1 Introduction

Microbiology is a *branch of science that deals with* ***microbes,*** *the microscopic for of life.*

Microbiology gives its focus on the occurrence and distribution of microorganisms in natu their form, structure, physiology, reproduction, metabolism and classification; their helpful harmful relationships with other living things; their significance and usefulness in science, medici and industry; their beneficial and harmful effects on human being and domestic animals an cultivated plants.

Microbes

Microbes are invisible creatures, too small to be seen with the naked eye. They can b seen only by magnifying their image with a microscope. They are the ***micro-organisms.*** The include ***viruses, bacteria, algae, fungi*** and ***protozoa.***

The science of viruses is called ***Virology;*** that of bacteria is called ***Bacteriology***; that o algae is called ***Phycology***; that of fungi is called ***Mycology*** and that of protozoans is calle ***Protozoology.***

Distribution

Microbes are widely distributed in the world. They are ***omnipresent***. They are prese everywhere in air, water, soil, in living plants and animals, in dead matter, etc. They are al distributed in Arctic regions, in hot tropical areas, forest, frosty atmosphere or even in whol arid region. They are present on our body, in our body, on our clothings, in the air we breathe, the food we eat, in the water we drink, in our mouth and in our intestine. Actually one third, the dry weight of human faeces is bacteria.

Levels of Organization

The microbes are either ***unicellular*** or ***multicellular*** or ***non-cellular*** forms. Protoz and bacteria are unicellular forms and are made up of single cells. The algae and fungi multicellular forms where the organism is assembled out of many cells. But viruses lack a cell

2 History of Microbiology

Anton Van Leeuwenhoek is the ***father of Microbiology***. He was an amateur lens grinder in Holland. He assembled simple microscopes and in 1674, through his own microscopes, he brought to light for the first time, the existence of a new world of micro-organisms in his mouth, food and stagnant water. He observed bacteria and named them as ***animalcules***.

	Wirsbery described the micro-organisms as ***infusoria.***
1767	***Linnaeus*** classified the micro-organisms as ***chaos infusoria.***
1773 and ***1788***	***Muller*** classified the bacteria and coined the terms ***vibrio*** and ***monas***.
1798	***Edward Jenner*** introduced ***vaccination*** for the prevention of small pox through inoculation of cowpox virus.
1829	***Ehrenberg*** established the genus ***Bacterium***, meaning ***'staff'*** in Greek. He also discovered two more genera, namely *Spirillum* and *Spirochete*.
1835	***Agostino Bassi*** showed that the silkworm disease, ***muscardine*** was caused by a fungus, *Botrytis*.
1836	***Theodor Schwann*** demonstrated that yeast causes ***fermentation.***
1857	***Nageli*** coined the term *Schizomycetes* and he correlated bacteria to fungi.
1822 -1895	***Louis Pasteur*** proposed the theory of ***spontaneous generation of life.***
1843 -1910	***Robert Koch*** discovered *Bacilli*.
1827 -1912	***Lord Lister*** employed practical applications of bacteriology in the field of surgery. For the first time, he used surgical appliances and antiseptic dressings to save the surgical wounds from the entrance of bacteria.

1866	***Louis Pasteur*** introduced the concept of ***pasteurization.***
1885	***Louis Pasteur*** discovered vaccine against rabies.
1875	***Weigert*** stained bacteria with ***methyl violet dye.***
1879	***Albert Neisser*** discovered the *Gonococcus*, the causative agent of ***gonorrhoea.***
1884	***Gram*** introduced the ***Gram staining technique*** for bacteria
1880	***Karl Joseph Eberth*** isolated the *Typhoid bacillus.*
1880	***Charles Louis Alphonse Laveran*** discovered the parasite of malaria.
1882	***Robert Koch*** isolated the *Tubercle bacillus* and proved that it was the causative agent of ***tuberculosis.***
1882	***Carl Friedlander*** discovered the bacillus of *Klebsiella pneumoniae*
1888	***Pasteur*** laid the foundation for Pasteur Institute in Paris, where microbiology was extensively studied.
	Elie Metchnikoff discovered that certain leucocytes eat bacteria and he established the theory of ***phagocytosis.***
1888	The science of ***Soil Microbiology*** was introduced.
1888	***Beijernick*** isolated and studied the root-nodule bacteria.
1893, 1994	***Winogradsky*** demonstrated that the aerobic bacterium *Clostridium* fixes atmospheric N_2 in the soil.
1897	***Edward Buchner*** showed that extracts of yeast cells could produce alcoholic fermentation.
1901	***Beijernick*** isolated three aerobic nitrogen fixing bacteria, namely *Azotobacter chroococcum* and *A. agile.*
1892	***Ivanowski*** discovered ***tobacco mosaic virus.***
	Emil Hansen began ***industrial fermentation*** with the help of pure culture of bacteria and yeasts as ***starters.***
1894	***Shibasaburo Kitasato*** and ***Alexandre Yersin*** discovered the *Plague bacillus.*
1898	***Kiyoshi Shiga*** discovered the *Dysentery bacillus.*
1898	***Ronald Ross***, discovered malarial parasite in the salivary glands of the *Anopheles* mosquito.
1929	***Sir Alexander Fleming*** discovered ***penicillin.*** He was called the ***father of antibiotics.***
1949	***Enders, Robbins*** and ***Weller*** cultured ***Poliomyelitis***, the polio causing virus.
1950	***Salk*** introduced ***polio vaccine.***
1973	***Waksman*** discovered ***streptomycin.***

1. Robert Koch (1843-1910)

• ***Koch***, a ***German physician*** and ***pathologist*** was awarded the Nobel prize in 1905 for his research in ***tuberculosis.***

• He identified the causative agent of ***tuberculosis***, namely the tubercle bacilli, *Mycobacterium tuberculosis* in 1882. He also identified the protein derived from these bacteria namely ***tuberculin***.

• He also identified the causative agent of ***cholera***, the cholera bacillus and named *Cholera vibrio* in 1883.

• He studied the life cycle of *Anthrax bacillus* and its causative role in the disease ***anthrax***.

• He introduced ***staining techniques*** and demonstrated the methods of obtaining bacteria in pure culture using solid media.

2. Edward Jenner (1749 - 1823)

• ***Edward Jenner,*** an English physician who had suffered the pain of variolation at younger age, developed a scientific and far safer method to smallpox immunisation. His work was based on his ***observations on milk maids.*** He found that those who often contracted ***cowpox**** were remarkably resistant to ***smallpox.***

• In 1786, ***Jenner*** inoculated a boy with material got from the lesions of a milk maid, who was attacked by cowpox. After some weeks, the boy was again inoculated with infectious pus taken from a patient suffering from small pox, but the boy did not get the disease. This made him to conclude that this process of inoculation leads to immunity against small pox.

Later, it was ***Louis Pasteur*** who coined the term ***vaccine*** for such process of ***prophylaxis*** in honour of ***Edward Jenner*** who prepared the first vaccine from ***cowpox*** (Lt. Vacca-cow).

• Thus the ***process of vaccination*** was introduced by ***Jenner.*** According to the ***World Health Organisation (WHO), Jennerian vaccination*** has eliminated small pox totally from the human population.

3. Louis Pasteur (1822- 1895)

- ***Louis Pasteur*** is a ***French Chemist.***
- He is the ***Father of Immunology.***
- He worked on the common diseases such as ***pebrine*** (disease of the silk worm) ***anthrax*** (disease of the cattle), ***chicken cholera*** (disease of the fowls) and ***rabies.***
- While inoculating chickens with an old culture of *Bacillus,* namely *Pasteurella aviseptica,* he happened to observe that the inoculated chickens failed to develop illness. This ***Pasteur*** had a chance observation which made him to demonstrate that aged cultures lose virulence but retain the capacity to induce immunity against cholera. Thus started the process ***attenuation**** and the aged cultures of chicken cholera became the first attenuated vaccine.
- Working on sheep, ***Pasteur*** observed that when sheep were inoculated with culture of ***anthrax*** bacteria, namely *Bacillus anthracis,* cultivated at 42°C the sheep did not develop

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Cowpox* *It is a mild viral pox of cattle. It is easily transmitted to dairy workers. The hands of the milk maids are frequently infected by this pox in the process of milking the cattle.*

Prophylaxis* *- Prevention of a disease.*

Attenuation* *- The process of weakening or reducing the virulence of pathogenic organisms without losing the capacity to induce immunity.*

the disease. But at their usual growth temperature of 37°C, these bacteria were highly pathogenic. From this, he inferred that the pathogenic bacteria lost their virulence on cultivation at high temperature.

❖ Following the work of ***Pasteur*** many methods have been now used to attenuate the virulence of pathogenic organisms so that they could be used for the purpose of vaccination. One such method is passing the micro-organisms through ***'unnatural hosts'***. For example, the virus causing ***rabies*** is passed through rabbit and the smallpox virus of human being is passed through ***cow*** many times. Thus the viruses lose their capacity to produce serious diseases but retained the capacity to induce immunity.

❖ The most interesting of ***Pasteur's*** work was the production of a ***vaccine*** for ***rabies***. He injected the spinal cord extracts from rabid dogs (dogs attacked by rabies) into normal healthy rabbits. From rabbits these extracts were prepared from the infected spinal cords and dried for several days at room temperature. From these extracts which contained the attenuated viruses, the rabies vaccine was prepared.

4. Alexandre Fleming (1881-1955)

Fleming a ***British bacteriologist*** received Nobel prize in 1945 for his discovery of ***penicillin***.

He discovered the ***antibiotic lysozyme.***

He discovered an ***antibacterial*** substance, namely ***penicillin*** from a fungus (mold).

3 Concepts of Microbiology

1. Theory of Spontaneous Generation

The formation of life from non-living substances is called *spontaneous generation* or *abiogenesis*. *Aristotle* (384–322 BC) suggested that maggots and grubs develop spontaneously in decaying matter. *Epicurus* (342–271 BC) wrote that worms and many other animals originated from soil and manure by the action of sun and rain.

The theory of spontaneous generation was disproved by *Redi* (1650), *Spallanzani* (1765) and *Louis Pasteur*. They experimentally proved that micro-organisms cannot arise spontaneously from decaying meat and flesh. Worms, maggots and micro-organisms develop from decaying flesh only from the eggs and spores already deposited by the insects, air, etc. When the decaying flesh is sealed from external agents, life cannot appear spontaneously from the flesh.

Thus, *Louis Pasteur* and others disproved the theory of spontaneous generation and they argued that life originated from pre-existing life only. This concept is called *biogenesis*.

2. Germ Theory of Fermentation

The anaerobic enzymatic conversion of organic compounds, especially carbohydrate, to simpler compounds, especially to ethyl alcohol, producing energy in the form of ATP is called *fermentation*.

Louis Pasteur defined fermentation as *'life without air'*. He proved that micro-organisms are involved in fermentation.

Theodor Schwann in 1836 demonstrated that yeast causes fermentation and produces alcohol.

Edward Buchner in 1897 showed that extracts of yeast cells could produce alcoholic fermentation.

Pasteur observed the formation of lactic acid from sugar by several different kinds of bacteria. He demonstrated that specific microorganisms were required for alcoholic, lactic, butyric and other fermentations. This is called *germ theory of fermentation*.

4 Scope of Microbiology

Microorganisms have gained tremendous significance. This has been partly due to increasing recognition of the economic importance of microorganisms and partly to development of new techniques of investigation. Today, microorganisms are the basic tool genetic engineering and biotechnology.

1. Biogeochemical Cycles

The flow of chemicals between living and non-living things is called ***biogeochemical cycles***. Microbes decompose dead plants and animals into simple chemical nutrients that can used by plants and photosynthetic organisms.

The plants use these simple chemical nutrients and incorporate them into complex organic compounds which are the ultimate source of food for all animals.

When these plants and animals die, the microorganisms decompose the complex organic compounds of dead bodies into simple chemical nutrients. These nutrients are again available the use of plants. This recycling process allows the earth, with its limited supply of nutrients, sustain a continuum of life.

2. Cellulose Digestion

Ruminants cannot digest cellulose present in plants because they do not contain the enzyme ***cellulase***. The stomach of ruminants contains microbes which help in the digestion of cellulose. If the ruminants are deprived of these microbes, the ruminants will starve and die.

3. Food Production

Some of our foods are actually the by-products of microbial growth. ***Cheese*** is produced by the growth of microorganisms such as *Leuconostoc citrovorum* and *Streptococcus lactis*. The type and flavour of the cheese is largely determined by the microorganism used for processing. The blue mold *Penicillium roqueforti* provides both the flavour and colour to Roquefort cheese.

Yoghurt* results from the growth of bacteria such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in milk.

The leavening of bread is accomplished by *Saccharomyces cerevisiae* (baker's yeast) which produces CO_2 as a waste product. This gas is trapped as tiny bubbles that cause ***dough**** to rise.

**Yoghurt* : *Fermented liquor made from milk.*

**•Dough* : *Mixture containing flour, water, etc. in the form of a paste for making*

The baker's yeast is also responsible for the production of alcoholic beverages.

Certain microbes ferment cabbage to *sauerkraut**. Still others change soyabean extract to *soy sauce*. Even chocolate is the result of the action of yeasts on the cocoa bean prior to roasting.

