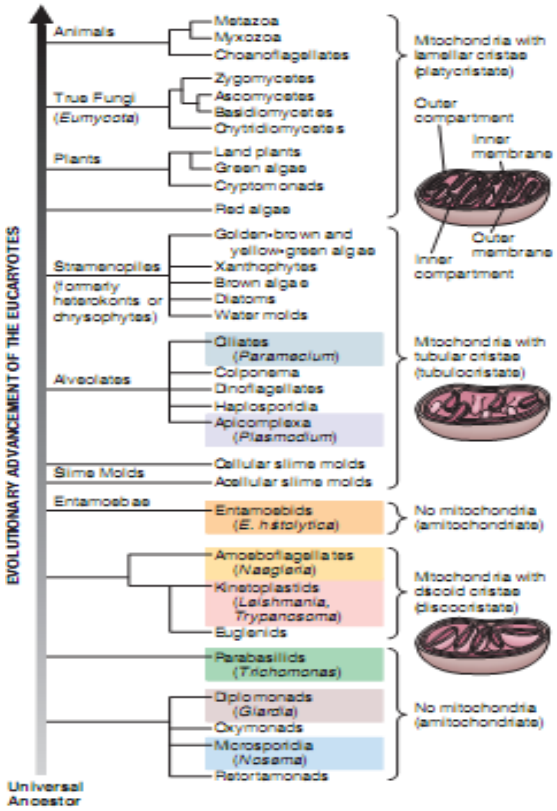
The red algae contain the red pigment phycoerythrin, one of the two types of phycobilins that they possess. The other accessory pigment is the blue pigment phycocyanin. The presence of these pigments explains how the red algae can live at depths of 100 m or more. The wavelengths of light (green, violet, 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 un- dergoes photodestruction in bright light, other pigments predominate and the algae take on shades of blue, brown, and dark green. The cell walls of most red algae include a rigid inner part composed of microfibrils and a mucilaginous matrix. The matrix is composed of sulfated polymers of galactose called agar, funori, porphysan, and carrageenan. These four polymers give the red algae their flexible, slippery texture. Agar is used extensively in the laboratory as a culture medium component.

Many red algae also deposit calcium carbonate in their cell walls and play an important role in building coral reefs.

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.



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

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	*Entamoeba*	Mammals	Intestine	Amebiasis
	Iodamoeba	Swine	Intestine	Enteritis
Sporozoa	*Babesia*	Cattle	Blood cells	Babesiosis
	Theileria	Cattle, sheep, goats	Blood cells	Theileriasis
	Sarcocystis	Mammals, birds	Muscles	Sarcosporidiosis
	Toxoplasma	Cats	Intestine	Toxoplasmosis
	Isospora	Dogs	Intestine	Coccidiosis
	Eimeria	Cattle, cats, chickens, swine	Intestine	Coccidiosis
	Plasmodium	Many animals	Bloodstream, liver	Malaria
	Leucocytozoon	Birds	Spleen, lungs, blood	Leucocytozoonosis
	Cryptosporidium	Mammals	Intestine	Cryptosporidiosis
Ciliates	*Balantidium*	Swine	Large intestine	Balantidiasis
Flagellates	*Leishmania*	Dogs, cats, horses, sheep, cattle	Spleen, bone marrow, mucous membranes	Leishmaniasis
	Trypanosoma	Most animals	Blood	Trypanosomiasis
	Trichomonas	Horses, cattle	Genital tract	Trichomoniasis (abortion)
	Histomonas	Birds	Intestine	Blackhead disease
	Giardia	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.

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).

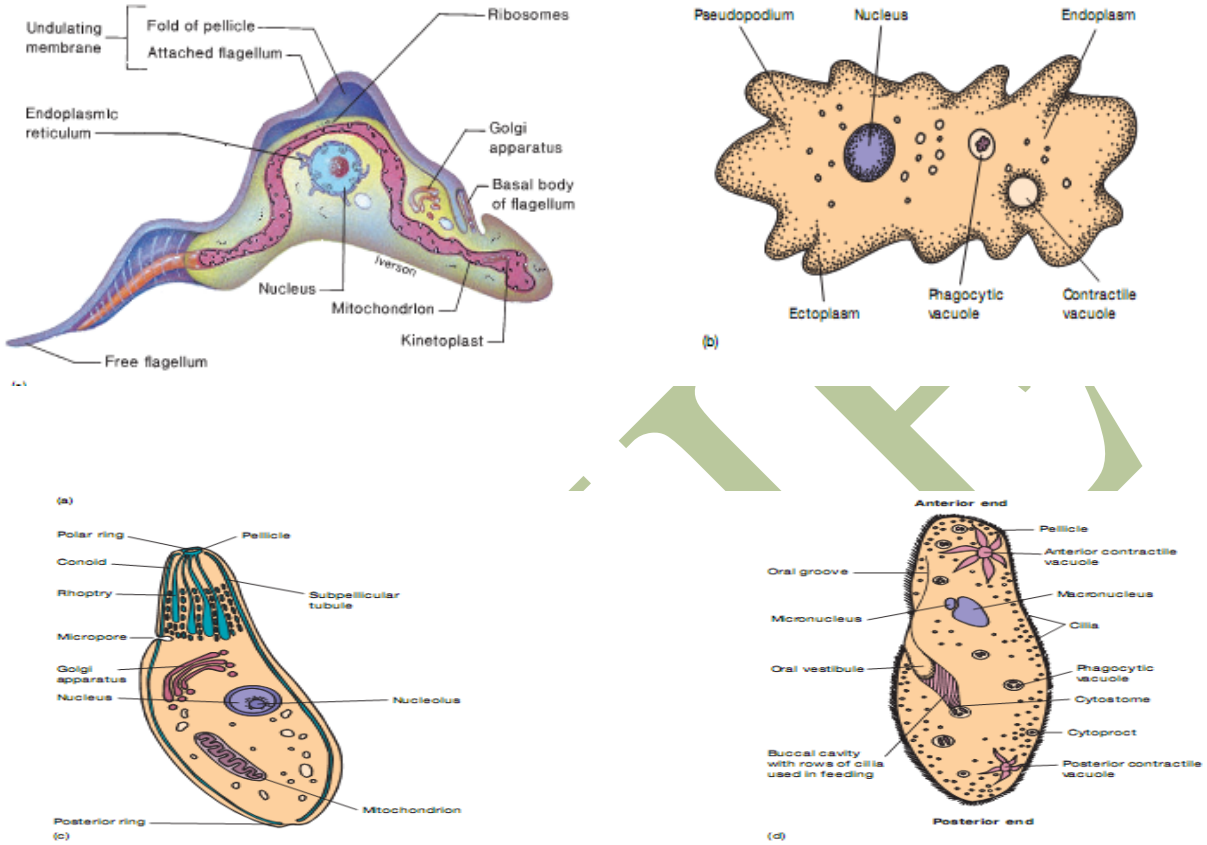
Classification

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

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: *Sarcomastigophora*	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: *Mastigophora*	One or more flagella; division by longitudinal binary fission; sexual reproduction in some groups	
Class: *Zoomastigophorea*	Chromatophores absent; one to many flagella; amoeboid forms, with or without flagella; sexuality known in some groups; mainly parasitic	*Trypanosoma* *Giardia* *Trichomonas* *Leishmania* *Trichonympha*
Subphylum: *Sarcodina*	Locomotion primarily by pseudopodia; shells (tests) often present; flagella restricted to reproductive stages when present; asexual reproduction by fission; mostly free living	
Superclass: *Rhizopoda*	Locomotion by pseudopodia or by protoplasmic flow with discrete pseudopodia; some contain tests	*Amoeba* *Elphidium* *Coccodiscus*
Phylum: *Labyrinthomorpha*	Spindle-shaped cells capable of producing mucous tracks; trophic stage as ectoplasmic network; nonamoeboid cells; saprozoic and parasitic on algae and seagrass	*Labyrinthula*
Phylum: *Apicomplexa*	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	*Plasmodium* *Toxoplasma* *Eimeria* *Cryptosporidium*
Phylum: *Microspora*	Unicellular spores with spiroplasm containing polar filaments; obligatory intracellular parasites	*Nosema*
Phylum: *Ascetospora*	Spore with one or more spiroplasms; no polar capsules or polar filaments; all parasitic in invertebrates	*Haplosporidium*
Phylum: *Myxozoa*	Spores of multicellular origin; one or more polar capsules; all parasitic, especially in fish	*Myxosoma*
Phylum: *Ciliophora*	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	*Didinium* *Stentor* *Vorticella* *Tetrahymena* *Paramecium* *Tokophrya* *Entodinium* *Nyctotherus* *Balantidium* *Ichthyophthirius*



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

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

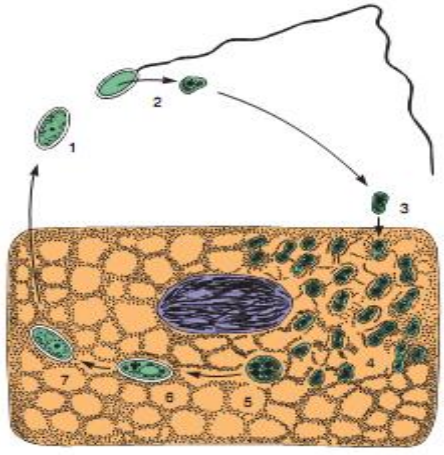
events producing many small infective organisms through the formation of uninuclear buds. Sexual reproduction involvesnthe fertilization of a large female macrogamate by a small, flagellated male gamete. The resulting zygote becomes a thickwalled cyst called an oocyst.Within the oocyst, meiotic divisions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



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 Ascetospora

Ascetospora is a relatively small phylum that consists exclusively of parasitic protists characterized by spores lacking polar caps or polar filaments. Ascetosporans 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 m 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

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 inside the cells and they are active, feed, respire, reproduce, grow and move.

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

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 nfor the synthesis of ribosomal protein, rRna and tRNA.

Structure of viruses

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

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

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

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,

Viruses are single stranded DNA(Ssdna) Eg-*Coliphage*

Viruses with double strandedDNA(DsDNA) Eg-*Vaccina*

Viruses with single stranded RNA(SsRNA) Eg-*TMV*

Viruses with double stranded RNA(DsRNA) Eg-*Reovirus.*

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 aatachment 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 (symmentry). 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 donot cause lysis of the host cell. They exhibit lysogenic life cycle.

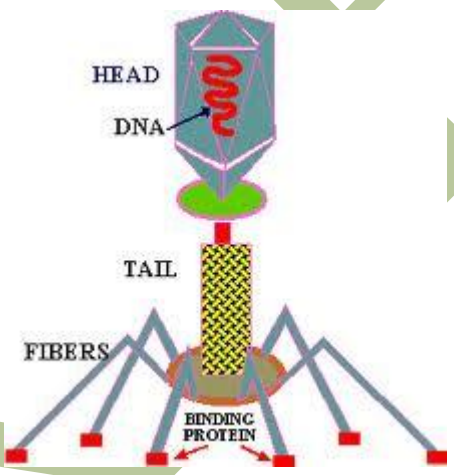
Structure of virus

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.

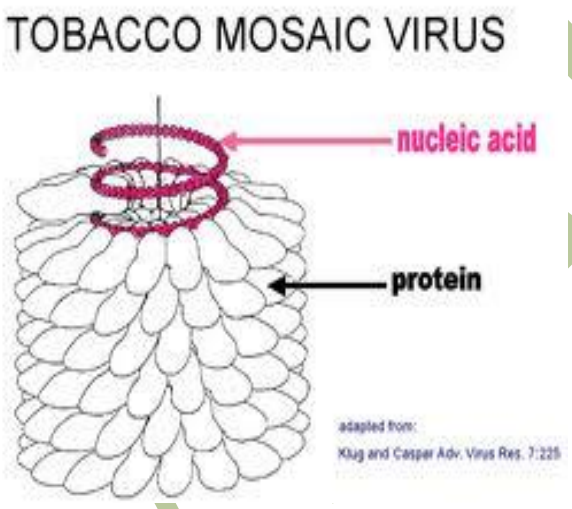


T4 bacteriophage

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.

The bacteriophage has a complex symmetry .It exhibits a combination of icosahedral and helical symmetry. The head is icohederal 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 th head are arranged in the form of an icohederon 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 facts. 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, Polyhederal, helical, cylindrical or rod-like.

Viruses occur in three main shapes, namely

A,Polyhedral

B,Helical

C,Complex

A, Polyhederal

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

In tetrahedral viruses, the capsid has 4 sides. In octahedral viruses,the capsid has 8 sides.In icosahederal viruses,the capsid has 8 sides.IN icosahederal 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.

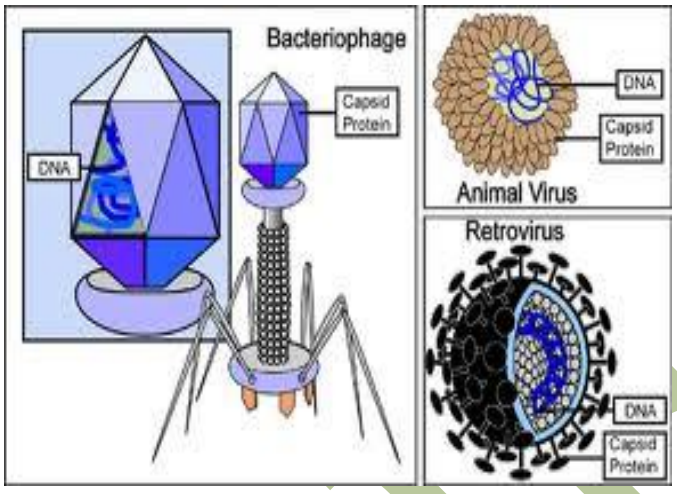
The capsid is made up of 2,130 monomers.The monomers are arranged in 130 turns.

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.



Classification

Viruses are grouped under Acaryota, Holmes(1948) included viruse 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

Viruses that infect animals are called animal viruses.They are include in the group Zoophaginae. Animal viruses contain DNA. Some animal viruses contain RNA. The ucleic 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 :

Poliovirus *yellow fever virus*

Vaccinia virus *rabies virus*

Adeno virus *mumps virus*

Herpes virus *Measles virus*

Reovirus *Influenza virus*

Dengue virus *HIV*

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: *Tobacco mosaic virus(TMV)* *Cucumo virus*

Tomato mosaic virus *Tobacco necrosis virus*

Potato virus *Alfalfa mosaic virus*

Papaya mosaic virus *cauliflower mosaic virus*

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 plae in contact with the bacterial cellwall.

2. Injection of phage DNA into the Bacterium

The tail secret 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;mit remains outside the bacterium.

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 nucleutides 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

All the T-series phages exhibit lytic cycle. These viruses infect bacteria and on the competition 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 curface of the bacterium.

The spikes anchor firmly

A hole is made in the cellwall 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 copies is enclosed by a protein coat producing new virus

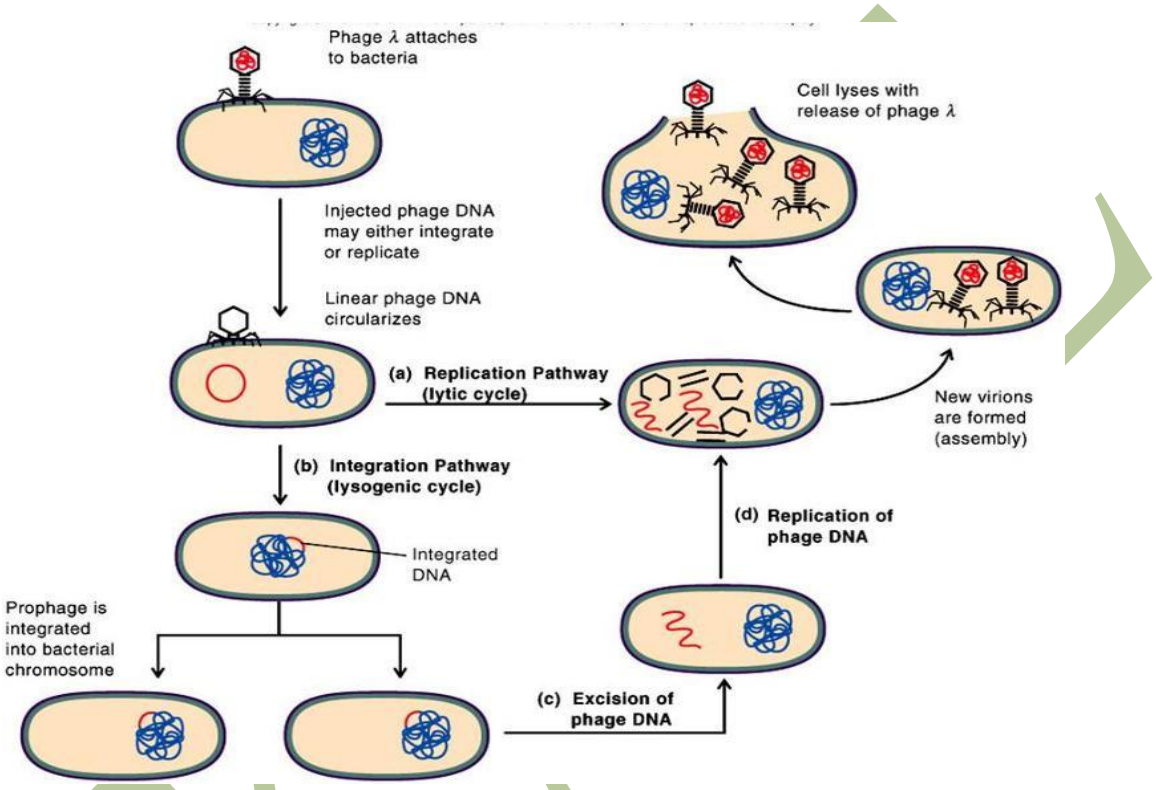
Soon the bacterial wall ruptures and the new virus particle are released

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

Lysogenic cycle

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

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

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

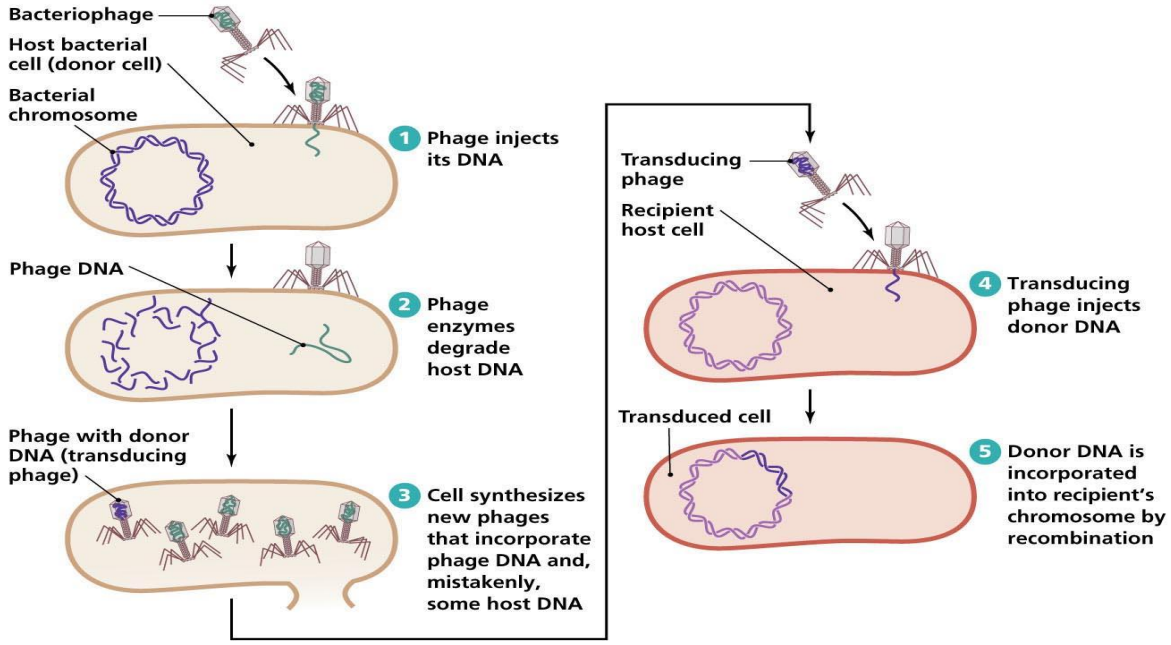
Non-envelopedviruses

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.



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

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.

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 organells 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.

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.

Unit III

SYLLABUS

Nutritional categories of microorganisms, media, types of media, methods of isolation, staining and types, purification and preservation

Nutritional Requirements of Microorganisms

Mineral Nutrients

The microbial nutrients can be classified as macro (major) nutrients, and micro (minor) nutrients or trace elements on the basis of their amount required.

Macro or Major Mineral Nutrients:

The microbial cells contain water accounting for some 80-90% of their total weight and, therefore, the water is always the major essential nutrient in quantitative terms.

The solid matter of cells contain, in addition to oxygen and hydrogen (derivable metabolically from water), the other macro (major) elements, namely, carbon, nitrogen, phosphorus, sulphur, potassium, magnesium, sodium, calcium and iron in order of decreasing abundance.

About 95% of cellular dry weight of microbial cells is accounted for only six macro (major) elements (O, H, C, N, P and S). However, approximate percentage of dry weight and general physiological functions of major mineral nutrients are given in Table.

Element (Nutrient)	Percentage of dry weight	Physiological functions
(*i*) Carbon (C)	50	Constituent of all organic cell materials.
(*ii*) Oxygen (O)	20	Constituent of cellular water and most organic cell materials; molecular oxygen serves as an electron receptor in aerobic respiration.
(*iii*) Nitrogen (N)	14	Constituent of proteins, nucleic acids, coenzymes.
(*iv*) Hydrogen (H)	8	Constituent of cellular water, organic cell materials.
(*v*) Phosphorus (P)	3	Constituent of nucleic acids, phospholipids, coenzymes.
(*vi*) Sulphur (S)	1	Constituent of some amino acids (cysteine and methionine), of some coenzymes (*e.g.*, CoA, cocarboxylase).
(*vii*) Potassium (K)	1	Important inorganic cation in cells, cofactor for some enzymatic reactions.
(*viii*) Sodium (Na)	1	Important inorganic cations in cells, important in membrane transport.
(*ix*) Calcium (Ca)	0.5	Important inorganic cation in cells, cofactor for some enzymatic reactions (*e.g.*, reactions by proteinases). It is essential component of endospores as calcium dipicolinate. Calcium concentrations affect membrane permeability and play a critical role in movement of flagella and cilia.
(*x*) Magnesium (Mg)	0.5	Important inorganic cation in cells, cofactor for some enzymatic reactions sometimes replacing Mg. Magnesium plays important role in protein synthesis; without it the ribosomal subunits do not associate and translation of nucleic acids into protein is not possible.
(*xi*) Iron (Fe)	0.2	Constituent of cytochromes and other haeme or non-haeme proteins, cofactor for a number of enzymatic reactions.

Carbon assumes great importance as the main constituent of all organic cell materials and represents about 50% of cell's dry weight. CO_2 is the most oxidized form of carbon and the photosynthetic microorganisms reduce CO_2 to organic cell constituents. On the other hand, all the non-photosynthetic microorganisms obtain their carbon requirement mainly from organic nutrients which contain reduced carbon compounds.

These organic compounds not only provide the carbon for synthesis but also meet the energy requirement by entering into energy yielding metabolic pathways and are eventually oxidised to CO_2.

Some microbes have the ability to synthesize all their cellular components using a single organic carbon source while others, in addition to this one major carbon source, also need other complex carbon containing components which they cannot synthesize.

These components are called growth factors and include vitamins. Some microbes can utilize more than one carbon compound and exhibit a great degree of versatility. The others, however, are specialized in this regard.

Sulphur and nitrogen are taken up by most organisms and are subsequently reduced within the cell and utilized in other biosynthetic processes. The sulphur and nitrogen requirements of most organisms can also be met with organic nutrients that contain these two elements in reduced organic combinations such as amino acids. A few microorganisms are capable of reducing elemental nitrogen to ammonia and this process of nitrogen assimilation is known as biological nitrogen fixation.

Most of the microorganisms need molecular oxygen for respiration. In these, the oxygen serves as terminal electron acceptor, and such organisms are referred to as 'obligate aerobes'.

As opposed to this there are a few organisms which do not use molecular oxygen as terminal electron acceptor. We recall that oxygen is a component of the cellular material of all the microorganisms. These microbes are called 'obligate anaerobes'.

In fact, molecular oxygen is toxic to these organisms. Aerobes which can grow in the absence of oxygen are called 'facultative anaerobes' and the anaerobes which can grow in the presence of oxygen are referred to as 'facultative aerobes'. In addition to these major classes, there are organisms which grow best at reduced oxygen pressure but are obligate aerobes and these are called 'Microaerophilic'.

Micro or Minor Mineral Nutrients or Trace Elements:

The microorganisms, in general do not use only macro (major) elements but also others like cobalt, copper, manganese, molybdenum, nickel, selenium, tungsten, vanadium and zinc which are required in residual fraction by nearly all microorganisms.

These elements are often referred to as minor (micro) nutrients or trace elements. The micronutrients or trace elements are nevertheless just as critical to cell function as are the macronutrients.