Mushrooms are now extensively used as food. *Agaricus campestris* is the common field edible mushroom.

4. Energy Production

Microbial methane generators are used to convert manure to combustible fuel for powering vehicles and heaters.

Biological fuel generators convert our wastes into usable energy to supply power to residential communities and industry.

5. Industrial Products

Microorganisms are extensively used in industries to produce products useful to mankind. These products include fermented foods, alcohol, alcoholic beverages such as wine, beer, whiskeys, etc., antibiotics, pharmaceuticals, steroids, vaccines, vitamins, organic acids, amino acids, enzymes, proteins, organic solvents, synthetic fuels, etc.

6. Microbes in Medicine

Microbes and their products are of enormous significance in medicine. Some important drugs synthesized by microorganisms are antibiotics such as penicillin, streptomycin, chloramphenicol, tetracyclines, neomycin, actinomycin, etc., ***ergotin****, ephedrine, vitamins, glycerin, steroids, etc.

7. Microbes in Pesticides

Certain microbes like bacteria, fungi, viruses, protozoa and even nematodes infect insects and kill them. They may be called microbial pesticides and are used as ***biopesticides***.

8. Microbes in Improvement of Soil

Most of the bacteria and fungi live saprophytically on dead organic matters of soil. They decompose complex organic matter into simpler substances. In fact, they bring about decay by their various digestive and respiratory processes. The simple substances form a part of ***humus***, an important constituent of soil.

Blue green algae such as *Nostoc, Anabaena*, etc. are often employed in the reclamation of alkaline user lands.

Certain microbes increase the fertility of soil by converting atmospheric nitrogen into ammonia, nitrites and nitrates. This is brought about by microbes like *Nitrosomonas, Nitrobacter, Rhizobium*, etc.

9. Microbes to Better Sanitation

Microbes like bacteria, algae, fungi and protozoa are used in improvement of sanitary methods. They act on the solids and semisolids of sewage and decompose them.

**...alted and allowed to get sour in the German way.*

10. Microbes in Retting of Fibres

Retting is the process in which plant fibres such as coconut husk, jute, hemp, flax, etc., are separated by the activity of microorganisms. The materials are immersed in water for a considerable period. During this period, they absorb water and swell. The water medium becomes rich in the bacterium *Clostridium butyricum* which hydrolyses the pectic substances. Consequently, the fibres get separated and are then used in preparing ropes, sacks, etc.

11. Microbes and Genetic Engineering

Genetic engineering technique involves the use of micro-organisms. By genetic engineering technique, recombinant DNA is produced.

Applying this technique nitrogen fixing genes (***Nif genes***) are transferred from nitrogen fixing bacteria to cultivated plants.

Insulin synthesizing genes are transferred from vertebrates to microbes and insulin is synthesized on a large scale.

12. Biomining

Microbes are now used in extracting valuable metals like uranium from rocks. *Pseudomonas* and baker's yeast are used to absorb or adsorb heavy metals.

Thiobacillus ferrooxidans unlocks energy from inorganic compounds like iron sulphide. During this process, it produces sulphuric acid and iron sulphate. Due to this activity, *Thiobacillus* is used in mining operations.

Use of microbes in mining reduces the production cost.

11 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 0.5 micron to 3 micron.
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.*** Other organelles such as mitochondria, lysosomes, Golgi body, endoplasmic reticulum, centrioles, etc. are absent.
10. Appendages like ***flagella, pili*** are present.
11. They are either ***Gram positive*** or ***Gram negative.***
12. They show ***absorptive*** mode of nutrition.
13. They multiply by ***binary fission.***
14. Some produce ***endospores.***

Structure of Bacteria

Bacteria are unicellular, microscopic, prokaryotic organisms lacking chlorophyll.

Bacteria were omnipresent. They range in size from 0.5mm to 600mm.

The bacteria are either ***spherical*** or ***rod*** shaped or ***spiral*** or ***curved.***

The spherical bacterium is called ***coccus.*** Coccus means a ***berry.***

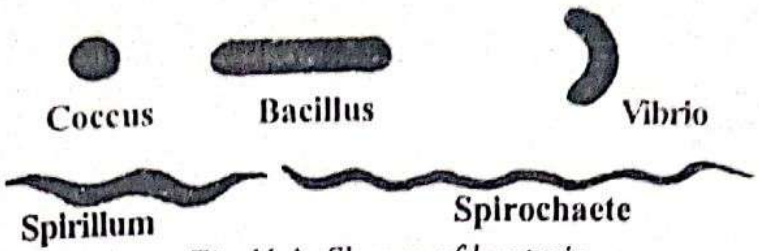

Fig.11.1: Shapes of bacteria.

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 fours, 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.***

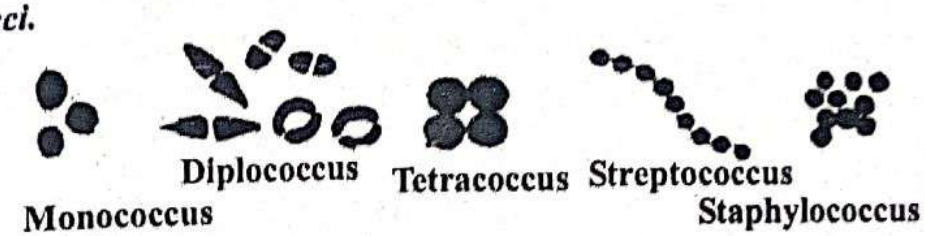

Fig.11.2; Cocci (Spherical).

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.

The individual rod-like bacteria are simply called ***bacilli***. The bacilli arranged in pairs are called ***Diplobacilli***. A chain of bacilli is called ***Streptobacillus***. A bunch of bacilli is called ***Staphylobacillus***.

Fig.11.3; Bacilli (Rods).

The ***spiral*** bacteria are spirally curved. They may be slightly curved like a comma Eg. *Vibrio* or spirally coiled. Eg. *Spirillum*.

In addition there are ***filamentous bacteria*** and fungus like bacteria. They are ***multicellular***.

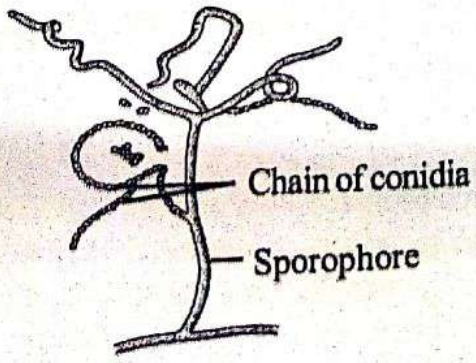

Fig.11.4: A filamentous bacterium.

There are two types of bacteria. They are *Gram positive bacteria* and *gram negative bacteria*. Gram positive bacteria retain violet colour on Gram staining. Gram negative bacteria appear in red colour.

The bacteria are *motile* or *non - motile*. They may or may not contain *flagella*.

When the flagellum is absent, the bacterium is called *atrichous*. When the bacterium contains only one flagellum at the end, it is called *monotrichous*. When the flagellum is present at both the ends, the bacterium is called *amphitrichous*. When there is a bunch of flagella at one end the bacterium is called *lophotrichous*. In some bacteria, the flagella are present all over the cell these bacteria are called *peritrichous*.

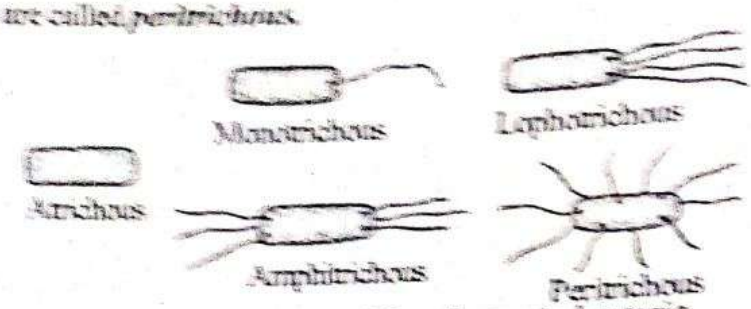

Fig.11.5: Types of flagellation in bacteria.

The flagella are present in bacilli and spiral bacteria. They are absent from cocci. They are whip-like. Each flagellum has three parts, namely a ***basal body***, a ***hook*** and a ***shaft***. The flagella are used for ***locomotion***.

The bacteria may be ***motile*** or ***non-motile***. The Bacilli and spirilla are motile. The cocci are non-motile. Motility is brought about by a ***flagella***.

Some hair-like structures are present on the bacteria. These are called *pili* or *fimbriae*. They are used for ***attachment***. Some pili are longer in some bacteria and they are called *sex pili*.

A bacterial cell is protected by a ***cell envelope***. The cell envelope is made up of a *capsule*, a ***cell wall*** and a ***plasma membrane***.

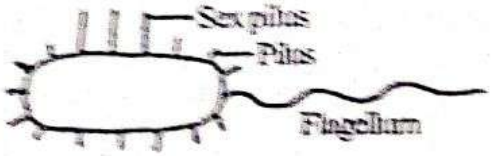

Fig.11.6: A bacterium with pili and flagellum.

In some cells a *slimy cover* is present instead of a capsule.

In some other cells an ***outer plasma membrane*** is present between the capsule and the cell wall.

The bacteria covered by a capsule are called ***capsulated bacteria***. The bacteria which do not contain a capsule are called ***non-capsulated bacteria***.

The cell envelope encloses the *cytoplasm*. It is colloidal in nature. It does not exhibit streaming movement. It contains *ribosomes* and *mesosomes*. Golgi bodies, endoplasmic reticulum and mitochondria are absent. The ribosomes are 70S type.

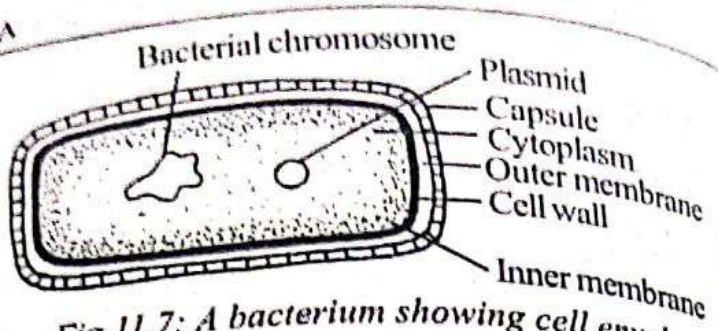

Fig.11.7: A bacterium showing cell envelope.

Mesosomes are pocket-like structures formed by the invagination of plasma mem
The cytoplasm contains a ***bacterial chromosome*** in the cytoplasm. It is a double
DNA. It is not surrounded by a nuclear membrane. Hence the nuclear material of a
called a ***nucleoid.***

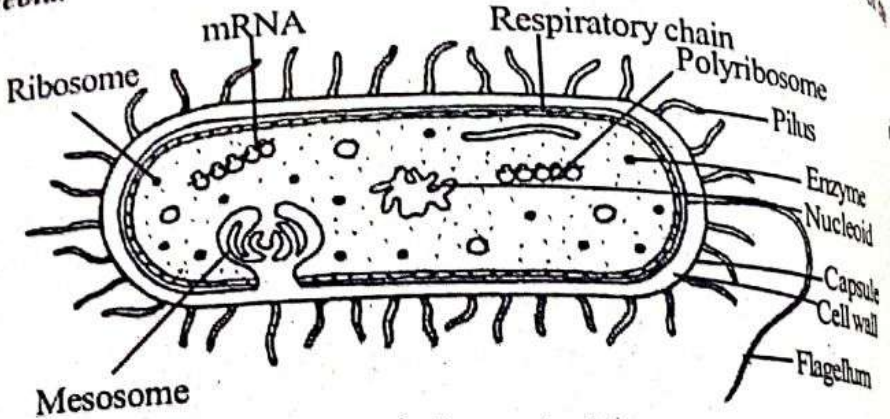

Fig.11.8: Structure of a typical bacterium. E. coli.

Some bacteria contain an extra chromosomal circular DNA called ***plasmid.***

Based on nutrition, the bacteria are classified into two types, namely autotrophs and ***heterotrophs.***

The autotrophs use the CO_2 as the source of carbon. The heterotrophs use organic as the source of carbon.

The bacteria may be ***aerobic*** or ***anaerobic*** or ***facultative anaerobic.***

Aerobic bacteria use O_2 for respiration. Anaerobic bacteria use CO_2. Facultative use O_2 when O_2 is available and use CO_2 when O_2 is not available.

Based on temperature tolerance, the bacteria are classified into three groups as

1. *Mesophilic bacteria*
2. *Thermophilic bacteria*
3. *Psychrophilic bacteria*

The ***Mesophilic bacteria*** grow well in temperature between 25°C and 40°C. The bacteria grow well above 40°C. ***Psychrophilic*** bacteria grow well in temperature less

Bacteria reproduce by ***binary fission, budding, fragmentation, endo conidiospores.***

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21 Yeast (Saccharomyces)

Division	:	*Amastigomycota (Yeast)*
Sub-division	:	*Ascomycotina*
Class	:	*Ascomycetes*
Sub-class	:	*Hemiascomycetes*
Order	:	*Endomycetales*
Family	:	*Saccharomycetaceae*
Genus	:	***Saccharomyces (Yeast)***

Saccharomyces are popularly known as '***Yeasts***'. ***Leeuwenhoek*** first described yeast in ***1680***.