They are metals playing the role of cell's catalysts and many of them are play a structural role in various enzymes. Following table summarizes the major micronutrients of living systems and gives examples of enzymes in which each plays a role. Some microorganisms, however, need additional specific mineral nutrients, for example, diatoms and some microalgae require silica, supplied as silicate, to impregnate their cell walls.

Micronutrient	Cellular function
(*i*) Cobalt (Co)	Vitamin B_{12}; transcarboxylase (propionic acid bacteria).
(*ii*) Copper (Cu)	Respiration (cytochrome *c* oxidase); photosynthesis (plastocyanin, some superoxide dismutases).
(*iii*) Manganese (Mn)	Acts as activator of various enzymes; occurs in some superoxide dismutases and in the photolytic (water-splitting) enzyme in oxygenic phototrophs (photosystem-II).
(*iv*) Molybdenum (Mo)	Present in some flavin-containing enzymes, nitrogenase, nitrate reductase, sulphide oxidase, some formate dehydrogenases.
(*v*) Nickel (Ni)	Present in most hydrogenase enzymes; coenzyme F_{430} of methano-genes; carbon monoxide dehydrogenase; urease.
(*vi*) Selenium (Se)	Occurs in formate dehydrogenase; certain hydrogenases; amino acid selenocysteine.
(*vii*) Tungsten (W)	In some formate dehydrogenases; oxotransferases of hyperthermo-philes.
(*viii*) Vanadium (V)	Vanadium nitrogenase; bromoperoxidase.
(*ix*) Zinc (Zn)	In carbonic anhydrase; alcohol dehydrogenase; RNA and DNA polymerases; many DNA-binding proteins.

Growth Factors

Besides the mineral nutrients, the microorganisms need some organic compounds. Most of the microorganisms are capable of synthesizing these organic compounds from simpler carbon resources; others cannot and need their supply from outside for their proper growth and development.

Organic nutrients of this type are known collectively as growth factors (essential metabolites) and can be categorized into three groups (amino acids, purines and pyrimidines and vitamins) on the basis of their chemical structure and metabolic function.

Amino acids and purines and pyrimidines are the constituents of proteins and nucleic acids, respectively. Vitamins, however, are the most commonly needed growth factor and form parts of the prosthetic groups or active centres of certain enzymes. Some important vitamins and their functions are summarized in the following table.

	Vitamin	Functions
(*i*)	Riboflavin (B_2)	Precursor of flavin mononucleotide (FMN) and flavin-adenine dinucleotide (FAD) which are involved in electron transport chain.
(*ii*)	Cobalamine (B_{12})	Reduction of and transfer of single carbon fragments; synthesis of deoxyribose.
(*iii*)	Biotin	In fatty acid biosynthesis; in β-decarboxylation; in some CO_2-fixation reactions.
(*iv*)	*p*-Aminobenzoic acid	Precursor of folic acid.
(*v*)	Folic acid	One–carbon metabolism; transfer of methyl group.
(*vi*)	Thiamine (B_1)	Transketolase; α-decarboxylations.
(*vii*)	Nicotinic acid (niacin)	Precursor of nicotinamide adenine dinucleotide (NAD^+); electron transfer in oxidation-reduction reactions.
(*viii*)	Lipoic acid	Acyl group transfer in decarboxylation of pyruvic acid and α-ketoglutaric acid.
(*ix*)	Pantothenic acid	Precursor of coenzyme A; activation of acetyl and other acyl derivations.
(*x*)	Vitamin B_6	Amino acid and keto acid transformations.
(*xi*)	Vitamin K	Electron transport; in synthesis of shingolipids.
(*xii*)	Hydroxamates	Solubilization of iron and transport into cell.

Since the growth factors fulfill specific needs in biosynthesis of certain molecules, they are needed in very small amounts; the vitamins even in less smaller quantities, because of the various coenzymes of which they are precursors, have catalytic roles and consequently are present at levels of a few parts per million in the microbial cell.

Microbiological Techniques

Concepts

1. Microorganisms require about 10 elements in large quantities, in part because they are used to construct carbohydrates, lipids, proteins, and nucleic acids. Several other elements are needed in very small amounts and are parts of enzymes and cofactors.
2. All microorganisms can be placed in one of a few nutritional categories on the basis of their requirements for carbon, energy, and hydrogen atoms or electrons.
3. Nutrient molecules frequently cannot cross selectively permeable plasma membranes
4. Through passive diffusion. They must be transported by one of three major mechanisms
5. Involving the use of membrane carrier proteins. Eukaryotic microorganisms also employ endocytosis for nutrient uptake.
6. Culture media are needed to grow microorganisms in the laboratory and to carry out specialized procedures like microbial identification, water and food analysis, and the isolation of particular microorganisms. Many different media are available for these and other purposes.
7. Pure cultures can be obtained through the use of spread plates, streak plates, or pour plates and are required for the careful study of an individual microbial species.

Media preparation

Microrganisms need nutrients, a source of energy and certain environmental conditions in order to grow and reproduce. In the environment, microbes have adapted to the habitats most suitable for their needs, in the laboratory, however, these requirements must be met by a culture medium. This is basically an aqueous solution to which all the necessary nutrients have been added. Depending on the type and combination of nutrients, different categories of media can be made.

Categories

Complex media are rich in nutrients, they contain water soluble extracts of plant or animal tissue (e.g., enzymatically digested animal proteins such as peptone and tryptone). Usually a sugar, often glucose is added to serve as the main carbon and energy source. The combination of extracts and sugar creates a medium which is rich in minerals and organic nutrients, but since the exact composition is unknown, the medium is called complex.

Defined media are media composed of pure ingredients in carefully measured concentrations dissolved in double distilled water i.e., the exact chemical composition of the medium is known. Typically, they contain a simple sugar as the carbon and energy source, an inorganic nitrogen source, various mineral salts and if necessary growth factors (purified amino acids, vitamins, purines and pyrimidines).

Selective/differential media are media based on either of the two categories above supplemented with growth-promoting or growth-inhibiting additives. The additives may be species- or organism-selective (e.g., a specific substrate, or an inhibitor such as cyclohexamide (artidione) which inhibits all eucaryotic growth and is typically used to prevent fungal growth in mixed cultures)

Differential media or indicator media: distinguish one microorganism type from another growing on the same media. This type of media uses the biochemical characteristics of a microorganism growing in the presence of specific nutrients or indicators (such as neutral red, phenol red, eosin y, or methylene blue) added to the medium to visibly indicate the defining characteristics of a microorganism. This type of media is used for the detection of microorganisms and by molecular biologists to detect recombinant strains of bacteria

Isolation of Pure Bacterial Cultures from Specimens

Selective Media

- A selective medium is prepared by the addition of specific substances to a culture medium that will permit growth of one group of bacteria while inhibiting growth of some other groups.
- The following are examples:
- Salmonella-Shigella agar (SS) is used to isolate *Salmonella* and *Shigella* species. Its bile salt mixture inhibits many groups of coliforms. Both *Salmonella* and *Shigella* species produce

colorless colonies because they are unable to ferment lactose. Lactose-fermenting bacteria will produce pink colonies.

- Mannitol salt agar (MS) is used for the isolation of staphylococci. The selectivity is obtained by the high (7.5%) salt concentration that inhibits growth of many groups of bacteria. The mannitol in this medium helps in differentiating the pathogenic from the nonpathogenic staphylococci, as the former ferment mannitol to form acid while the latter do not. Bismuth sulfite agar (BS) is used for the isolation of *Salmonella typhi*, especially from stool and food specimens. *S. typhi* reduces the sulfite to sulfide, resulting inblack colonies with a metallic sheen.

Differential Media

The incorporation of certain chemicals into a medium may result in diagnostically useful growth or visible change in the medium after incubation. The following are

examples:

Eosin methylene blue agar (EMB) differentiates between lactose fermenters and nonlactose fermenters. EMB contains lactose, salts, and two dyes—eosin and methylene blue. *E. coli*, which is a lactose fermenter, will produce a dark colony or one that has a metallic sheen. *S. typhi*, a nonlactose fermenter, will appear colorless.

MacConkey agar is used for the selection and recovery of *Enterobacteriaceae* and related gram-negative rods. The bile salts and crystal violet in this medium inhibit the growth of gram-positive bacteria and some fastidious gram-negative bacteria. Because lactose is the sole carbohydrate, lactose-fermenting bacteria produce colonies that are various shades of red, whereas nonlactose fermenters produce colorless colonies.

Hektoen enteric agar is used to increase the yield of *Salmonella* and *Shigella* species relative to other microbiota. The high bile salt concentration inhibits the growth of gram-positive bacteria and retards the growth of many coliform strains.

Enrichment Media

The addition of blood, serum, or extracts to tryptic soy agar or broth will support the growth of many fastidious bacteria. These media are used primarily to isolate bacteria from cerebrospinal fluid, pleural fluid, sputum, and wound abscesses. The following are examples:

Blood agar (can also be a differential medium): addition of citrated blood to tryptic soy agar makes possible variable hemolysis, which permits differentiation of some species of bacteria. Three hemolytic patterns can be observed on blood agar.

1. α-hemolysis—greenish to brownish halo around the colony (e.g., *Streptococcus gordonii*, *Streptococcus pneumoniae*).

2. β-hemolysis—complete lysis of blood cells resulting in a clearing effect around growth of the colony (e.g., *Staphylococcus aureus* and *Streptococcus*

pyogenes).

3. Nonhemolytic—no change in medium (e.g., *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*).Chocolate agar is made from heated blood, which provides necessary growth factors to support bacteria such as *Haemophilus influenzae* and *Neisseria*

gonorrhoeae.

Characteristic Media

- Characteristic media are used to test bacteria for particular metabolic activities, products, or requirements. The following are examples:
- Urea broth is used to detect the enzyme urease. Some enteric bacteria are able to break down urea, using urease, into ammonia and CO2.

- Triple sugar iron (TSI) agar contains lactose, sucrose, and glucose plus ferrous ammonium sulfate and sodium thiosulfate. TSI is used for the identification of enteric organisms based on their ability to attack glucose, lactose, or sucrose and to liberate sulfides from ammonium sulfate or sodium thiosulfate.

- Citrate agar contains sodium citrate, which serves as the sole source of carbon, and ammonium phosphate, the sole source of nitrogen. Citrate agar is used to differentiate enteric bacteria on the basis of citrate utilization.

- Lysine iron agar (LIA) is used to differentiate bacteria that can either deaminate or decarboxylate the amino acid lysine. LIA contains lysine, which permits enzymedetection, and ferric ammonium citrate for the detection of H2S production.Sulfide, indole, motility (SIM) medium is used for three different tests. One can observe the production of sulfides, formation of indole (a metabolic product fromtryptophan utilization), and motility. This medium is generally used for the differentiation of enteric organisms.

Methods of Sterilization

Sterilization is a term referring to any process that eliminates (removes) or kills all forms of microbial life, including transmissible agents (such as fungi, bacteria, viruses, spore forms, etc.) present on a surface, contained in a fluid, in medication, or in a compound such as biological

culture media. Sterilization can be achieved by applying the proper combinations of heat, chemicals, irradiation, high pressure, and filtration

Sterilization implies the complete destruction of all microorganisms including spores, this is accomplished by the use of heat, chemicals, radiation, filtration

Heat: Denatures and coagulates vital proteins. There are various forms of heat sterilisation.

Red Heat :Inoculating wires or loops are sterilised by holding them in a Bunsen flame until they are red hot.

Moist Heat : Bacteria are more readily destroyed by moist heat (steam) than dry heat. Usually used for the sterilisation of culture media, aqueous solutions and the destruction of discarded cultures. Air must first be removed in order to achieve the 121 °C necessary for successful sterilisation. This is accomplished by the use of an autoclave (the technical version of a pressure cooker), which follows automatic cycles of heating under pressure for the required time.

Dry Heat :Usually employed for materials which could either be corroded by steam or must remain dry before use. These include metal instruments, glass petri dishes, flasks and pipettes and cotton wool. In practice, dry heat sterilisation requires longer time intervals and higher temperatures than steam sterilisation, e.g. steam sterilisation 121°C for 15mins or dry heat sterilizations 160°C for 120 minutes.

Chemical :Usually employed for delicate equipment such as optical instruments and electrical devices which would be damaged by heat. Due to the toxicity of the chemicals used, this is not the most popular form of sterilisation. Chemicals employed include: gaseous ethylene oxide, which alkylates amino, sulfhydryl, carboxyl and hydroxyl groups of microbial cell compounds; formaldehyde, used as a fumigant; and hydrogen peroxide vapour used in aseptic packaging.

Radiation

Employed for heat-sensitive materials and for environmental samples such as soil and sediment where structural changes caused by heat need to be avoided. Two forms of radiation are used

Ultra violet radiation

Initiates the excitation of atoms which in nucleic acids leads to fatal mutations. UV light cannot penetrate materials so is used mainly for surface treatments e.g. Laminar flow benches, and air and water.