Yeast is an ***unicellular fungus***. It grows in sugary substances, fruit juices and on the surface of fruits. They are commonly called '***sugar fungus***'. They are saprophytes. Few species are ***parasitic***. A few of them live as ***symbionts*** in ***insects***.

Yeast is used in baking industry. Hence yeast is commonly called ***baker's yeast***.

Yeasts are ***haploid***. But a few are ***diploid***.

Yeast is ***unicellular*** and ***microscopic***. The cells are ***oval*** or ***spherical*** or ***ellipsoidal*** in shape.

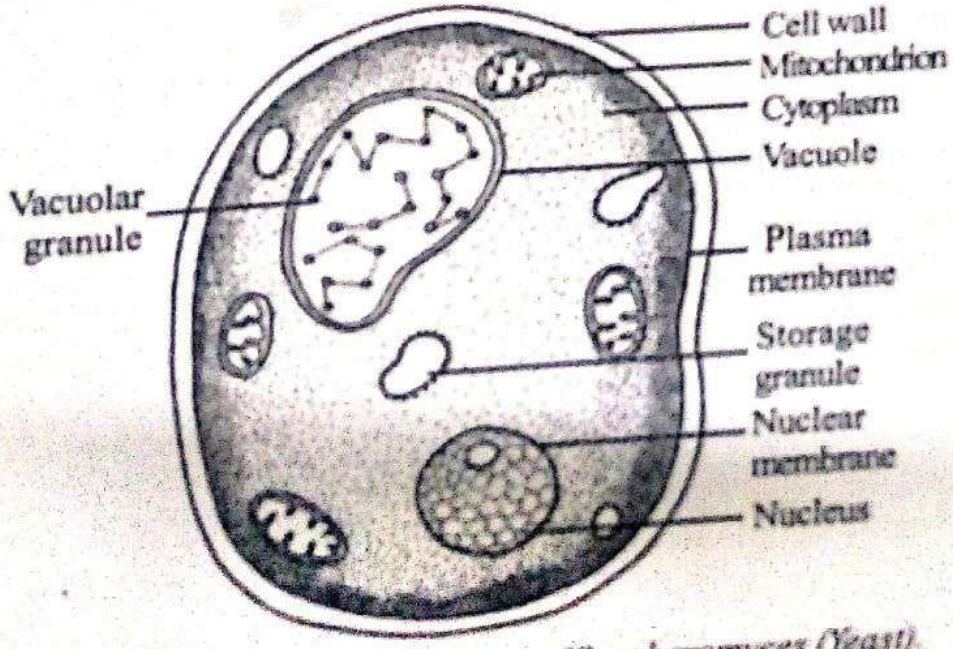

Fig. 21.1: Cell structure of Saccharomyces (Yeast).

24 Viruses

Viruses are defined as '*sub-microscopic, self reproducing 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 forms of life. They are ***parasitic***.

They live inside the cells. When they live inside cells they are active and they feed, respire, reproduce, grow and move. When they live outside the cells they remain inactive and they do not feed, respire, reproduce, grow and move. Thus, the viruses resemble the living organisms in the intracellular state and the non-living chemicals in the extracellular state. Hence they are called ***living chemicals.***

Viruses are so small that they even pass through filters. Hence viruses are called ***filterable molecules.***

Discovery

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

Characteristics of Viruses

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

1. Viruses are extremely smaller in size. They are smaller than bacteria. They are invisible under the light microscope. They are only slightly larger than a larger protein and a nucleic acid. They range in size from 100 A^0 to 2,500 A^0.
2. They ***live inside living cells***. When they live inside cell, they are active and they feed, respire, reproduce, grow and move. When they live out side, they remain inactive and behave as non-living things. So they are called as ***living chemicals.***
3. They are potentially ***infectious.***
4. They have a ***single nucleic acid*** either DNA or RNA (except RNA-DNA viruses).

5. Viruses do not contain cellular structures such as, plasma membrane, mitochondria, Golgi complex, lysosomes, ribosomes, etc.

6. They can be crystallised.

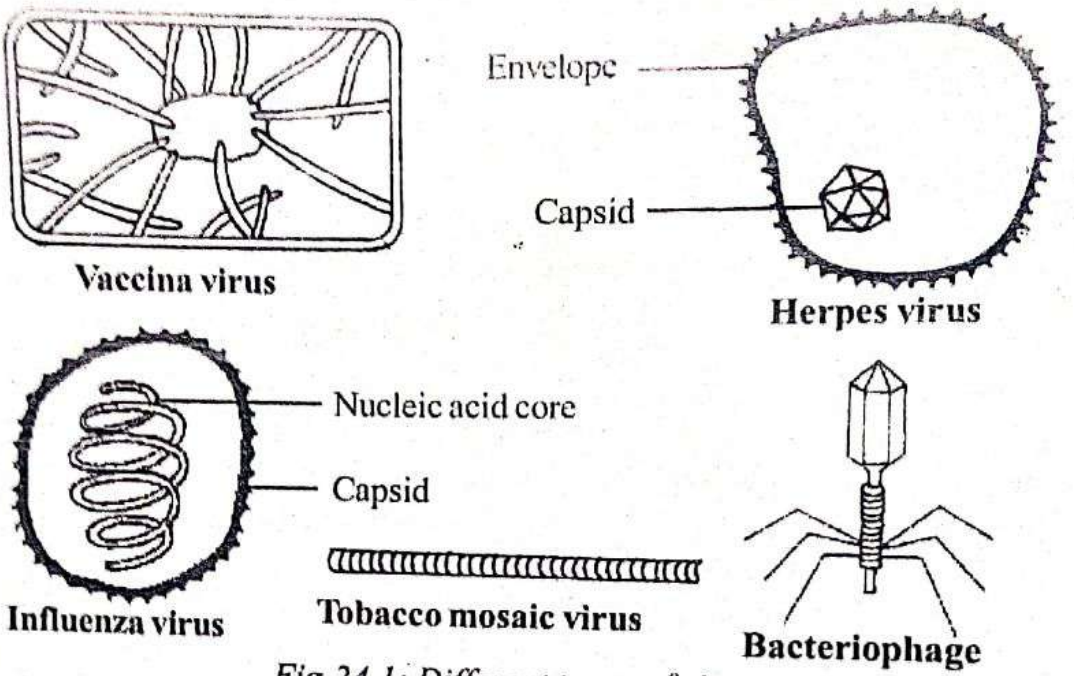

Fig.24.1: Different types of viruses.

7. Viruses do not have the power for growth and division. A fully formed virus does not increase in size by the addition of new molecules. Similarly a virus itself cannot divide. Only its nucleic acids DNA or RNA, divide.

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

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

10. Viruses are intracellular obligatory parasites. They live inside the cells of plants, animals and bacteria.

Structure of Viruses

Viruses are submicroscopic infective molecules that multiply only intracellularly and are potentially pathogenic. They are briefly called ***living molecules.***

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

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

The shape of viruses varies. They may be rod shaped (Eg. *Poxviruses*), bullet shaped, brick shaped, spherical, oval or irregular. Some are like a piece of rope.

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

**nm = nanometer = 1 millimicron = one thousand of a micron = one millionth of a millimetre.*

26 T_4 Bacteriophage

Bacteriophage is a ***bacterial virus.*** It is a virus living inside bacterial cells. Bacteriophage means ***bacteria eating agent***. It was first described by ***Twort*** in 1915.

The phages were numbered as per the sequence of discovery such as Type 1(T_1), Type 2 (T_2) ..... (T_7).

T_2, T_4 and T_6 are structurally similar and are called ***T-even phages.***

The common bacteriophage is ***T_4 bacteriophage***. It is parasitic on human ***colon bacteria*** ***Escherichia coli.*** It is also known as ***coliphage.***

The T_4 phage is ***tadpole-shaped***. It consists of 3 parts, namely a ***head***, a ***neck*** and a ***tail***.

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

A ***double stranded*** DNA is present inside the head. The DNA is highly coiled and tightly packed. It contains more than 75 genes.

The neck is very short and it contains ***collar***. It connects the head with the tail.

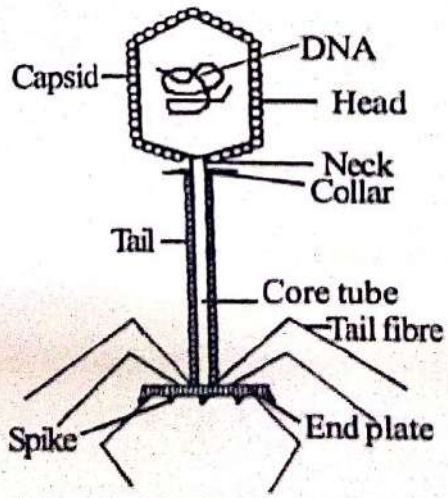

Fig.26.1: A sectional view of T_4 bacteriophage.

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 core tube is covered by a *sheath* or tube made up of about 144 protein *contractile* subunits.

The free end of the core tube has a *hexagonal end plate*. The end plate has 6 *spikes* and 6 *fibres*. The spikes are used for penetration and the fibres are used for attachment on the host.

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

The bacteriophage has 2144 capsomeres. Of these, 2000 capsomeres are present in the head and 144 capsomeres are in the tail. The 2000 capsomeres of the head are arranged in the form of an *icosahedron* having 20 triangular sides called *facets* and 12 corners called *vertices*. There are two types of capsomeres, namely *pentons* and *hexons*. Pentons are 12 in number and are located on the head. The hexons are located on the facets.

The 144 capsids of the tail are helically arranged to form the core tube.

Highlights

Bacteriophage

1. ***Bacteriophage*** is a ***bacterial virus.***
2. It means ***bacteria eating agent.***
3. The common bacteriophage is ***T_4 bacteriophage.***
4. It is parasitic on human ***colon bacteria, Escherichia coli.***
5. It is also known as ***coliphage.***
6. The T4 phage is ***tadpole-shaped.***
7. It consists of ***head, neck*** and ***tail.***
8. The head is polyhedral. It has double ***stranded DNA.***
9. The neck is very short and it contains ***collar.***
10. The tail consists of a central hollow ***core tube.***
11. It has a ***complex symmetry.***

13 Nutritional Types of Bacteria

Bacteria need enough suitable nutrients for its growth and reproduction. Enough moisture and temperature, suitable pH and enough nutrients such as a carbon source, a nitrogen source, electron donors and trace elements are necessary for the growing bacteria. Bacteria are extraordinarily diverse in their specific nutrient requirements. The various nutritional types of bacteria are discussed below:

On the basis of nutrition, bacteria are divided basically into two groups. They are ***autotrophs*** and ***heterotrophs.*** The bacteria which utilize carbon dioxide as the source of carbon are called ***autotrophs.*** The bacteria which use organic compounds as the source of carbon are known as ***heterotrophs.***

The autotrophic bacteria which use sunlight as the energy source are called ***photoautotrophs*** or ***photosynthetic bacteria.***

Green sulphur bacteria contain a pigment ***bacterioviridin.*** They use sunlight as the source of energy to prepare food from CO_2 and H_2S.

$$6CO_2 + 12H_2S \xrightarrow{Light} C_6H_{12}O_6 + 6H_2O + 12S.$$

The autotrophic bacteria which derive energy by oxidizing certain inorganic compounds are known as ***chemoautotrophs*** or ***chemolitho-trophs*** or ***chemosynthetic bacteria.*** Nitrifying bacteria, sulphur bacteria, iron bacteria, etc. are examples of chemosynthetic bacteria.

The nitrifying bacteria oxidize ammonium salts into nitrites to draw energy to make food from CO_2.

$$NH_3 + 3O_2 \longrightarrow 2HNO_2 + 2H_2O + Energy\ (70K\ cal).$$

The sulphur bacteria oxidize hydrogen sulphide to get energy for chemosynthesis.

$$H_2S + 2O_2 \longrightarrow H_2SO_4 + Energy.$$

The iron bacteria oxidize ferrous iron compounds into ferric hydroxides to get energy for chemosynthesis.

$$Fe^{2+} + H^{+} + \frac{1}{2} O_2 \longrightarrow Fe^{3+} + \frac{1}{2} H_2O + Energy.$$

The carbon bacteria in the damp coal oxidizes carbon monoxide into carbon dioxide to get energy for doing chemosynthesis.

$$CO + \frac{1}{2} O_2 \longrightarrow CO_2 + Energy\ (74\ K\ cal).$$

The heterotrophic bacteria make use of organic compounds such as simple sugars and amino acids as the source of carbon. The bacteria which lives in the cells of other organisms by feeding on their cellular components are called ***parasitic bacteria.*** Those living on dead organic matter to draw energy and carbon source are called ***saprophytic bacteria*** or ***organotrophs.*** The bacteria which live in association with other organisms are known as ***symbiotic bacteria.*** Eg. *Rhizobium* in the root nodules of legumes.

Some heterotrophic bacteria use sunlight as the energy source and organic compounds as the source of carbon. Such bacteria are known as ***photoheterotrophs*** or ***photo-organotrophs.*** Eg. *Non-sulphur purple bacteria.*

Some heterotrophic bacteria use organic compounds as the source of carbon as well as energy. Such bacteria are known as ***chemoheterotrophs*** or ***chemo-organotrophs.***

34 Bacterial Growth

Bacterial growth refers to the *increase in cell number* or *cell population* which is brought about by cell multiplication.

Methods of Bacterial Growth

Bacteria grow by the following methods of reproduction:

1. *Binary fission*
2. *Budding*
3. *Filamentation*
4. *Sporulation*

1. Binary Fission

Binary fission refers to *the division of the parent cell into two daughter cells* in which binary fission both cytoplasm and nuclei divide. Eg. *Bacillus, Streptococcus, etc.*

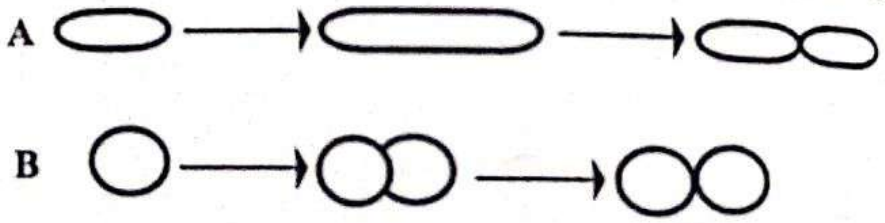

Fig.34.1: Binary fission in A. Bacillus, B. Prosthecobacter

2. Budding

In budding, a bacterial cell produces a small projection on its surface. It increases in size and separates into a daughter cell. Eg. *Rhodopseudomonas.*

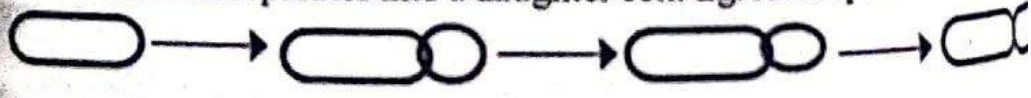

Fig.34.2: Budding in Rhodopseudomonas.

3. Filamentation

In filamentous bacteria, the filaments break into fragments and each fragment daughter filament. This process of growth is called ***filamentation*** or *fragmentation*

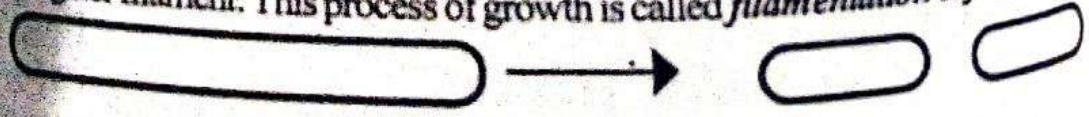

Fig.34.3: Filamentation in Nocardia.

Sporulation

Certain bacteria grow by producing spores. The hypha at its tip produces many spores. The spores separate and develop into new colonies. Eg. *Streptomyces.*

Fig.34.4: Sporulation in Streptomyces.

Growth Rate

During growth, a cell divides into two and the two then divide into four and the four into 8 and so on. In this way the cells grow by geometric progression.

1 ⟶ 2 ⟶ 4 ⟶ 8 ⟶ 16 ⟶ 32

The time required by a cell to divide or the time required for the population to double is called ***generation time.*** The generation time varies from species to species and it is affected by nutrients, temperature, etc. The generation time for *E.coli* in milk at 37^0C is 12 minutes.

Number of cells (N) derived from a single cell after n generations will be

$N=2^n$

Similarly after n generations, the number of cells derived from an initial cell population of O will be

$N=2^n NO$

Rate of Growth of a Culture

Different parameters are used to express the rate of growth in bacterial culture. They are (i). Growth rate constant denoted by 'k'; and (ii). Mean doubling time or generation time denoted by 'g'.

Growth rate of a cell refers to how rapidly a cell increases in mass. The rate of increase in bacteria at any particular time is proportional to the number or mass. The rate of increase in bacteria at any particular time is proportional to the number or mass of bacteria present at that time.

Rate of increase of cells = k (No. of cells or mass of cells).

Here 'k' is the proportionality constant and is an index of the rate of growth; 'k' is called the ***growth rate constant.*** Since we assume growth to be balanced 'k' also to the amount of that cellular component. In mathematical terms,

$\frac{dN}{dt} = kN$; $\frac{dX}{dt} = kX$; and $\frac{dZ}{dt} = kZ$

Where N is the number of cells per ml, X is the mass of cells/ml, Z is the amount of any cellular component/ml, t is the time and k is the growth rate constant.

Upon integration, the above equation yields

$\ln Z - \ln Z_0 = k(t-t_0)$

and in converting natural logarithm (In) to logarithms to the base 10,

$\log_{10} Z - \log_{10} Z_0 = k (t-t_0)$

where the values of Z and Z_0 correspond to the amount of any bacterial component of the culture at times t and t_0 respectively. If one considers the number at time t and t_0, then the equation becomes

$$\log_{10}N - \log_{10}N_0 = \frac{k}{2.303}(t-t_0)$$

By measuring the value of Z and Z_0 or by knowing the number N and N_0, one can compute the value of 'k', the growth rate constant. For example, if a culture contains 10^4 cells/ ml at t_0 and 10^{12} cells/ml 4 hours later, the specific growth rate of the culture is

$$k = \frac{(\log 10N - \log 10N_0)\,2.303}{t-t_0}$$

$$\text{i.e } k = \frac{(12-4)\,2.303}{4} = \frac{8 \times 2.303}{4} = 2 \times 2.303$$

$$= 4.606 \text{ hours}^{-1}$$

Generation Time or Mean Doubling Time

The generation time is defined as the time required for cell components of the culture or the number or mass to increase by a factor of 2. It is denoted by the symbol 'g'. Generation time also refers to the time that a bacterial cell takes to go through its life cycle.

Generation time are usually shorter for prokaryotes than eukaryotes and shorter for smaller than for larger cells, since the growth rate is proportional to the energy metabolism of the cell. The faster the cell metabolizes nutrients, the shorter its generation time.

The growth rate constant 'k' and the doubling time 'g' are related to one another.

The relationship is derived from the equation:

$$\ln Z - \ln Z_0 = k(t-t_0)$$

If the time interval $(t-t_0)$ considered is equal to 'g', then Z will be twice Z_0. Making these substitution, one obtains

$$k = \frac{\ln_2}{g} = \frac{0.693}{g}$$

In the case of the example we have chosen, the mean doubling time g, of the culture is :

$$g = \frac{0.693}{4.606} = 0.15 \text{ hour or } 9 \text{ minutes.}$$

This is relatively a very high growth rate for a bacterium. Since the rate of increase is proportional to numbers or mass, the doubling time 'g' is constant during a period of bacterial growth.

Using the parameters 'k' and 'g' one can also obtain the 'n' i.e the number of generations required to produce a particular number of bacteria or bacterial population. It is derived as follows:

$$N = N_0 \times 2^n \text{ (or)}$$

$$\log 10N = \log 10^{No} + n \log 10^2$$

$$\text{Therefore } n = \frac{\log 10N - \log 10N_0}{\log 10^2}$$

If we now substitute the value of $\log 10^2$, which is 0.301, in the above equation, we can simplify the equation to

$$n = \frac{\log 10N - \log 10N_0}{0.301} \quad \text{(or)}$$

$$n = 3.3(\log 10N - \log 10\,N_0)$$

For the problem given in our text the value of n, is

$$n = 3.3\,(12\text{-}4) = 3.3\,(8)$$

$$= 26.4 \text{ generations.}$$

That mean we want 26.4 generations to pass through to bring the bacterial population whose generation time is 9 minutes from 10^4 cells/ml to 10^{12} cells/ml.

Using the above relationship we can calculate generation time, 'g' and the growth rate 'R' as follows:

$$g = \frac{t}{n} = \frac{t}{3.3(\log 10N - \log 10\,N_0)} \quad \text{and}$$

$$R = \frac{3.3(\log 10N - \log 10N_0)}{t}$$

where t denotes time interval.

Growth Curve

Growth curve is a *graph obtained by plotting the number of cells against time factor.* A typical growth curve is obtained, when a known concentration of bacteria is inoculated into a suitable culture medium (***batch culture***). The bacteria grow by dividing binary fission. The bacterial cells are counted at regular intervals of one hour. The number of bacteria are plotted against time.

A typical bacterial growth curve shows four distinct phases viz, ***lag phase, log phase, stationary phase*** and ***decline phase***. Lag phase represents an initial period of no growth in terms of increase in cell numbers. In this phase the cells are metabolically active, capable of repairing cell damage and synthesizing enzymes.

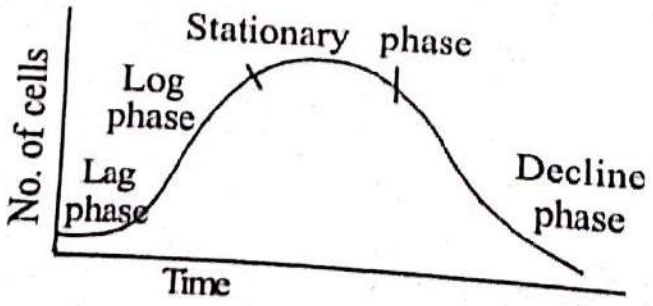

Fig. 34.5: A growth curve.

Log phase is a period of rapid growth. In this phase, the bacterial population increases exponentially and is also called ***growth phase.***

Log phase is followed by a stationary phase during which no new growth occurs.

Lastly, there is decline in the viable population in which all microbial cells die. This phase is called ***decline phase*** or ***death phase.***

a *continuous* and there will be no stationary

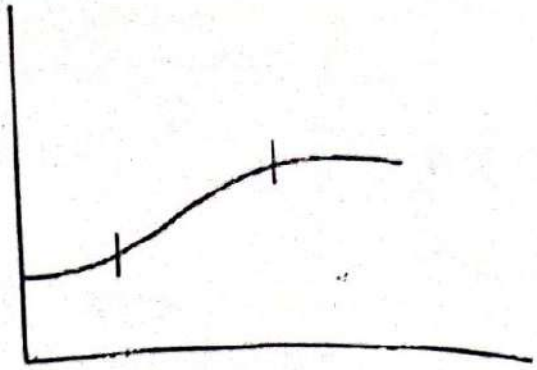

Fig. 34.6: Continuous growth curve.

Measurement of Bacterial Growth

Growth in bacteria and other unicellular organisms is measured by measuring increases in ***mass*** or increases in ***numbers*** in relation to time.

1. Measurement of Cell Mass

It is an indirect method of measurement of bacterial growth. The cell mass can be measured in the following ways:

a. Dry Weight Method

In this method, a known volume of culture is removed from the medium, washed, filtered, dried and weighed accurately. Such determinations are time consuming and insensitive.

b. Cell Volume Method

This method involves placing a standard volume, say 10ml, of the liquid culture in a calibrated centrifuge tube called a ***Hopkin's tube.*** It is centrifuged at a standard speed and the pellet is measured in volume. From a knowledge of the average volume of the individual cells, estimation of numbers is possible.

c. Chemical Method

Chemical estimates of cell masses are made by measuring the amount of some chemical component, say protein, DNA, RNA, free amino acid, phosphorus, etc. that are present in fairly constant amounts in living cells. Such methods are hardly applicable to bacteria and much used in measuring heavy growth of filamentous microorganisms.

d. Turbidimetric Method

This is a widely used technique. In this, we measure turbidity or scattering of light in liquid culture due to evenly dispersed cells suspended in it. This technique is based on the fact that small particles scatter light proportionally, to their concentration. When a beam of light is passed through a suspension of bacteria, the reduction in the amount of light transmitted is used to represent the bacterial mass. Such measurements are usually made in the ***colorimeter*** or ***spectrophotometer.***

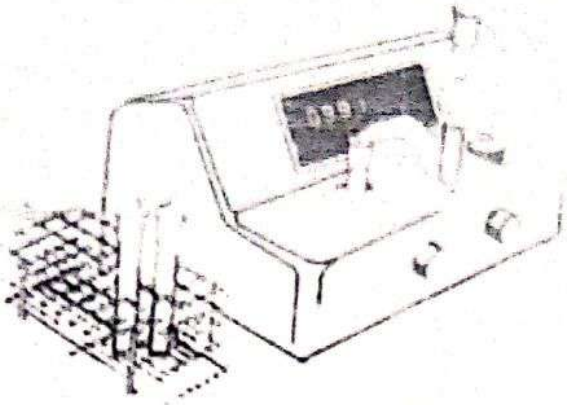

Fig.34.7: Reading the absorbance of a culture in a turbidimeter.

These instruments are convenient for estimating *cell concentration.* When calibrated against acterial suspension of known concentration, they provide an accurate and rapid way to estimate e dry weight of bacteria per unit volume of culture.

More sensitive instrument for measuring scattering is called ***nephelometer.*** It has a ght sensing device kept at right angles to the incident beam of light and hence directly measures e scattered light.

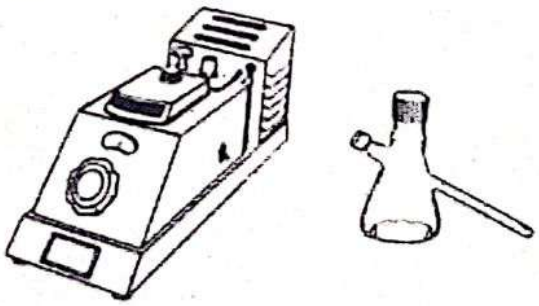

Fig.34.8: A nephelometer flask and a Klett-Summerson colorimeter.