Ionizing Radiation: Can penetrate samples, causing ionization within cells. Gamma radiation (γ) generated through a ^{60}Co -source is used to sterilise complex matrices such as soil and foodstuff. Microorganisms show increased resistance to radiation under anoxic conditions (2-5x) and also in frozen samples.

Filtration : Filtration sterilization operates through the exclusion rather than destruction of microorganisms. It is safe for the user and is employed for sensitive liquids and gases. Three types of filters are currently in use

Depth Filters : These are made of columns packed with fibrous materials such as glass wool or cotton wool. The twisting and turning fibres entrap particles and so act as filters; they show little resistance to flow and are used mainly for gases or as pre-filters for membrane filters which are easily clogged.

Membrane Filters

Act by screening out particles. Their effectiveness depends on the size of the membrane pores and the electrostatic attractions present. The most commonly used filters in microbiology are usually made of cellulose acetate or cellulose nitrate.Size of filter pores required to screen out: Yeast 0.45 - 1.2 µm,. bacteria 0.2 µm Viruses and mycoplasmas 0.01-0.1µm.Membrane filtration is usually employed for heat-sensitive substances, e.g. vitamin solutions; the filters are heat-sterilised before use.

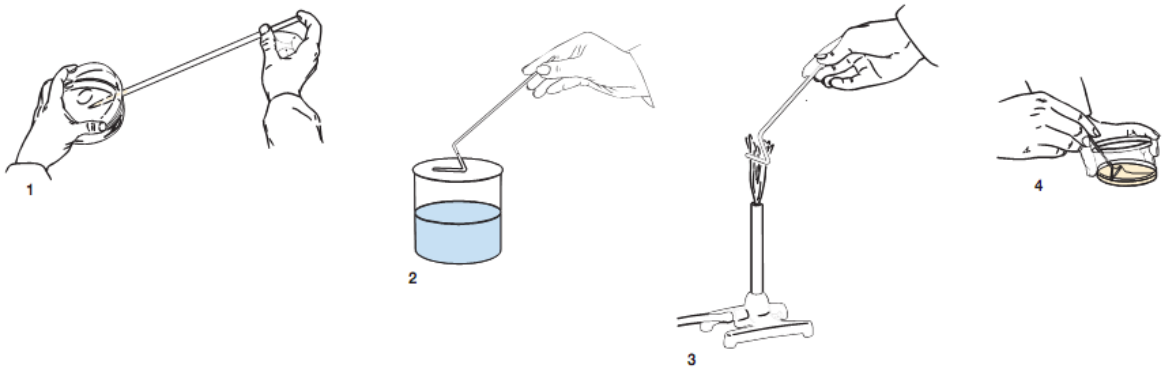
Techniques of pure culture

Isolation of Pure Cultures

In natural habitats microorganisms usually grow in complex, mixed populations containing several species. This presents a problem for the microbiologist because a single type of microorganism cannot be studied adequately in a mixed culture. One needs a pure culture, a population of cells arising from a single cell, to characterize an individual species. Pure cultures are so important that the development of pure culture techniques by the German bacteriologist Robert Koch transformed microbiology.

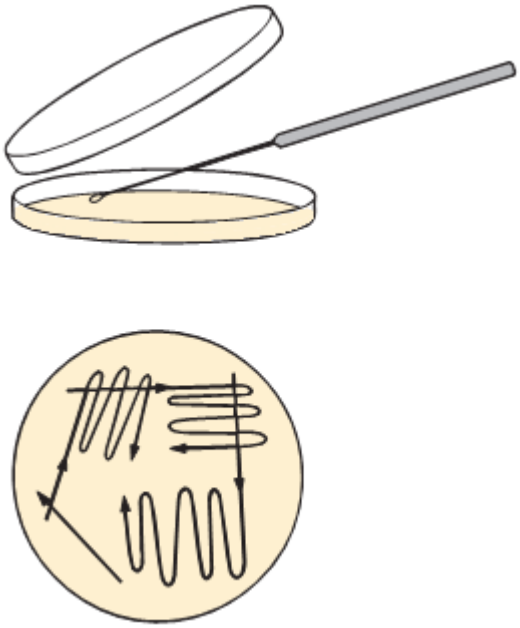
The Spread Plate and Streak Plate

If a mixture of cells is spread out on an agar surface so that every cell grows into a completely separate colony, a macroscopically visible growth or cluster of microorganisms on a solid medium, each colony represents a pure culture. The spread plate is an easy, direct way of achieving this result. A small volume of dilute microbial mixture containing around 30 to 300 cells is transferred to the center of an agar plate and spread evenly over the surface with a sterile bent-glass rod. The dispersed cells develop into isolated colonies. Because the number of colonies should equal the number of viable organisms in the sample, spread plates can be used to count the microbial population.

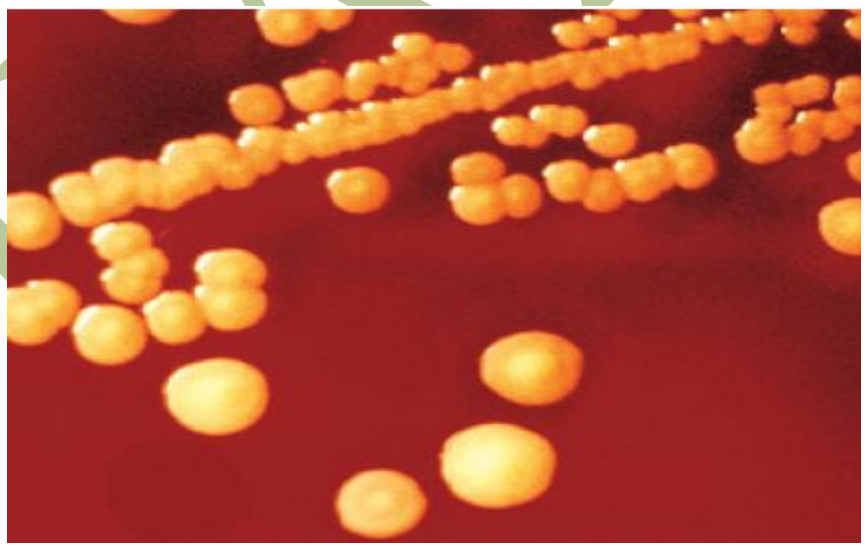


Spread-Plate Technique. The preparation of a spread plate. (1) Pipette a small sample onto the center of an agar medium plate. (2) Dip a glass spreader into a beaker of ethanol. (3) Briefly flame the ethanol soaked spreader and allow it to cool. (4) Spread the sample evenly over the agar surface with the sterilized spreader. Incubate.

Pure colonies also can be obtained from streak plates. The microbial mixture is transferred to the edge of an agar plate with an inoculating loop or swab and then streaked out over the surface in one of several patterns. At some point in the process, single cells drop from the loop as it is rubbed along the agar surface and develop into separate colonies. In both spread-plate and streak-plate techniques, successful isolation depends on spatial separation of single cells.



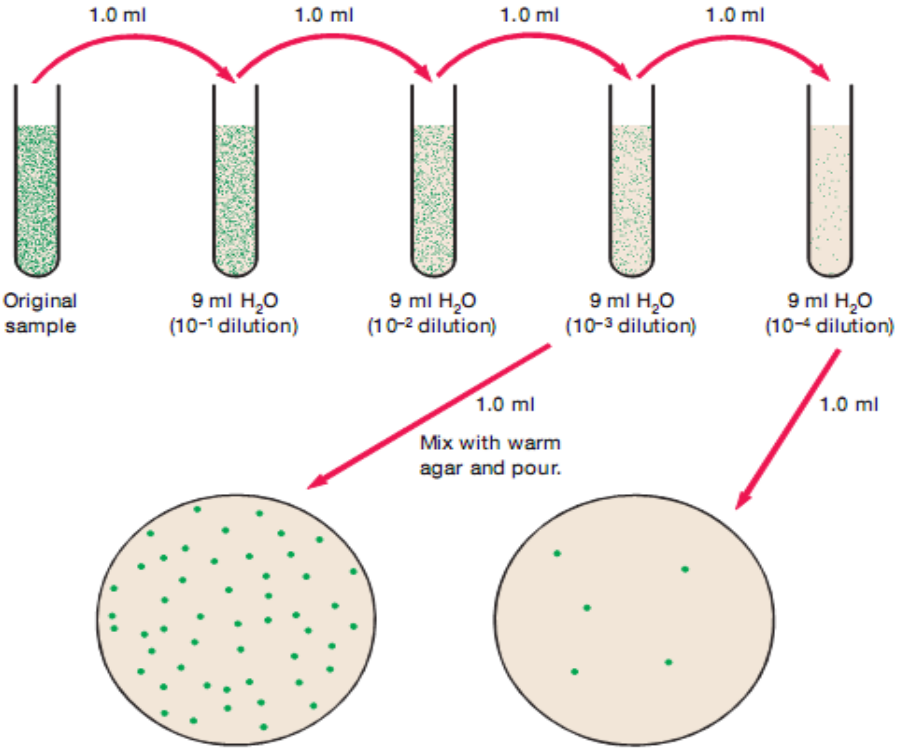
Streak-Plate Technique. Preparation of streak plates. The upper illustration shows a petri dish of agar being streaked with an inoculating loop. A commonly used streaking pattern is pictured at the bottom.



Bacterial Colonies on Agar. Colonies growing on a streak plate. A blood-agar plate has been inoculated with *Staphylococcus* *aureus*. After incubation, large, golden colonies have formed on the agar.

The Pour Plate

Extensively used with bacteria and fungi, a pour plate also can yield isolated colonies. The original sample is diluted several times to reduce the microbial population sufficiently to obtain separate colonies when plating. Then small volumes of several diluted samples are mixed with liquid agar that has been cooled to about 45°C, and the mixtures are poured immediately into sterile culture dishes. Most bacteria and fungi are not killed by a brief exposure to the warm agar. After the agar has hardened, each cell is fixed in place and forms an individual colony. Plates containing between 30 and 300 colonies are counted. The total number of colonies equals the number of viable microorganisms in the diluted sample. Colonies growing on the surface also can be used to inoculate fresh medium and prepare pure cultures.



The Pour-Plate Technique. The original sample is diluted several times to thin out the population sufficiently. The most diluted samples are then mixed with warm agar and poured into petri dishes. Isolated cells grow into colonies and can be used to establish pure cultures. The surface colonies are circular; subsurface colonies would be lenticular or lens shaped.

The preceding techniques require the use of special culture dishes named petri dishes or plates after their inventor Julius Richard Petri, a member of Robert Koch's laboratory; Petri developed these dishes around 1887 and they immediately replaced agar-coated glass plates. They consist of two round halves, the top half overlapping the bottom (figure 5.8). Petri dishes are very easy to use, may be stacked on each other to save space, and are one of the most common items in microbiology laboratories.

Staining

Staining is an auxiliary technique used in microscopy to enhance contrast in the microscopic image. Stains and dyes are frequently used in biology and medicine to highlight structures in biological tissues for viewing, often with the aid of different microscopes. Stains may be used to define and examine bulk tissues (highlighting, for example, muscle fibers or connective tissue), cell populations (classifying different blood cells, for instance), or organelles within individual cells.

Simple stain techniques. Staining can be performed with basic dyes such as crystal violet or methylene blue, positively charged dyes that are attracted to the negatively charged materials of the microbial cytoplasm. Such a procedure is the simple stain procedure.

Gram stain procedure. This differential technique separates bacteria into two groups, Gram-positive bacteria and Gram-negative bacteria. Crystal violet is first applied, followed by the mordant iodine, which fixes the stain.Then the slide is washed with alcohol, and the Gram-positive bacteria retain the crystal-violet iodine stain; however, the Gram-negative bacteria lose the stain. The Gram-negative bacteria subsequently stain with the safranin dye, the counterstain, used next. These bacteria appear red under the oil-immersion lens, while Gram-positive bacteria appear blue or purple, reflecting the crystal violet retained during the washing step.

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

Preparation and Staining of Specimens

Although living microorganisms can be directly examined withthe light microscope, they often must be fixed and stained to increasevisibility, accentuate specific morphological features, and preserve them for future study.

Fixation

The stained cells seen in a microscope should resemble living cells as closely as possible. **Fixation** is the process by which the internal and external structures of cells and microorganisms are preserved and fixed in position. It inactivates enzymes that might disrupt cell morphology and

toughens cell structures so that theydo not change during staining and observation. A microorganism usually is killed and attached firmly to the microscope slide during fixation.

There are two fundamentally different types of fixation.