2. Measurement of Cell Number

Counting the bacterial cells using a microscope is direct count. ***Total count*** is made directly th a microscope. This is a direct method and not precise as culture methods. However, it is less me consuming and easy to perform.

Generally specimens that contain large numbers of bacteria (more than 10^4 cells per ml) are uted from 1:10 to $1:10^5$ or more depending on sample and counting method, to make the mber more manageable and simplify the counting. The following methods are used to measure cell number.

Using Counting Chamber

A number of counting chambers are available for counting the number of cells under the roscope. They are ***Petroff-Hausser slide, Haemocytometer*** or special counting chamber.

In this method, a measured volume of a sample is spread over a measured area of the slide the cells therein are counted under a microscope. The counted number is multiplied by an propriate factor to calculate the actual number of cells in the whole volume of culture/sample.

The counting chambers available have depressions of the known depth and volume, marked off into squared areas. The organisms in an area are counted (say 50 or 100 squares) and the total number of bacteria in the sample can be calculated from the proportion of the total volume. This method is applicable to count upto 1×10^7 cells/ml of suspension.

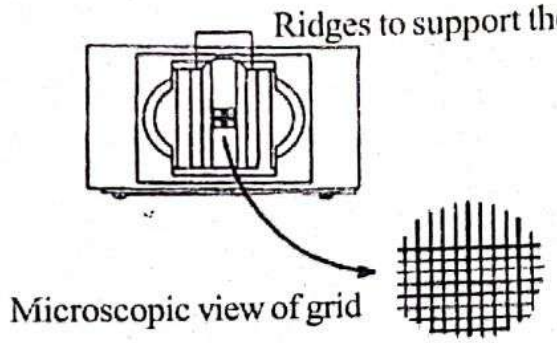

Fig.34.9: Diagram of a Petroff - Hausser bacterial counting chamber.

b. Spread Method or Smear Count

In this method, a known volume of sample is smeared over an exact area on a slide (say 1 square centimetre), dried, fixed and stained with ***methylene blue*** or with any other dye. The cells are counted in a known portion of the total area. Knowing the diameter of the microscopic field from previous measurements (by means of stage micrometer), one can calculate the number of organism per ml of culture.

c. Membrane Filter Count

In this method, a measured sample of fluid may be passed through sterile, porous membrane filters and the microorganisms on the filter are then counted directly. The organisms must not be too numerous and must be uniformly distributed. They are stained in situ on the membrane and then counted in calibrated fields. Before counting, the filter is made transparent by saturating it with immersion oil. This gives a total count.

d. Electronic Colony Counter

This method gives an accurate counting of thousands of cells, alive or dead (total count) in a few seconds. This instrument works on the principle of electronic gating or electronic eye. Basically, it depends on interruptions of an electronic beam that traverses a space between two closely adjacent electrodes. Each particle, as it passes between the electrodes, causes an interference with the electron beam due to different conductivities of cells and fluid. The interruptions are taken up by instruments and recorded electronically.

The petriplate in which bacteria to be counted is placed on the machine and each distinct colony that is separated from its neighbours is counted as if it were a single cell. This operation is performed automatically and records the total plate count on the display panel.

e. Plate or Colony Count

The enumeration of unicellular organisms can also be made by plate count, because single viable cells separated from one another in space give rise to separate colonies. The theoretical assumption in this is that one bacterial cell or clump of cells gives rise to one colony. By counting the number of macroscopically visible colonies that develop on the agar plate one can know the bacterial count.

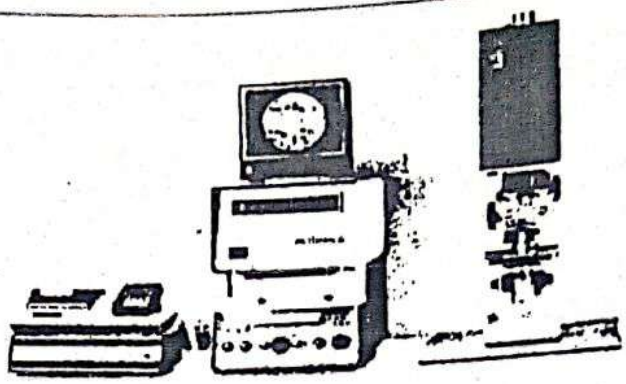

Fig.34.10: An electronic colony counter. (Courtesy of the New Brunswich Scientific company)

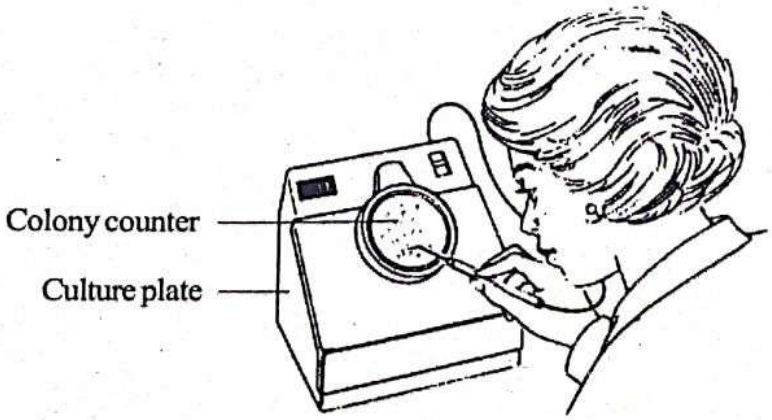

Fig.34.11: Quebec colony counter.

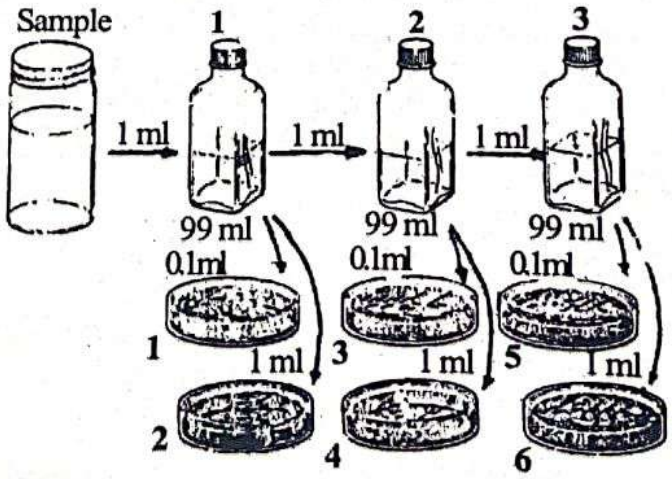

Fig.34.12: Diluting the sample for the pour plates by quantitative plating method.

35 Culture Medium

Culture medium is any solid or liquid material that supports the growth of ... The culture medium is composed of ***beef extract, peptone, yeast extract, ... water.*** In addition, it also contains ***bovine rumen fluid, blood, serum, ...***

A typical culture medium is prepared by mixing beef extract, peptone, ... water. This medium is liquid in nature and it is called ***nutrient broth.***

Beef extract	-	3g
Peptone	-	5g
Sodium chloride	-	5g
Water	-	1000ml

When agar (15g) is added to the above components, the medium becomes called ***nutrient agar.***

It is a complex medium for the growth of heterotrophic bacteria.

The culture media are classified variously. Based on consistency, they are classified into the following three types :

1. *Liquid medium or broth*
2. *Semi solid medium*
3. *Solid medium*

Based on composition, the culture medium is classified into five types.

1. *Natural or Empirical medium*
2. *Living medium*
3. *Synthetic medium*
4. *Complex medium*
5. *Minimal medium*

Based on the uses, the culture medium is classified into the following types.

1. *Selective medium*
2. *Differential medium*
3. *Enrichment medium*
4. *Enriched medium*
5. *Assay medium*
6. *Transport medium*
7. *Maintenance medium*
8. *Enumeration medium*
9. *Characterisation medium*

1. Liquid Medium or Broth

Broth is a ***liquid culture*** medium. It is also called ***broth***. During preparation of the medium, the solidifying agent is not added. This medium is composed of the following components:

Beef extract	-	3g
Peptone	-	5g
Sodium chloride	-	5g
Water	-	1000ml

The broth medium is used to study the ***growth rate*** and the ***sedimentation rate*** of bacterial cells.

2. Semi - Solid Medium

The semi-solid medium remains in the semi-solid condition and it is prepared by adding small amount of ***agar (3.75gms)*** to the ***broth.*** It is used to study ***bacterial motility.***

3. Solid Medium

The solid medium is ***solid*** in consisteney. It is also called ***agar medium***. It is prepared by adding large amount of agar (15gms). It is composed of the following components :

Beef extract	-	3g
Peptone	-	5g
Sodium chloride	-	5g
Agar	-	15gms
Water	-	1000ml

It is used for colony characterization, colony identification, isolation of bacterial cells and demonstration of antibiotic sensitivity.

4. Natural or Empirical Medium

When a natural product is used as such for growing bacteria, the medium is called ***natural medium***. Eg. *Milk, wine, blood, vegetable juices, yeast extract, coagulated egg, meat extract*, etc. This medium is used on the basis of experience and observation and hence the name empirical medium.

5. Living Medium

Living medium consists of living cells which are used for the growth of bacteria.

6. Synthetic Medium

Synthetic medium is synthesized by adding inorganic and organic compounds in definite proportions. Hence the chemical composition of this medium is well known. ***Broth*** is a synthetic medium.

7. Complex Medium

This medium contains many ingredients of unknown composition. This medium contains, sources of energy, vitamins, nitrogen and carbon. It is composed of sugar, yeast, beef extract, vitamins, salts, peptone, etc. It is easy to prepare. It is used for the culture of a wide variety of microorganisms. *Agar medium, broth.*

8. Minimal Medium

Minimal medium lacks certain growth factor. This medium is used in genetic experiments.

9. Selective Medium

Selective medium is a ***culture medium*** which allows the growth of a particular variety of bacteria. Other bacteria cannot grow.

[illegible] that medium which supports the growth of a particular bacterium from a mixture and hence becomes it's favourable medium for growth of a specific organism.

The medium which is used for isolating a specific organism from a mixed culture population is called selective medium. Selective medium contains certain chemicals which suppress or kill unwanted types of microorganisms. For example Mac Conkey's Agar medium contains crystal violet. Crystal violet kills Gram positive bacteria. When crystal violet is added to the medium containing both Gram positive and Gram negative bacteria, the Gram positive bacteria are killed and the Gram negative bacteria remain in the culture medium.

Many types of selective media are available. They are

Mac Conkey's agar.

Desoxycholate agar.

Phenylethanol agar.

Columbia CNA agar, etc.

Mac Conkey's agar medium is a typical selective medium. It is made up of the following components:

Peptone	20g
Lactose	10g
Neutral red solution (1%)	10ml
NaCl	5g
Bile salt	1.5g
Agar	15.5g
Crystal violet	1.00g
Distilled water	1000ml

Mac Conkey's agar is used for the culture and isolation of Gram negative lactose fermenting bacteria.

Desoxycholate agar is used for the isolation of Gram negative enteric bacillus.

Phenylethanol agar is used for the isolation of Gram positive Staphylococci and Streptococci.

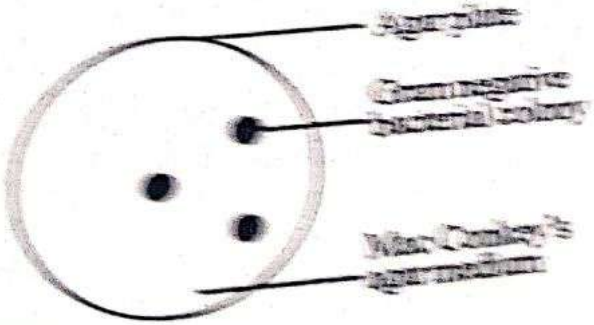

Fig 35.6: Selective medium

Columbia CNA agar is used for the isolation of Gram positive cocci.

Uses of selective medium: 1. It helps in the growth of a specific micro-organism.

2. It kills the unwanted micro-organism.

38 Culture Techniques

A microorganism can be cultured in a culture medium. The culture techniques are of different
es. They are :

1. *Batch culture*
2. *Continuous culture*
3. *Synchronous culture* and
4. *Fed-batch culture*

1. Batch Culture

Growth of microorganisms in a limited volume of liquid medium is called ***batch culture.*** nly one batch of bacteria is cultured in the medium, it is called ***batch culture.*** This is the plest method of culture of microorganisms.

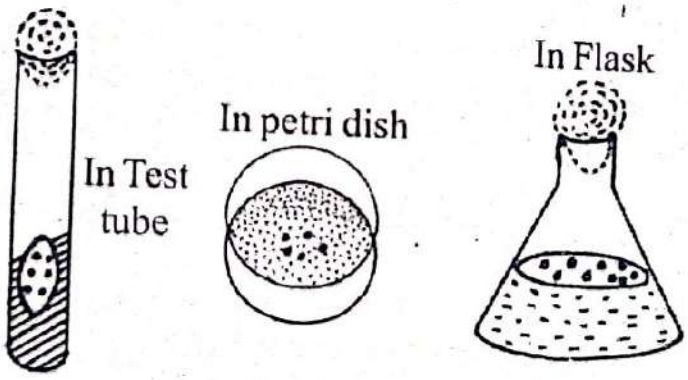

Fig.38.1: Batch culture.

In this method, the microorganism is grown on a ***limited*** amount of medium containing all utrients at optimum environmental conditions.

The vessel used in the culture is known as ***fermentor*** or ***bioreactor***. The vessels used may est tube or a petri dish or a conical flask.

In a batch system, the microorganism will pass through four stages, of growth such as, ***lag e, log phase, stationary phase*** and ***decline phase.***

The growth of microorganism continues until either one of the essential nutrients is exhausted ic by-products accumulate to inhibit growth.

In the ***lag phase,*** there is no increase in the number of cells. However, the cells increase volume. There is no division at this stage. The cells prepare for division. The cells are ctive physiologically. But the population remains stationary.

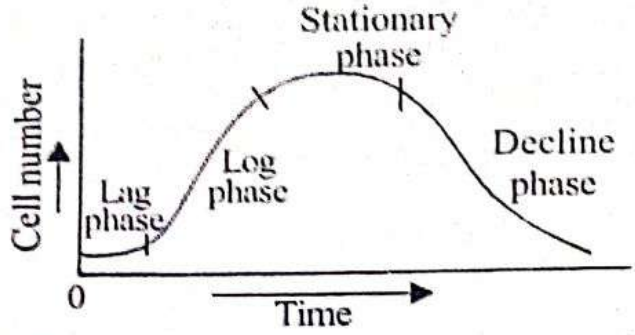

Fig.38.2: Typical bacterial growth curve in batch culture.

In the ***log phase,*** the cells divide rapidly at a constant rate. The cells divide in a geometric progression.

The time required by a cell to divide is called ***generation time.*** The generation time varies from species to species. For *E.coli,* it is 12 minutes in milk at 37°C.

In the ***stationary phase***, the growth slows down. The cells begin to die. There is a balance between dead and newly formed cells.

In the ***decline phase***, the cells die continuously. The death is due to the exhaustion of nutrients and the accumulation of toxic by products.

The growth stages of batch culture can be represented in the form of a curve called ***growth curve.***

2. Continuous Culture

Continuous culture refers to the *growth of the microorganism in a medium at a constant rate continuously.*

Continuous culture is possible when the nutrient is supplied continuously and the toxic by products and dead cells are removed regularly.

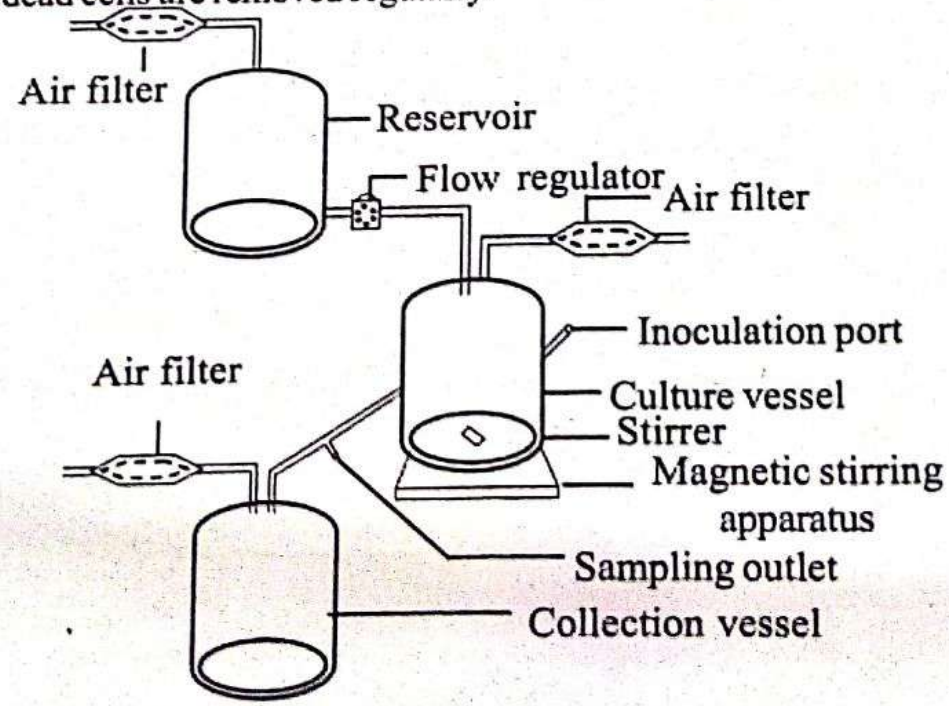

Fig.38.3: Working principle of continuous culture of microorganisms.

39 Methods of Culturing Bacteria

Bacteria can be cultured in the following methods in the laboratory:

1. *Broth culture*
2. *Agar plate culture*
3. *Agar slant culture*
4. *Agar stab culture*
5. *Roll tube culture*
6. *Deep media culture*

Broth Culture

Cultivation of bacteria in a liquid medium is called ***broth culture. Broth*** is a liquid dium. The broth contains ***beef extract, peptone, yeast extract, sodium chloride*** and ***water.***

Beef extract contains carbohydrates, organic nitrogen compounds, vitamins and salts. tone contains ***protein.*** Yeast extract supplies Vitamin B, growth factors, organic nitrogen carbon compounds.

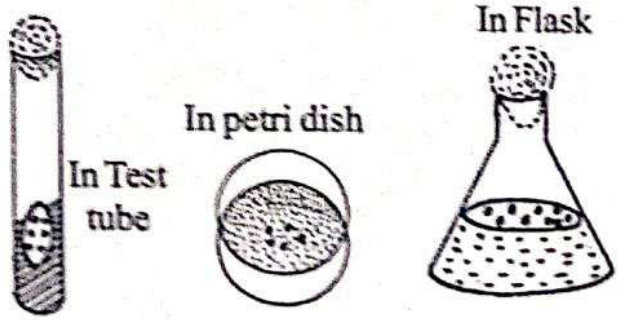

Fig.39.1: Broth culture.

Broth culture can be done in a petridish or in a test tube or in a conical flask.

The medium is poured into a test tube or petridish in an aseptic condition. ***Inoculation*** roducing the bacterium into the medium) is done with a wire-loop after flaming.

The culture tube is incubated at 37°C in an incubator for 24 to 48 hours.

Cloudiness in the broth is an indication of bacterial growth. In broth culture, the bacteria ain floating.

2. Agar Plate Culture

Cultivation of bacteria in a solid medium in a petri dish is called ***agar plate culture.*** It is also called ***plate culture.***

In agar plate culture, agar medium is used. It is a ***solid*** medium. Agar medium is nothing but ***broth plus agar.*** Broth is a liquid medium containing beef extract, peptone, yeast extract, sodium chloride and water. Agar is added as a solidifying agent.

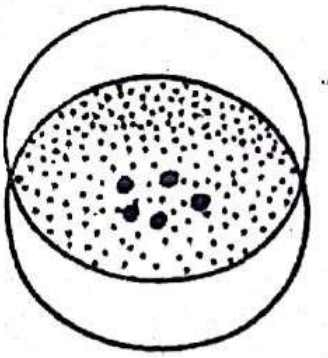

Fig.39.2: Agar plate culture.

About 12 to 15 ml of melted agar medium is poured into a petridish. It is allowed to solidify. Then the bacterium is inoculated and the agar plate is incubated at 37°C for 24 to 48 hours.

Agar plate is used for the cultivation of aerobic bacteria. In plate culture, the bacteria grow in the spot where they were inoculated.

3. Agar Slant Culture

Cultivation of bacteria on a solid agar medium in a test tube kept in a slanting position is called ***slant culture.***

In slant culture, agar medium is used. It is a ***solid*** medium. It contains ***broth plus agar.***

About 12 to 15 ml of melted agar medium is poured into a sterilized test tube. The mouth of the tube is plugged with cotton and it is kept in a slanting position. The medium is allowed to solidify in this position. The medium hardens with a slope.

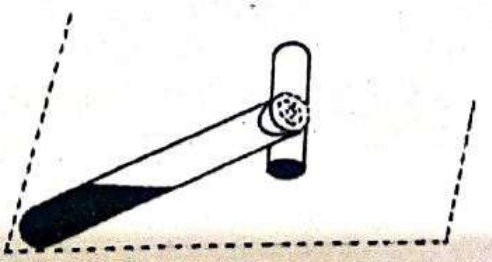

Fig.39.3: Agar slant culture.

After solidifying, the medium is inoculated with the bacterium. It is incubated at 37°C for 24 to 48 hours.

Agar slant is used for culturing aerobic bacteria.

4. Agar Stab Culture

*Cultivation of bacteria in a **solids** agar medium in a test tube kept in a vertical position* is called ***agar stab culture.***

Isolation and Purification Technique (Isolation of pure culture)

Natural environments such as, soil, water, air and human body contain species of microbes. They are mixed populations. *The isolation of one kind of from a mixture* is called ***isolation and purification technique*** or pure cult A pure culture is obtained by any one of the following methods:

1. *Serial dilution technique*
2. *Streak plate culture*
3. *Pour plate culture*
4. *Spread plate technique*
5. *Enrichment culture*
6. *Selective medium-culture*
7. *Differential medium-culture*
8. *Single cell isolation*

1. Serial Dilution Technique

The dilution of sample in successive stages is called ***serial dilution*** The mixture of microorganism (natural sample or mixed culture) is dil *test tubes of sterile medium until the last tube contains only a single org* technique, the dilution factor increases in a regular fashion. Eg. 1/10, 1/100, Serial dilution technique is used to isolate a single bacterium from a mixture

In this method, 1ml of sample is mixed to 9 ml of sterile water in a test tube. Th fold dilution and this dilution factor is represented as ***1/10*** or 10^{-1}.

Now from this 10 fold dilution, 1ml of sample is taken and is added to 9 ml st taken in a second test tube. Now the second tube contains a 100 fold dilution an factor is represented as ***1/100*** or 10^{-2}.

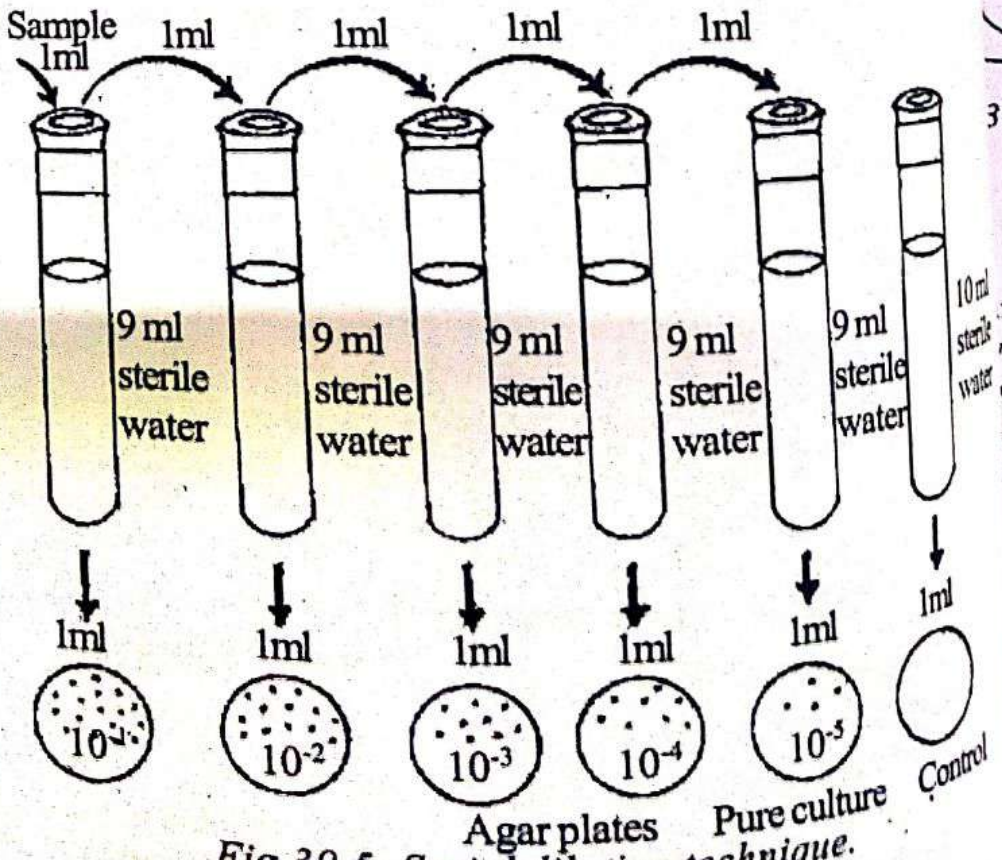

Fig.39.5: Serial dilution technique.

ikewise from tube two, 1 ml of sample is taken and is added to 9 ml of sterile water taken rd test tube. Now the third tube contains a 1000 fold dilution and the dilution factor is ented as *1/1000* or 10^{-3}.

ikewise, test tubes 4 and 5 are prepared. Test tube 4 provides 10^{-4} dilution and test tube 5 es 10^{-5} dilution. A 6th tube is prepared as a control containing 10 ml of sterile water only. hen from each tube, 1ml of diluted sample is taken and is added to an agar plate (a sh containing 10 to 15 ml of melted agar medium). The 6 agar plates are incubated at 30°C hours.

he agar plate containing 20 to 200 colonies are taken as pure culture of that organism.

eak Plate culture

***treak plate culture** is culturing the bacterial sample on an agar plate in the form of egular long lines.*

is a technique of ***isolating*** bacteria from a mixed culture. It is a ***pure culture*** method. this method, the agar medium is taken in a petridish. The medium is uniformly distributed ing the petridish.