(1) Bacteriologists heat-fix bacterial smears by gently flame heatingan air-dried film of bacteria. This adequately preserves overallmorphology but not structures within cells. (2) Chemical fixationmust be used to protect fine cellular substructure and the morphologyof larger, more delicate microorganisms. Chemical fixatives penetrate cells and react with cellular components, usually proteinsand lipids, to render them inactive, insoluble, and immobile. Common fixative mixtures contain such components as ethanol, acetic

acid, mercuric chloride, formaldehyde, and glutaraldehyde.

Dyes and Simple Staining

The many types of dyes used to stain microorganisms have twofeatures in common.

(1) They have **chromophore groups,**groups with conjugated double bonds that give the dye its color.

(2) They can bind with cells by ionic, covalent, or hydrophobicbonding. For example, a positively charged dye binds to negatively charged structures on the cell. Ionizable dyes may be divided into two general classes based

on the nature of their charged group.

1. **Basic dyes**—methylene blue, basic fuchsin, crystal violet,safranin, malachite green—have positively charged groups(usually some form of pentavalent nitrogen) and are generally sold as chloride salts. Basic dyes bind tonegatively charged molecules like nucleic acids and manyproteins. Because the surfaces of bacterial cells also are negatively charged, basic dyes are most often used in

bacteriology.

2. **Acid dyes**—eosin, rose bengal, and acid fuchsin—possess negatively charged groups such as carboxyls (—COOH)and phenolic hydroxyls (—OH). Acid dyes, because oftheir negative charge, bind to positively charged cell structures. The pH may alter staining effectiveness since the nature and degree of the charge on cell components change with pH. Thus anionicdyes stain best under acidic conditions when proteins and many other molecules carry a positive charge; basic dyes are most effective at higher pHs. Although ionic interactions are probably the most common means of attachment, dyes also bind through covalent bonds or because of their solubility characteristics. For instance, DNA can be stained by the Feulgen procedure in which Schiff's reagent iscovalently attached to its deoxyribose sugars after hydrochloricacid treatment. Sudan III (Sudan Black) selectively stains lipids because it is lipid soluble but will not dissolve in aqueous portions

of the cell. Microorganisms often can be stained very satisfactorily by

Simple staining, in which a single staining agent is used. Simple staining's value lies in its simplicity and ease of use. One covers the fixed smear with stain for the proper length of time,washes the excess stain off with water, and blots the slide dry.Basic dyes like crystal violet, methylene blue, and carbolfuchsinare frequently used to determine the size, shape, and arrangement of bacteria.

Differential Staining

Differential staining procedures divide bacteria into separategroups based on staining properties. The **Gram stain,** developedin 1884 by the Danish physician Christian Gram, is the mostwidely employed staining method in bacteriology. It is a differentialstaining procedure because it divides bacteria into two

classes—gram negative and gram positive.

Gram-positive and gram negative bacteria

In the first step of the Gram-staining procedure, the smear is stained with the basic dye crystal violet, the primary stain. It is followed by treatment with an iodine solutionfunctioning as a **mordant.** That is, the iodine increases the interactionbetween the cell and the dye so that the cell is stainedmore strongly. The smear is next decolorized by of the Gram stain; gram-positive bacteria retain the crystal violet,whereas gram-negative bacteria lose their crystal violetand become colorless. Finally, the smear is counterstained with a simple, basic dye different in color from crystal violet. Safranin, the most common counterstain, colors gram-negativebacteria pink to red and leaves gram-positive bacteria dark purple

Acid-fast staining is another important differential staining procedure. A few species, particularly those in the genus *Mycobacterium* do not bind simple stains readilyand must be stained by a harsher treatment: heating with a mixtureof basic fuchsin and phenol (the Ziehl-Neelsen method).Once basic fuchsin has penetrated with the aid of heat and phenol,acid-fast cells are not easily decolorized by an acid-alcoholwash and hence remain red. This is due to the quite high lipid contentof acid-fast cell walls; in particular, mycolic acid—a groupof branched chain hydroxy lipids—appears responsible for acidfastness.Non-acid-fast bacteria are decolorized by acid-alcoholand thus are stained blue by methylene blue counterstain. Thismethod is used to identify *Mycobacterium tuberculosis* and *M.leprae* the pathogens responsible for tuberculosisand leprosy, respectively.

Staining Specific Structures

Many special staining procedures have been developed over theyears to study specific bacterial structures with the light microscope.One of the simplest is **negative staining,** a technique thatreveals the presence of the diffuse capsules surrounding manybacteria. Bacteria are mixed with India ink or Nigrosin dye andspread out in a thin film on a slide. After air-drying, bacteria appearas lighter bodies in the midst of a blue-black background becauseink and dye particles cannot penetrate either the bacterialcell or its capsule. The extent of the light region is determined bythe size of the capsule and of the cell itself. There is little distortion of bacterial shape, and the cell can be counterstained for evengreater visibility .

Purification of the isolates

The morphology of colonies should be observed with optical microscope. According to different morphology of the colonies, the single colony of predominant bacteria can be picked and inoculated to the medium slant. Then purified colonies were obtained by repeated streaking of the single colony on fresh agar plates and their morphology was recorded as the basis for classification in detail (Christine, 2002). In the experiment, there were no strict anaerobe bacteria on the vacuum dryer flat.

Preservation and pure culture maintenanace

Once a microorganism has been isolated and grown in pure culture, it becomes necessary to maintain the viability and purity of the microorganism by keeping the pure cultures free from contamination. Normally in laboratories, the pure cultures are transferred periodically onto or into a fresh medium (sub culturing) to allow continuous growth and viability of microorganisms. The transfer is always subject to aseptic conditions to avoid contamination. Since repeated sub culturing is time consuming, it becomes difficult to maintain a large number of pure cultures successfully for a long time. In addition, there is a risk of genetic changes as well as contamination. Therefore, it is now being replaced by some modern methods that do not need frequent sub culturing. These methods include refrigeration, paraffin method, cryopreservation, and lyophilization (freeze drying).

Preservation of microbes

Microorganisms require special preservation methods in order to ensure optimal long-term viability and genetic stability.

The following points highlight the top five methods of preserving microbial culture. The methods are: 1. Agar Slant Cultures 2. Agar Slant Culture Covered with Oil (Parafin Method) 3. Saline Suspension 4. Preservation at Very Low Temperature 5. Preservation by Drying in Vacuum 6. Preservation by Freeze Drying (Byophilization).

Agar Slant Cultures

All microbiology laboratories preserve micro-organisms on agar slant. The slants are incubated for 24hr or more and are then stored in a refrigerator. These cultures are periodically transferred to fresh media. Time intervals at which the transfers are made which varies with the origin and condition of growth.

Agar Slant Culture Covered with Oil (Parafin Method)

The agar slants are inoculated and incubated until good growth appears. They are then covered with sterile mineral oil to a depth of 1 cm above the tip of slant surface. Transfers are made by removing a loop full of the growth, touching the loop to the glass surface to drain off excess oil, inoculating a fresh medium and then preserving the initial stock culture.

This is a simple and most economical method of preserving bacteria and fungi where they remain viable for several years at room temperature. The layer of paraffin prevents dehydration of the medium and by ensuring an aerobic condition, the microorganism remain in dormant state.

Saline Suspension

Sodium chloride in high concentration is frequently an inhibitor of bacterial growth. Bacteria are suspended in 1% salt solution (sublethal concentration in screw cap tubes to prevent evaporation). The tubes are stored at room temperature. Whenever needed the transfer is made on agar slant.

Preservation at Very Low Temperature

The organisms are suspended in nutrient broth containing 15% glycerol. The suspension is frozen and stored at -15°C to -30°C. The availability of liquid nitrogen (temp -196°C) provides another main preserving stock culture. In this procedure culture are frozen with a protective agent (glycerol or dimethane sulphoxide) in sealed ampoules. The frozen cultures are kept in liquid nitrogen refrigerator.

Preservation by Drying in Vacuum:

The organisms are dried over calcium chloride in vacuum and are stored in the refrigerator.

Refrigeration

Pure cultures can be successfully stored at 0-4°C either in refrigerators or in cold-rooms. This method is applied for short duration (2-3 weeks for bacteria and 3-4 months for fungi) because the

metabolic activities of the microorganisms are greatly slowed down but not stopped. Thus their growth continues slowly, nutrients are utilized and waste products released in medium. This results in, finally, the death of the microbes after sometime.

Paraffin Method

Preservation by overlaying cultures with mineral oil .This is a simple and most economical method of maintaining pure cultures of bacteria and fungi. In this method, sterile liquid paraffin in poured over the slant (slope) of culture and stored upright at room temperature. The layer of paraffin ensures anaerobic conditions and prevents dehydration of the medium. This condition helps microorganisms or pure culture to remain in a dormant state and, therefore, the culture can be preserved form months to years (varies with species). The advantage of this method is that we can remove some of the growth under the oil with a transfer needle, inoculate a fresh medium, and still preserve the original culture. The simplicity of the method makes it attractive, but changes in the characteristics of a strain can still occur.

Cryopreservation

Cryopreservation (i.e., freezing in liquid nitrogen at -196°C or in the gas phase above the liquid nitrogen at -150°C) helps survival of pure cultures for long storage times. In this method, the microorganisms of culture are rapidly frozen in liquid nitrogen at -196°C in the presence of stabilizing agents such as glycerol or Dimethyl Sulfoxide (DMSO) that prevent the cell damage due to formation of ice crystals and promote cell survival. This liquid nitrogen method has been successful with many species that cannot be preserved by lyophilization and most species can remain viable under these conditions for 10 to 30 years without undergoing change in their characteristics, however this method is expensive

Lyophilization (Freeze-Drying)

In this method, the culture is rapidly frozen at a very low temperature (-70°C) and then dehydrated by vacuum. Under these conditions, the microbial cells are dehydrated and their metabolic activities are stopped; as a result, the microbes go into dormant state and retain viability for years. Lyophilized or freeze-dried pure cultures and then sealed and stored in the dark at 4°C in refrigerators. Freeze-drying method is the most frequently used technique by culture collection centers. Many species of bacteria preserved by this method have remained viable and unchanged in their characteristics for more than 30 year

Quality control after preservation

After every preservation of a microbial strain controls are necessary. At least viability and purity, and where appropriate, the identity of the preserved culture have to be checked immediately after

preservation. Details of these controls are inserted in the protocols of the preservation. If available a registration form for the freeze-drying process per batch (e.g. vacuum, product temperature, shelf temperature, condenser temperature, time) is filed.

Any remark on the viability or properties of a batch has to be archived and remain available to compare with future controls.

Unit IV

SYLLABUS

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 streile broth medium and incubation of the culture under optimum temperature, PH, and gases conditions. under these conditions , the cells while 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.

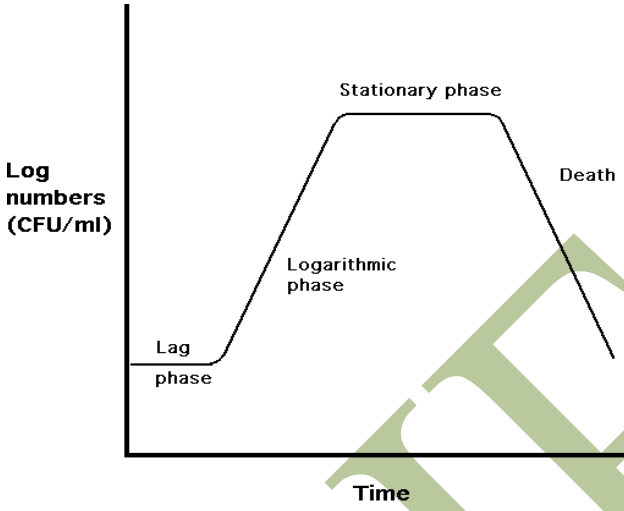
21. **Lag phase :** during this stage the cells are adjusting to their new environment cellular metabolism is accelerated , resulting in raid biosynthesis of cellular macromolecules, primarily enzymes, in preparation for the next phase of the cycle .Although the cells are increasing in size , there is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

22. **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 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 .

23. **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.

Determinination 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.



Hypothetical bacterial growth curve.

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.