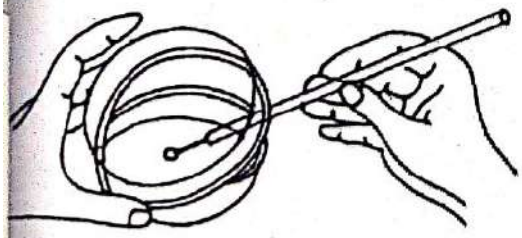

Fig.39.6: Streaking an agar plate.

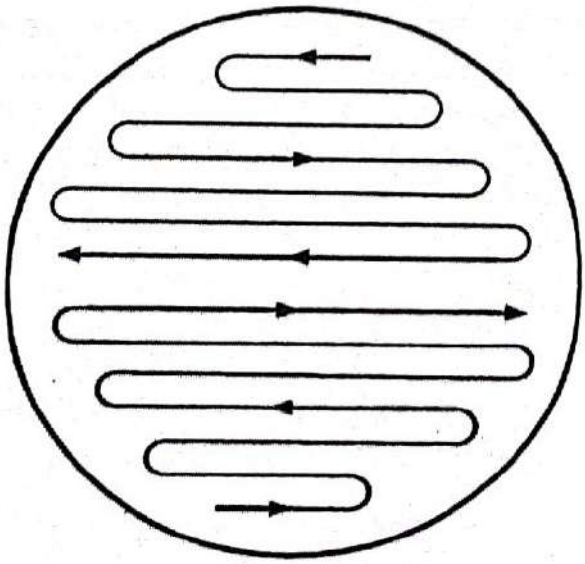

Fig.39.7: Streak plate method.

e sample is taken on an ***inoculation needle.*** It is streaked on the agar plate. The may be a ***continuous streak*** or ***quadrant streak*** or ***radiant streak*** or ***T-streak*** e successive streaks thin out the culture. Streaking isolates the individual cells and are d in different regions of the agar medium.

e streaked plates are kept upside down in the incubator for 24-48 hours

e isolated cells will grow into colonies on different regions of the agar medium.

Plate Culture

s an ***isolation*** and ***purification technique.***

pour plate method, the sample is serially diluted in an agar tube and the contents are nto petridishes and incubated.

Four tubes A,B,C and D are serially arranged.
Agar medium is poured into all tubes.
One loopful of sample is transferred to tube A. It is vigorously shaken.

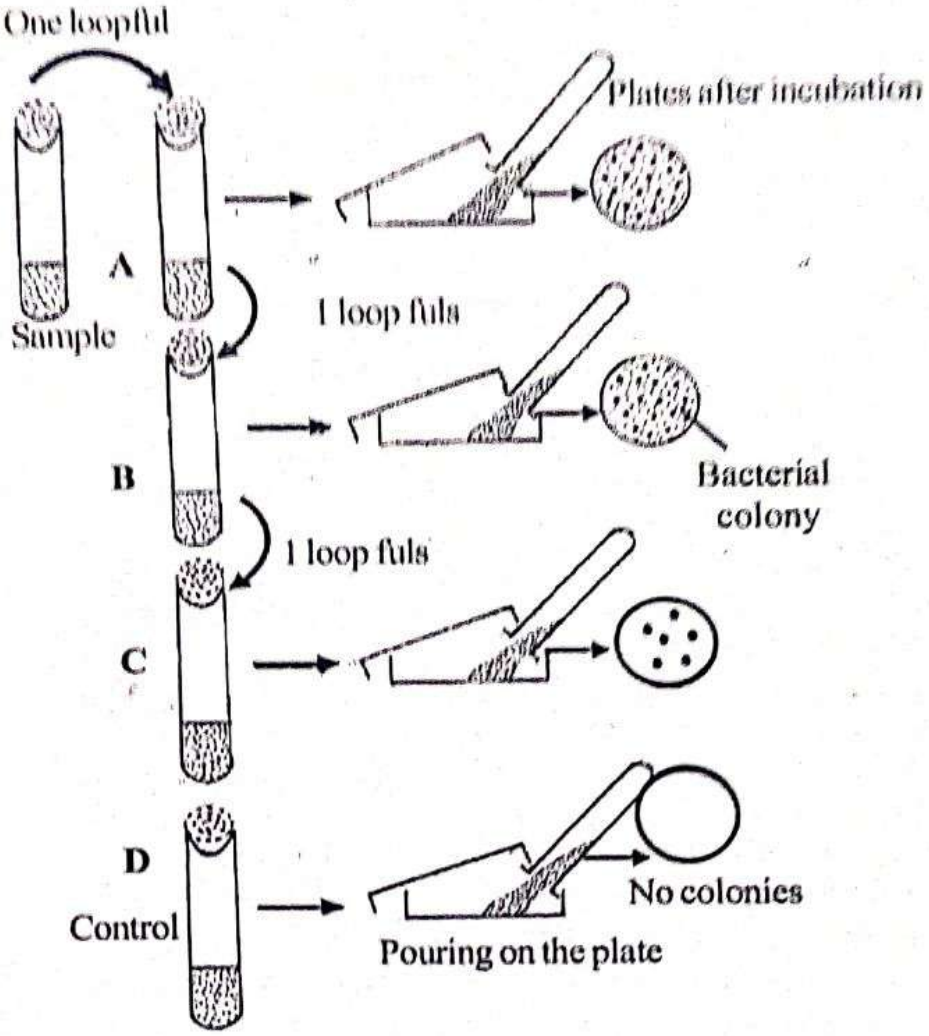

Fig.39.8: Steps in pour plate method.

A loopful of sample is taken from tube A and transferred to tube B. It is vigorously shaken.
Now a loopful is taken from tube B and is transferred to tube C and shaken well.
Tube D serves as ***control***. It is added with agar medium alone.
Now the contents of tubes A,B,C and D are poured into petridishes A,B,C and D.
After solidifying, the petridishes are incubated at 25°C for 24-48 hours.
The plates are observed for colonies.

4. Spread Plate Technique

It is an ***isolation technique***. It is a modification of pour plate technique. In this method, the mixed culture is serially diluted in sterile distilled water. A small amount of the diluted mixture

is then pour on the surface of an agar plate and it is spread evenly using a sterile bent glass rod called *spreader*. The isolated cells grow into colonies.

Fig 39.9: A.Glass spreader and B. Method of spreading

5. Enrichment Culture

It is an isolation technique. In this method, a particular nutrient, which favours the growth of the desired bacterium, is added to the medium. When the mixed culture is placed in this enriched medium, the desired bacterium will grow dominantly.

The soil bacterium *Nitrobacter* oxidizes nitrates to nitrites. An enrichment culture of *Nitrobacter* is obtained by adding ***sodium nitrite*** in the medium inoculated with soil.

6. Selective Medium - Culture

It is an isolation technique. A selective medium contains a chemical, which suppresses or kills unwanted species. But the medium allows the desired bacterium to grow. For example, ***crystal violet*** inhibits gram positive bacteria. When crystal violet is added to the medium, the medium will select the gram negative bacteria.

7. Differential Medium - Culture

The differential medium gives different appearances to different species. For example, in ***eosin-methylene blue agar*** medium, *E.coli* will produce colonies with a brilliant ***green metallic sheen*** and *Acrobacter aerogenes* will produce pink colonies with dark centres.

8. Single Cell Isolation

It is an isolation technique. In this method individual cells are picked out using a *micromanipulator* and a microscope.

41 Maintenance of Bacterial Culture

Storing bacterial culture alive for future use is called ***maintenance of bacterial culture.*** It is the preservation of bacterial culture. The preserved culture is called ***stock culture collection.*** Some cultures can be preserved for 80 years.

Different methods are employed for maintenance of bacterial cultures. They are

1. Periodic transfer to fresh media
2. Maintaining with mineral oil
3. Maintenance in formaldehyde
4. Lyophilization
5. Preservation by liquid nitrogen at very low temperature
6. Soredelli's method of preservation
7. Storage in sterile soil
8. Storage in silica gel

1. Periodic Transfer to Fresh Media

The culture can be stored alive by transferring the culture to fresh medium at regular interval. The transfer can be done once in a ***month.*** For better results, the medium should favour ***slow rate of growth.***

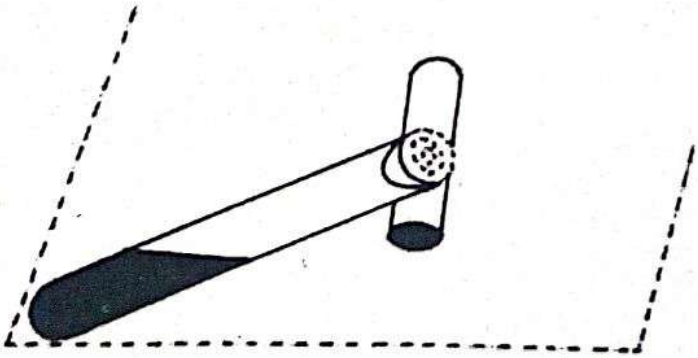

Fig.41.1: Agar slant.

The medium used for this method is called ***nutrient agar***. Nutrient agar contains peptone, beef extract, NaCl, agar and distilled water.

The culture is maintained in ***agar slant***. Agar slant is an agar nutrient culture medium kept in a slanting position.

2. Maintaining with Mineral Oil

Bacterial culture can be stored alive for 2 years in ***mineral oil***.

The culture is kept in ***agar slant***. Mineral oil is poured into the agar slant tube. It must be above the tip of the slanted surface. The oil covered slant is stored.

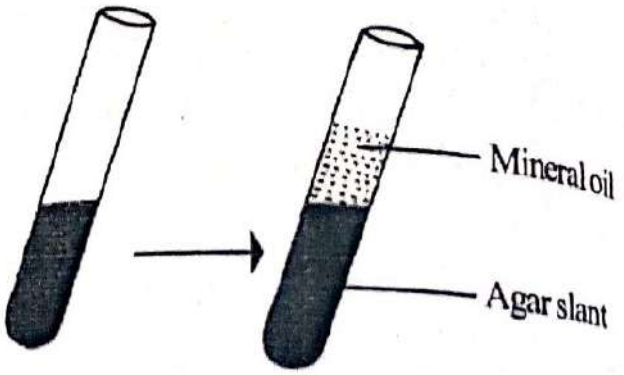

Fig.41.2: Agar slant with mineral oil.

3. Maintenance in Formaldehyde

Agar plate cultures can be preserved by placing a drop of formaldehyde on the lid. It is stored in a refrigerator at 4°C.

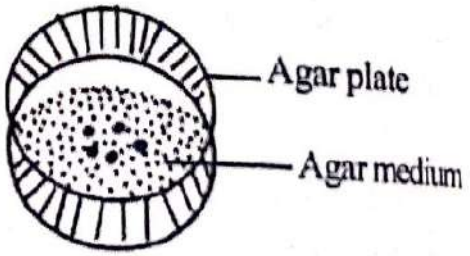

Fig.41.3: Agar Plate.

4. Lyophilization

Lyophilization or ***freeze- drying*** can be used to preserve many kinds of bacteria that would be killed by ordinary drying. In this process a dense cell suspension is placed in vials and frozen at -60°C to -78°C.

The vials are then subjected to rapid ***dehydration*** under high vacuum. This causes little damage to delicate cell structures .

The vials are then sealed off under a vacuum and stored in a refrigerator. With this method one can preserve a culture for more than 30 years. This method has the following advantages:

1. It requires minimum storage space.
2. Hundreds of lyophilized cultures can be stored in a small area.
3. Culture vials can be sent conveniently through the mail.

42 Microbial Nutrition

Nutrition refers to *the nourishment of organisms*. It includes the type of food and the mode of intake of food.

Microbes are broadly classified into three types based on their nutrition. They are ***autotrophs, heterotrophs*** and ***mixotrophs***.

1. Autotrophs

Autotrophs synthesize their food using inorganic substances Eg. Green plants, *Euglena, Volvox,* some bacteria, etc.

Auto = self, ***trophe*** = nourish Autotrophs are ***self feeders***. They are also called ***lithotrophs***. They depend on only inorganic substances and water for their energy.

The autotrophs are of two types, namely ***photosynthetic autotrophs*** and ***chemosynthetic autotrophs.***

1. Photosynthetic Autotrophs

Photosynthetic autotrophs use the energy of sunlight. They are also called ***photolithotrophs***. They use CO_2 for their carbon source. The process of synthesis is called ***photosynthesis***. In photosynthesis CO_2, mineral salts, water, sunlight and chlorophyll are used.