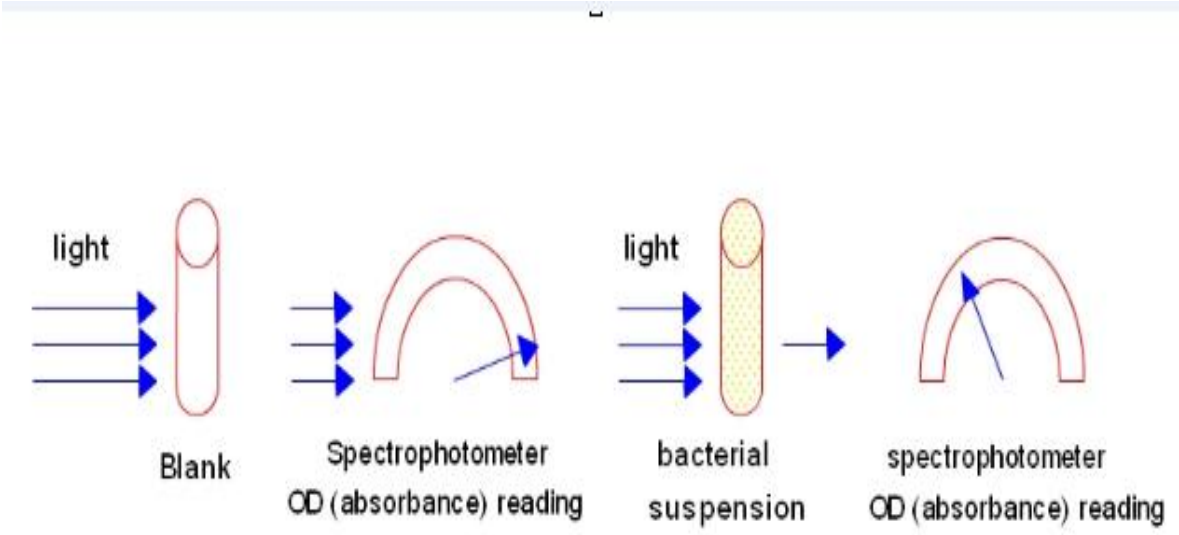
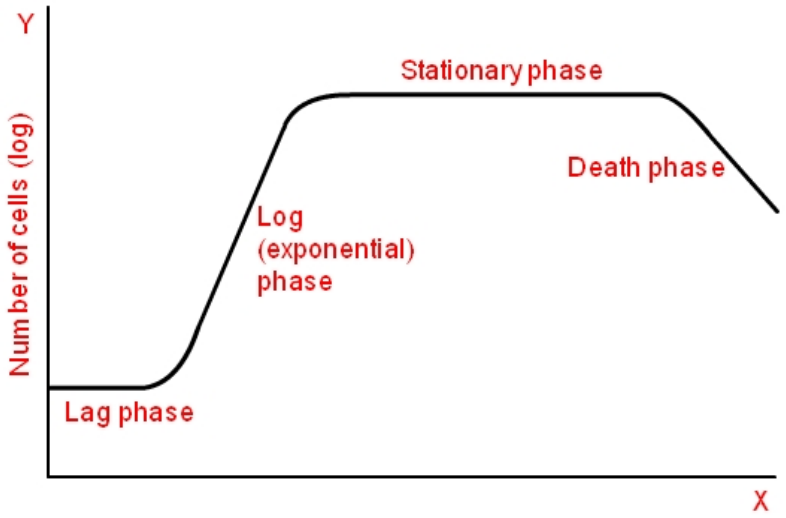


Fig1 . Absorbance Reading of the Bacterial Suspension



The growth curve has four distinct phases (Fig 2)

1. 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.

2. 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.

3. 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.

4. 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 in 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

(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 that 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	No. 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 109 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 = 2n

No = # of cells in population initially Nt = # of cells in population at time t

Nt = No X 2n

logNt = logNo + nlog2

logNt - logNo = n log2

b) For example: What is the generation time if 100 bacterial cells growing logarithmically for 5 hours produced 1.7x106 cells?

n = log(1.7x106) – log 100 = 14 generations in 5 hours log2

g = 5 hours/14 generations = 0.357 generations/hour

3. Determination of g using growth curve data

a) Plot time on X axis and CFU/ml on Y axis (log scale)

b) Pick a point on the Y axis in log growth

c) 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)

d) Multiple the point that you picked in (b) by 2 (because we want to know when the population doubles)

e) 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)

f) The generation time is the difference between the X values from (e) and (c)

1.E+08

2.E+08

CFU/ml 1.E+07

1.E+06

0 1 2 3 4 5 6 7

hours

generation time

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 >106/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)

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:

colony forming units

= total CFU/ml ~ total bacteria /ml

(ml plated) (dilution plated)

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)

C. 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 107 – 109 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 waterexits 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 = aw

(1) aw = vapor pressure of solution

vapor pressure of water

(2) aw = (% relative humidity of solution) / 100 where relative means relative to water

2. environment with low aw = 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 [] inside

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

(2) algae/fungi: sucrose and polyols

(3) Halobaterium: 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 aw /high osmotic pressure: dried foods, salt

lakes, salted foods, preserves

3. high aw = 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 aw /low osmotic pressure: water, blood

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

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

. Temperature

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

2. Why does temperature matter?

a) Enzyme activity

(1) low temp = slow rate of enzyme reactions

(2) high temp = denaturation of enzymes

b) Membrane structure

(1) low temp = not fluid enough

(2) high temp = disrupted

3. Cardinal temperatures (Fig. 6-14)

a) minimal

b) optimal

c) maximal

4. Range

a) stenothermal = small temp range

b) euthermal = large temp range

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

a) Psychrophiles

(1) min = <0□ C / opt = < 15□ 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

(2) location – hot springs, hot water lines, compost bins, soils exposed to direct sunlight

(3) adaptations –

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

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

(ii) 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

(2) Usually detoxified by superoxide dismutase (SOD) then catalase

3. Cultivation of anaerobes

a) "Anaerobic media" which contains reducing agents such as thioglycollate or cysteine to eliminate any O_2 by conversion to H_2O

b) Elimination of O_2 in container or work area (glove box)

(1) by flushing with nitrogen

(2) applying vacuum

(3) GasPak jar (Fig. 6-18): O_2 consuming gas removes O_2

4. Cultivation of aerobes

a) Vigorous shaking

b) Bubbling in sterile air

5. Location of organisms depends on the O_2 available and the presence of other organisms that consume or generate O_2

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 O_2 to generate singlet O_2

□ □ singlet O_2 □ is a higher energy form of O_2 □ 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 I n 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, 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 mycoplasms 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 aw or water activity. The aw of pure

water is 1.0 while that of a saturated salt solution is 0.75. Most spoilage bacteria require a minimum aw of 0.90. Some bacteria can tolerate an aw above 0.75 as can some yeasts and most molds. Most yeasts require 0.87 water activity. An aw 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 400C (1050F) are called thermophiles while those growing below 250 C (750F) 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.

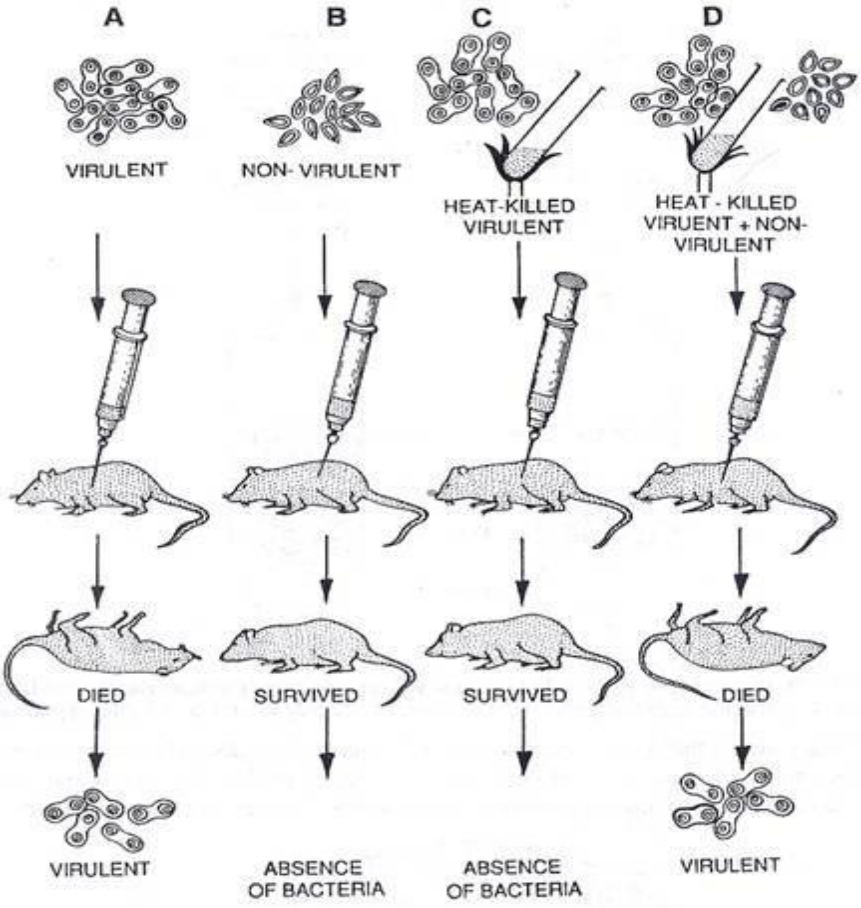
The phenomenon of transformation was first recorded by Griffith (1928). Avery, Macleod and McCarty (1944) demonstrated that the transforming principle being DNA in the sequence of events in bacterial transformation.

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.

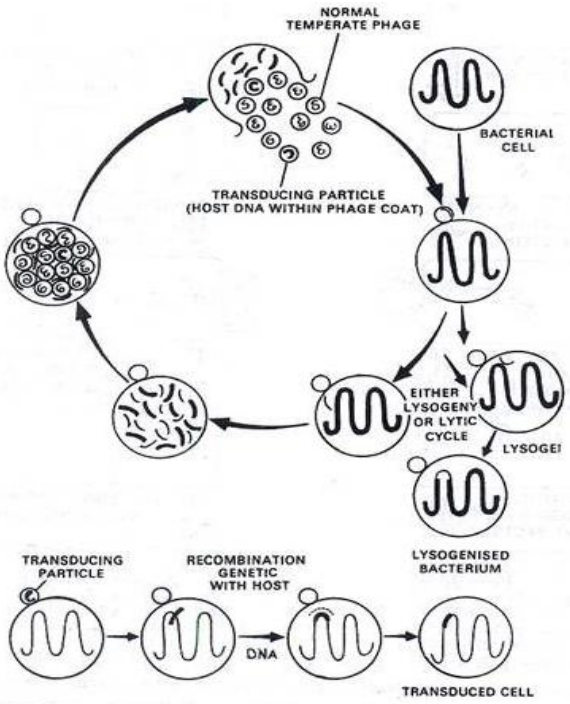
When injected into mice (A) only capsulated smooth cells (virulent) produced the disease, but not the non-virulent rough cells (B). On the other hand when the heat killed capsulated (virulent) smooth cells were mixed with non-virulent rough cells (D) and then were injected in the mice the disease was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



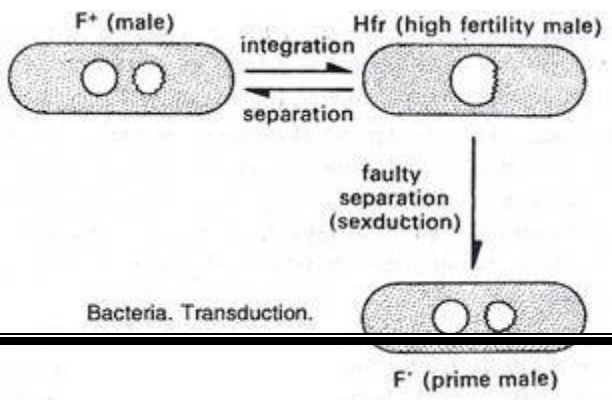
2. Bacterial transduction:



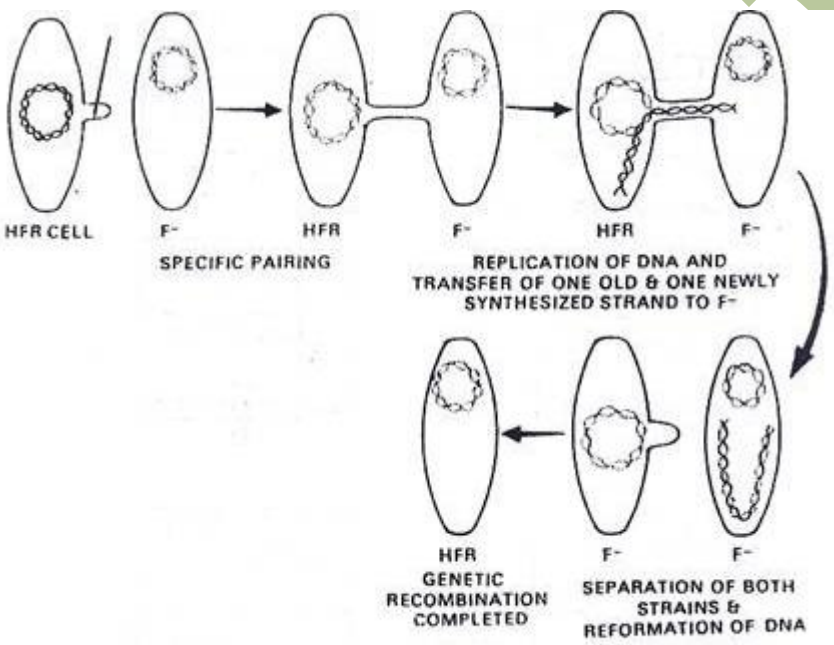
Bacteria. Upper diagram: transduction mechanism where phage particles containing host can be formed. Lower diagram: genetic recombination with transducing particles.

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.



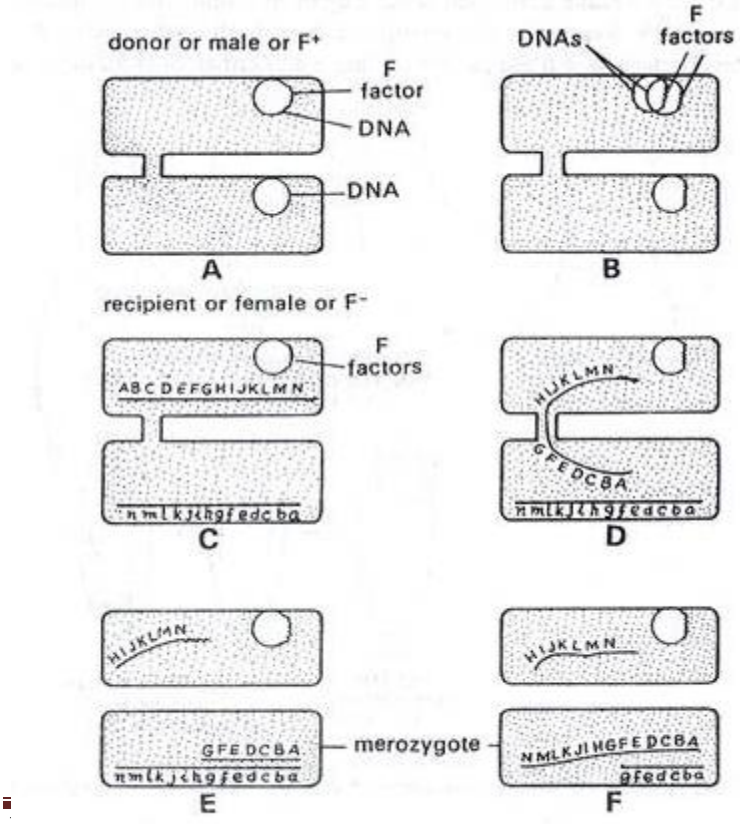
Bacteria, Diagrammatic representation of possible mechanism of conjugation.

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.



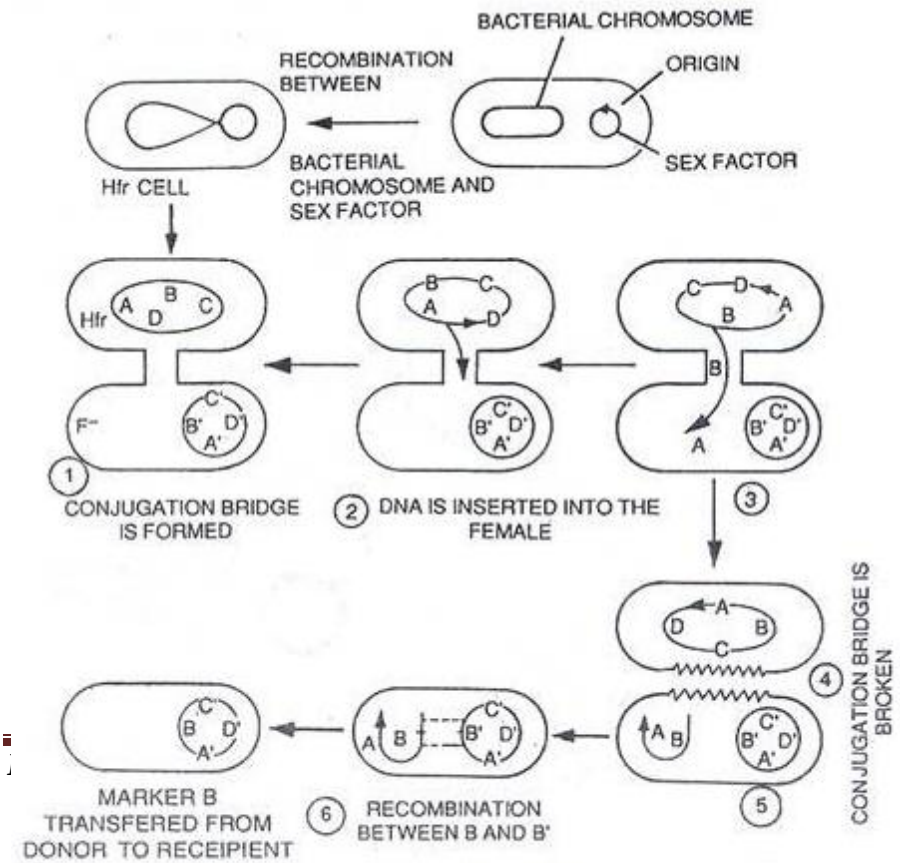
Bacteria. Conjugation between sterile male and female of *Escherichia coli*.

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 2.5µm 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).



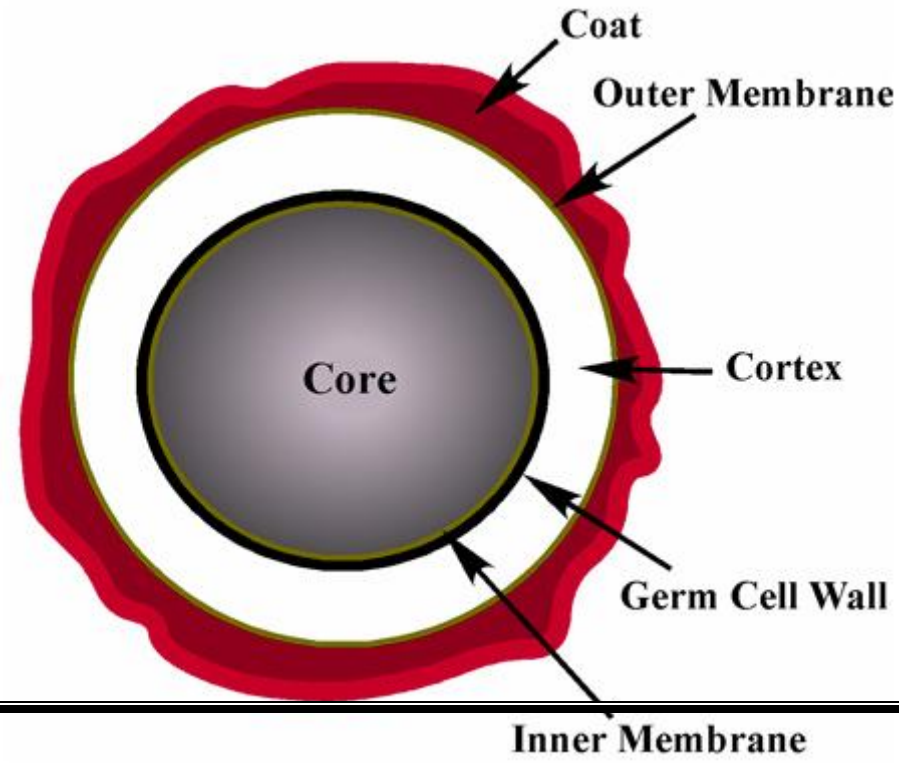
Bacterial conjugation

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.

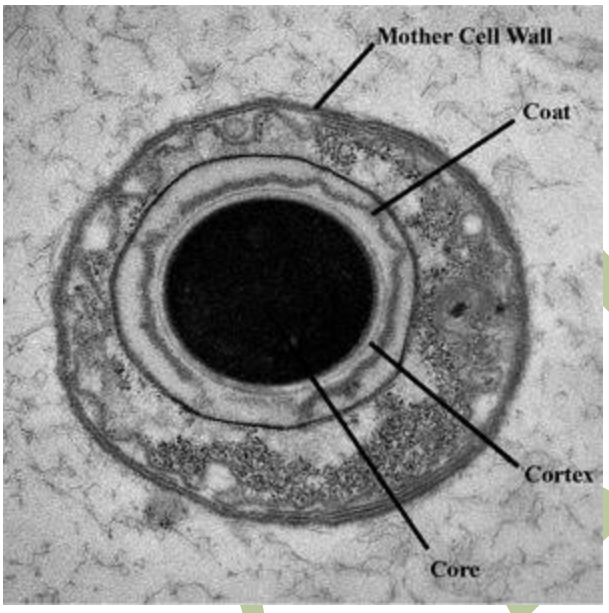
Sporulation in bacteria

Endospore Development

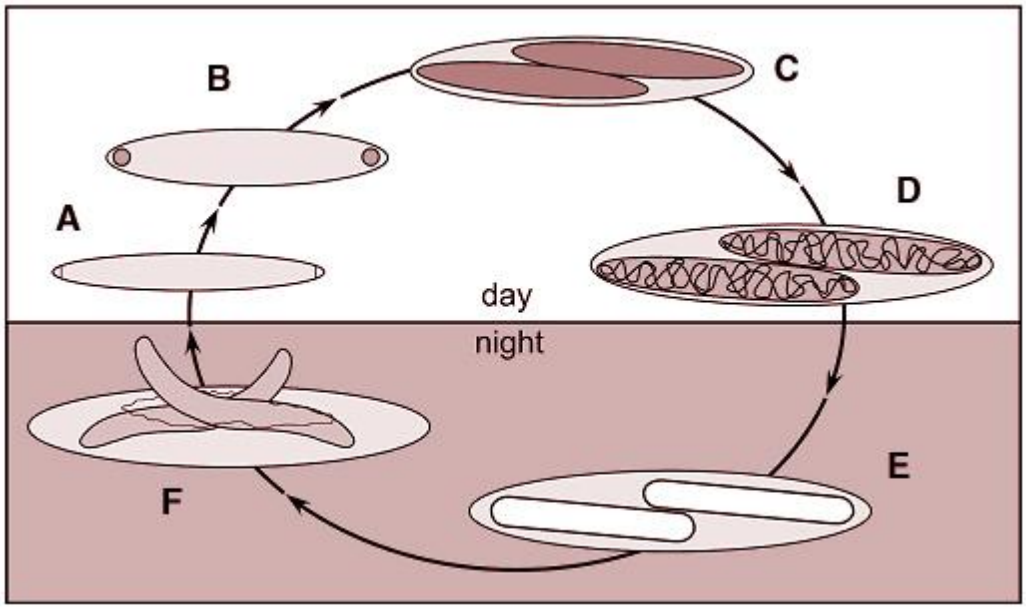
The process of forming an endospore is complex. The model organism used to study endospore formation is *Bacillus subtilis*. Endospore development requires several hours to complete. Key morphological changes in the process have been used as markers to define stages of development. As a cell begins the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

Endospore formation in some *Epulopiscium*-like symbionts follows a daily cycle:

A) Polar septa are formed at the poles of the cell.

B) Forespores become engulfed.

C) Forespores gradually increase in size within the mother cell through the day.

D) In late afternoon, final preparations for endospore dormancy.

E) Endospores mature and remain dormant throughout most of the night.

F) Just before sunrise, the endospores germinate and are released from mother cell to repeat the cycle.

Possible Questions

2 marks

1. Give examples for microbial secondary metabolites.
2. Define conjugation.
3. Define transduction.
4. Define transformation.
5. Define endospores.
6. Define chemostat and turbidostat.
7. List out the factors affecting microbial growth.

8 marks

1. Give a detailed note on growth factors of bacteria.
2. Describe in detailed about Bacterial Reproduction.
3. Describe in detail: i) Chemostat ii) Turbidostat
4. Write in detail about bacterial transduction.
5. Describe in detail about microbial growth curve.
6. Elaborate about bacterial transformation.
7. Give a detailed account on batch and continuous culture.
8. Describe about endospore formation in bacteria.
9. Give a detailed account on measurement of microbial growth.
10. Give a detailed account on factors affecting growth of bacteria.

Unit V

SYLLABUS

Water microbiology: bacterial pollutants of water, colliforms and non coliforms, sewage composition and its disposal.

Food microbiology: Important microorganisms in food Microbiology: moulds, yeasts, bacteria, major food borne infections and intoxications, preservation of various types of foods, fermented foods.

Water microbiology

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.

However, the presence of other disease causing microbes in water is unhealthy and even life threatening. 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* known as O157:H7 can be fatal. The contamination of the municipal water supply of Walkerton, Ontario, Canada in the summer of 2000 by strain O157:H7 sickened 2,000 people and killed seven people.

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. *Cryptosporidium* contamination of

the drinking water of Milwaukee, Wisconsin with in 1993 sickened more than 400,000 people and killed 47 people.

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 limnitic 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*). Also, in 2001, researchers demonstrated that the ancient form of microbial life known as archaebacteria is one of the dominant forms of life in the ocean. The role of archaebacteria in the ocean food chain is not yet known, but must be of vital importance.

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).

	Microorganisms	Disease
Bacteria	*Salmonella typhi*	typhoid
	Salmonella choleraesuis	typhoid, gastroenteritis
	Salmonella enteritidis	typhoid, gastroenteritis
	Shigella sp.	dysentery
	Vibrio cholerae	cholera
	Camplobacter jejuni	enteritis
	intestinal pathogenic coliform	gastroenteritis
	Mycobacterium tuberculosis	tuberculosis
Virus	*rotavirus*	gastroenteritis
	poliovirus	infantile paralysis
Protozoa	*Cryptosporidium*	typhoid
	Giardia	typhoid
	Entamoeba	dysentery
Algae	*Microcystis*	liver disorder
	Aphanizomenon	nervous disorder
	Anabaena	nervous disorder
	Cylindrospermopsis	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,it's presence or absence classifies drinking water as unfit or fit for human consumption respectively.*E coli* is one such indicator bacteria. It's 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	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

Standard for bacteria in drinking water, water environment and effluent

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 utilized 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) Depiction of dissolved oxygen in water leading to anoxic (oxygen-less) condition which may ultimately kill O_2 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.

Treatment of Sewage:

Our objectives behind the sewage treatment would be to kill pathogenic microorganisms, prevent anoxia, raise the pH to alkaline side, increase photosynthetic rate, reduce organic content, etc. When these objectives are achieved by the way of treating the sewage, the conditions prevailing in a natural water reservoir are induced in sewage water and the latter can be reused.

Sewage treatment processes are many and varied. We will discuss only those sewage treatment processes which are generally applied in single dwelling unit situations and municipal situations.

Food microbiology – important microorganisms

Moulds

Microorganisms importance in Food Microbiology. Moulds – General characteristic of moulds, classification and identification of moulds Microorganisms important 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, sonti, 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 chinks. 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 Characterstics: 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. 23 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) sporan- giospores. 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. 24 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 zygospores 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 zygospores are covered by a tough wall and can survive drying for long periods. 3. Ascospores: The Ascomycetes (septate) form sexual spores known as ascopores, 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 basidiospore. 25 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 370 C 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 temoeratures below freezing. Growth has been reported at as low as -5 to - 100 C. 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 of 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, apophysis, 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. 27 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. Geotrichum (Oospora or Oidium) This genus is included with the yeast like fungi by some writers and with the molds by others. Species may be white, yellowish, orange, or red, with the growth appearing first as a firm, felt like mass that later becomes soft and creamy. Geotrichum candidum (Oospora lactis), often called the "dairy mold,” gives white to cream-colored growth. Neurospora (Monilia): This genus has been described under various names

because of the confusion concerning its classification. It is classed among the perfect molds (producing sexual spores) and call the genus Neurospora. Neurospora (Monilia) sitophila, the most important species in foods, sometimes is termed the "red bread mold" because its pink, loose-textured growth often occurs on bread. 29 30 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. 31 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 ma 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

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, pearshaped, 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. Sexual reproduction of "true" yeasts (Ascomycotina) results in the production of ascospores, the yeast cell serving as the ascus. The formation of ascospores follows conjugation of two cells in most species of true yeasts, but some may produce ascospores without conjugation, followed by conjugation of ascospores or small daughter cells. The usual number of spores per ascus and the appearance of the ascospores are characteristic of the kind of yeast. The 33 ascospores may differ in color, in smoothness or roughness of their walls, and in their shape (round, oval, reniform, bean- or sickle-shaped, Saturn- or hat~ shaped, hemispherical, angular, fusiform, or needle-shaped). "False" yeasts, which produce no ascospores or other sexual spores, belong to the Fungi Imperfecti. Cells of some yeasts become chlamydospores by formation of a thick wall about the cell, for example, Candida, Rhodotorula, and Cryptococcus. 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. 34 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 aw, yeasts may be classified as ordinary if they do not grow in high

concentrations of solutes, i.e., in a low aw, and as osmophilic if they do. Lower limits of aw for ordinary yeasts range from 0.88 to 0.94. Osmophilic yeasts have been found growing slowly in media with an aw 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 aw 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 300 C 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. 35 Classification and identification of yeasts The true yeasts are in the subdivision Ascomycotina, and the false, or asporogennous, 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. 36 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 kloeckeri is now D. hansenii. Genus Kluyveromyces. These yeasts reproduce by multilateral budding, and ascopores 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. 37 Genus Hansenula These yeasts resemble Pichia in appearance but are usuua1ly 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. Groups of Yeasts Film yeasts, in the genera Pichia, Hansenula, Debaryomyces, Candida, and Trichosporon, grow on the surface of acid products such as sauerkraut and pickles, oxidize the organic acids, and enable less acid-tolerant organisms to continue the spoilage. Hansenula and Pichia tolerate high levels of alcohol and may oxidize it in alcoholic beverages. Pichia species are encouraged to grow on Jerez and Arbois wine, to which they are supposed to impart distinctive flavors and esters. Debaryomyces is very salt tolerant and can grow on cheese brines with as much as 24 percent salt. The film yeasts produce little or no alcohol from sugars. 38 Apiculate or lemon-shaped yeasts, in Saccharomycodes, 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

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. Cultural characteristics important in food bacteriology: 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 40 (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. 41 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 250 C, but growth can occur over a temperature range of 0 to 450 C 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 thermosaccharoolyticum 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. 42 carotovora causes rotting in a large number of plants. E. carotovora subsp. atroseptica produces a black rot in potatoes. E. carotovora subsp. betavasculorum 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 43 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 thermoduric 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., 44 (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, grampositive, 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. lute us 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. 45 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 homofermentative. 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. Pediococci 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 pyoverdin of Pseudomonas fluorescens and white, cream-colored, reddish, brown, or even black (P. nigrifaciens*) colors of other species. 46 (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 aw (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 font endospores. It resemble 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, coagulasepositive 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 is homofermentative. The streptococci important in foods are divided in food 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 450 C. The viridans groups includes S. thermophilus, a coccus important in cheeses making at 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 450

C but not at 100 C. The lactic group contains the important dairy bacteria, S. lactis and S. cremoris*, grow at 100 C but not at 450 C. 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. 47 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 450 C. 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 80 C and most of them at as high as 48 to 500 C. 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 48 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 sliminese 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. Clostridum 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. 49 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, Clostridum, 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. Thermoduric Bacteria: Thermoduric 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 Byssochlamys fulva, Aspergillus and Penicillium are thermoduric. Some thermoduric 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 sps 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 sps., Sarcina, pediococcus, Alcaligenes. 50 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.

Campylobacter jejuni – Foodborne Infection

Found

Intestinal tracts of animals, birds, raw milk, untreated water, and sewage sludge.

Transmission

Contaminated water, raw milk, and raw or undercooked meat, poultry, or shellfish.

Symptoms

Fever, headache, and muscle pain followed by diarrhea (sometimes bloody), abdominal pain, and nausea that appear two to five days after eating; may last seven to 10 days.

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. For more information, read: How to get started with the canning preservation method.

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: 10 refreshing, easy pickling recipes.

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. For more information, read: Winter food storage guide in a root cellar or other cellaring methods.

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 Rumptof

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.

Nutritive value of fermented foods

Fermented foods can be more nutritious than their unfermented counterparts. This can come about in at least three different ways.

1. Microorganisms not only are catabolic, breaking down more complex compounds, but they also are anabolic and synthesize several complex vitamins and other growth factors.

2. The fermented foods can be improved nutritionally has to do with the liberation of nutrients locked into plant structures and cells by indigestible materials. This is especially true in the case of

certain grains and seeds. Milling process do much to release nutrients from such items by physically rupturing cellulosic and hemicellulosic structures surrounded the endosperm, which is rich in digestible carbohydrates and proteins. Fermentation, especially by certain bacteria, yeast and molds, breaks down indigestible coatings and cell walls both chemically and physically.

3. The fermentation can enhance nutritional value, especially of plant materials, involves enzymatic splitting of cellulose, hemicellulose, and related polymers that are not digestible by humans into simpler sugars and sugar derivatives. Cellulosic materials in fermented foods can be nutritionally improved for humans by the action of microbial enzymes.

Possible Questions

2 marks

1. Give examples for fermented food.
2. List out the important microorganisms in food industry.
3. What are coliforms?
4. Name any two food borne diseases.
5. List out various techniques to preserve foods.
6. List out the diseases caused by polluted water.

6 marks

1. What is sewage waste? Explain about disposal of sewage.
2. Give a detailed account on major food borne infections.
3. Elaborate about important microorganisms of food industry.
4. What are fermented foods? Explain its types.
5. Write in detail about the role of moulds and yeast in food Microbiology.
6. Explain in detail about the Bacterial pollutants of water.
7. Discuss in detail about fermented milk products.
8. Explain in detail about the Bacterial pollutants of water.
9. Discuss in detail about fermented milk products.
10. Write in detail about the food preservation techniques.
11. Give a detailed note on Sewage composition.

Reg. No. : ----------

[18BTU203]

KARPAGAM ACADEMY OF HIGHER EDUCATION, COIMBATORE
FIRST INTERNAL ASSESMENT, DECEMBER 2018
(For the Candidates admitted from 2018 and onwards)
Second Semester
DEPARTMENT OF BIOTECHNOLOGY

GENERAL MICROBIOLOGY

Date: **I B.Sc., Biotechnology**
Time: 2 hours **Maximum: 50 marks**

PART A – (20 x 1 = 20 marks)
Choose the correct answer

1. Microbiology is a branch of science that deals with _____?
   a. Virus b. Plants c. Microbes d. Bacteria

2. Who is the father of microbiology?
   a. Muller b. Antony Van Leeuwenhoek c. Louis Pasteur d. Robert Koch

3. Microbes are_____?
   a. Prokaryotes b. Eukaryotes c. Both a and b d. Unicellular

4. Microbes are_____?
   a. Prokaryotes b. Multicellular c. Plants d. Unicelluar

5. Germ plasm theory states that infectious disease are caused by _____?
   a. Bacteria b. Microbes c. Fungus d. Virus

6. The formation of life from non-living substances is called _____?
   a. Mith b. Genetics c. spontaneous generation theory d. Symbiosis

7. The technique used to avoid all microorganisms is accomplished by_____.
   a. Sterilization b. Disinfection c. Incineration d. Boiling

8. Who discovered the concept of pasteurization?
   a. Muller b. Leeuvanhoek c. Robert Koch d. Louis Pasteur

9. Who discovered bacilli?
   a. Robert Koch b. Muller c. Pasteur d. Weigert

10. Extra chromosomal DNA of bacteria are known as _____?
    a. Capsule b. Plasmid c. Cell wall d. Nucleus

11. Fungi are a group of _____ organisms?
    a. Eukaryotic ,heterotropic b. autotropic c. aerobic d. Anarobic bacteria

12. Protozoa also known as _____
    a. Bacteria b. Protoctistia c. Fungus d. Virus

13. Ribosome is the site of
    a. Transcription b. Translation c. Degradation d. energy generation

14. Gram staining was developed by
    a. Muller b. Christian Gram c. Louis Pasteur d. Robert Koch

15. Viruses can be cultivated on
    a. lab media b. living cells c. broth d. agar

16. Type of bacteria are capable of synthesizing their own food from inorganic substances?
    a. Autotrophic b. Heterotrophic c. Sporophytes d. Parasites

17. Which type of bactreria are capable of synthesizing their own food from hosts?
    a. Aerobic b. Anaerobic c. Parasites d. Autotropic bacteria

18. Autoclaving is an example of _____ sterilization
    a. moist heat b. dry heat c. red heat d. all of these

19. Sterilization of heat sensitive material is achieved by_____
    a. dry heat b. moist heat c. red heat d. radiation

20. Locomotion organ present in bacteria is____
    a. flagella b. cilia c. ribosome d. mitochondria

Part –B **3 X 2 = 6 marks**
Answer All the Questions

21. Define Microbiology.
22. What is pasteurization?
23. Draw the structure of bacteria.

Part –C **3 X 10 = 30 marks**
Answer All the Questions. Choosing either a or b.

24. a. Extend notes on the classification of microorganisms.
    (Or)
    b. Explain in detail about history and scope of Microbiology.

25. a. Differentiate prokaryotes from eukaryotes.
    (Or)
    b. Elaborate note on Current classification of bacteria.

26. a. Give a detailed note on bacterial morphology.
    (Or)
    b. Diagrammatically explain the structure of fungi.