2. Chemosynthetic Autotrophs

The organisms which derive energy by oxidising certain inorganic compounds are called ***chemoautotrophs*** or ***chemosynthetic bacteria.*** Nitrifying bacteria, sulphur bacteria, iron bacteria, etc. are examples of chemosynthetic bacteria.

The nitrifying bacteria oxidize ammonium salts into nitrites to draw energy to make food from CO_2.

$$NH_3 + 3O_2 \longrightarrow 2HNO_2 + 2H_2O + \text{Energy (70K cal)}.$$

The sulphur bacteria oxidize hydrogen sulphide to get energy for chemosynthesis.

$$H_2S + 2O_2 \longrightarrow H_2SO_4 + \text{Energy}.$$

The iron bacteria oxidize ferrous iron compounds into ferric hydroxides to get energy for chemosynthesis.

$$Fe^{2+} + H^+ + \tfrac{1}{2}\, O_2 \longrightarrow Fe^{3+} + \tfrac{1}{2}\, H_2O + \text{Energy}.$$

38 Culture Techniques

A microorganism can be cultured in a culture medium. The culture techniques are of different ...es. They are :

1. *Batch culture*
2. *Continuous culture*
3. *Synchronous culture* and
4. *Fed-batch culture*

1. Batch Culture

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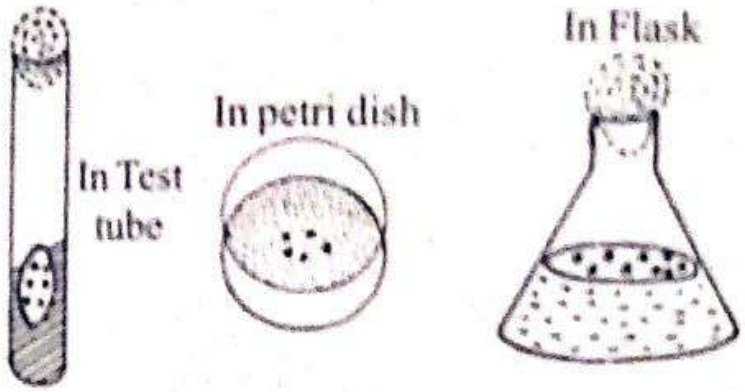

Fig. 38.1: Batch culture.

In this method, the microorganism is grown on a ***limited*** amount of medium containing all ...nutrients at optimum environmental conditions.

The vessel used in the culture is known as ***fermentor*** or ***bioreactor***. The vessels used may ...a test tube or a petri dish or a conical flask.

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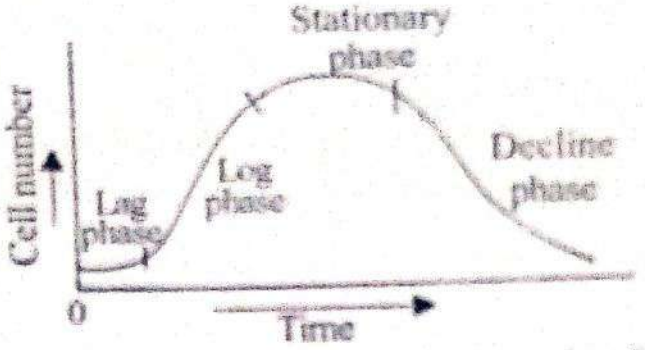

Fig.38.2: Typical bacterial growth curve in batch culture.

In the ***log phase***, the cells divide rapidly at a constant rate. The cells divide in a geometric progression.

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In the ***stationary phase***, the growth slows down. The cells begin to die. There is a balance between dead and newly formed cells.

In the ***decline phase***, the cells die continuously. The death is due to the exhaustion of nutrients and the accumulation of toxic by products.

The growth stages of batch culture can be represented in the form of a curve called ***growth curve.***

2. Continuous Culture

Continuous culture refers to the *growth of the microorganism in a medium at a constant rate continuously.*

Continuous culture is possible when the nutrient is supplied continuously and the toxic by products and dead cells are removed regularly.

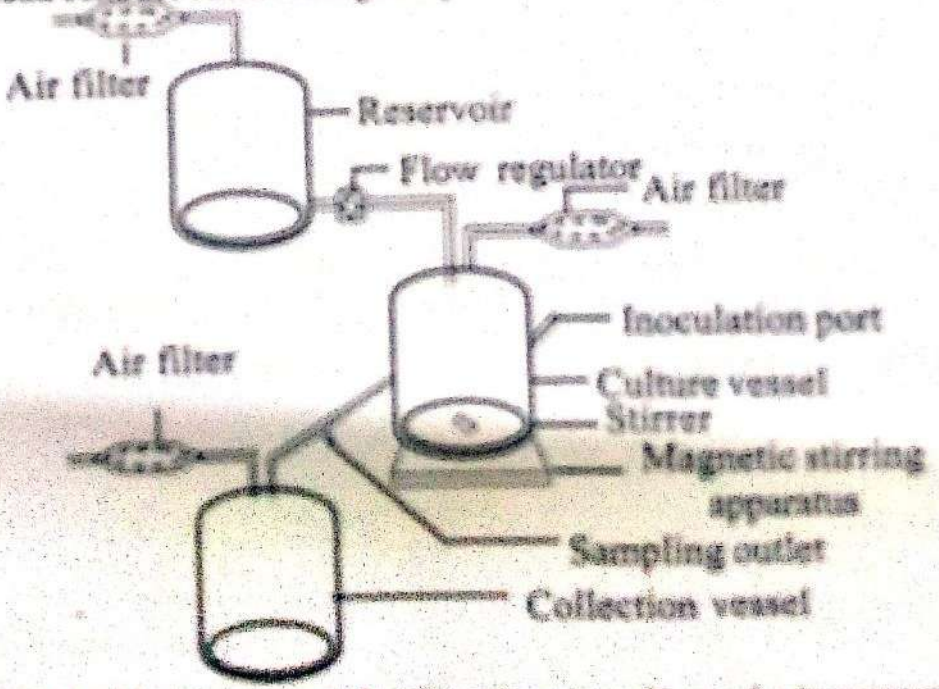

Fig.38.3: Working principle of continuous culture of microorganisms.

4.2

Bacterial Reproduction and Growth

Most bacteria reproduce by an asexual process called **binary fission.** In this sequence of events, the chromosome duplicates, the cell elongates, and the plasma membrane pinches inward at the center of the cell. When the nuclear material has been evenly distributed, the cell wall thickens and expands inward to separate the dividing cell. No mitotic structures (e.g., spindle, aster) are present as in eukaryotic cells. However, in 1997, researchers demonstrated that two different proteins appear to guide the two chromosomes to opposite poles of the cell.

Reproduction by binary fission lends a certain immortality to bacteria because there is never a moment at which the first bacterium has died. Bacteria mature, undergo binary fission, and are young again. It is conceivable that the original bacterium, though billions of years old, is still among us.

Once the division is complete, bacteria grow and develop the features that make each species unique. The interval of time until the completion of the next division is known as the **generation time** (or **doubling time**). In some bacteria, the generation time is very short; for others, it is quite long. For example, for *Staphylococcus aureus*, the generation time is about 30 minutes; for *Mycobacterium tuberculosis*, the agent of tuberculosis, it is approximately 18 hours; and for the syphilis spirochete, *Treponema pallidum*, it is a long 33 hours. The generation time helps determine the amount of time that passes before disease symptoms appear in an infected individual.

Generation time: the time period that passes between binary fissions in bacteria.

One of the most remarkable generation times is the 20 minutes for *Escherichia coli* growing under optimal conditions. If you were to begin with a single rod at 8:00 A.M. this morning, two would be present by 8:20, four by 8:40, and eight by 9:00 A.M. You would have sixty-four rods by 10:00 A.M. and 512 by 11:00 A.M. By 6:00 tonight, the culture would contain just over a billion rods. Indeed, one enterprising mathematician has calculated that if binary fission were to continue for 36 hours, or until 8:00 tomorrow night, there would be enough bacteria to cover the face of the Earth!

esh'er-ik'e-a

Fortunately, the reproductive potential of a bacterium is never realized because of the limitations of the external environment. Thus, we need never worry about being smothered with bacteria. Apparently bacteria are subject to the same controls as all other organisms on Earth, as we shall see next.

THE BACTERIAL GROWTH CURVE

A typical **growth curve** for a population of bacterial cells (FIGURE 4.14) illustrates some of the dynamics that affect the population over the course of time. The population's history may begin when several bacteria enter the human respiratory tract or are transferred to a tube of growth medium in the laboratory. Four distinct phases of the curve are recognized: the lag phase, the logarithmic phase, the stationary phase, and the decline phase.

Growth medium: material for the cultivation of microorganisms.

The **lag phase** encompasses the first few hours of the curve. During this time, bacteria adapt to their new environment. In the respiratory tract, scavenging white blood cells may engulf and destroy some bacteria; in growth media, some organisms may die from the shock of transfer or the inability to adapt to the new environment. However, the biochemical activity in the remaining bacteria is intense as they store nutrients, synthesize enzymes, and prepare for binary fission. The curve remains at a plateau, balanced by reproduction in some cells and death in others.

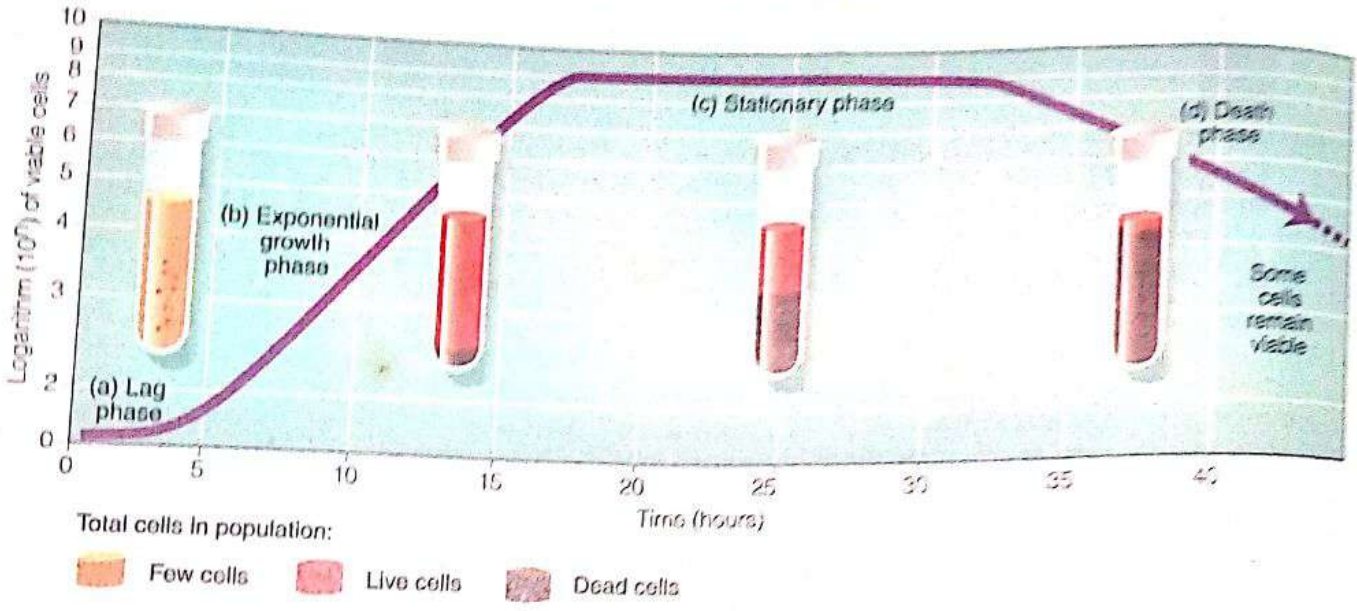

FIGURE 4.14

The Growth Curve for a Bacterial Population

(a) During the lag phase, the population numbers remain stable as bacteria prepare for division. (b) During the logarithmic (exponential growth) phase, the numbers double with each generation time. Environmental factors later lead to cell death, and (c) the stationary phase shows a stabilizing population. (d) The decline phase is the period during which cell death becomes substantial.

Logarithmic phase: the phase of a bacterial growth curve at which reproduction and growth are at their highest rates.

The population then enters an active stage of growth called the **logarithmic phase** (or log phase); the term **exponential growth phase** is also used. The mass of each cell increases rapidly, and reproduction follows. As each generation time passes, the number of bacteria doubles, and the graph rises in a straight line if logarithms (powers of 10) of the numbers are used for the curve. However, a J-shaped curve develops if the actual numbers are used (FIGURE 4.15).

In humans, disease symptoms usually develop during the log phase because the bacteria and their toxins are causing tissue damage. Coughing or fever may occur, and fluid may enter the lungs if the air sacs are damaged. If the bacteria produce toxins, tissue destruction may become apparent. But vulnerability to antibiotics is also highest at this stage. In the laboratory, the population growth may be so vigorous that visible colonies appear on solid media, each colony consisting of millions of organisms (FIGURE 4.16). Broth media may become cloudy with growth. Because the population is at its biochemical optimum, research experiments are generally performed during the log phase.

Stationary phase: the phase of a bacterial growth curve at which the reproduction rate equals the death rate.

After some hours or days, the vigor of the population changes and, as the reproductive and death rates equalize, the population enters another plateau, the **stationary phase**. In the respiratory tract, antibodies from the immune system are attacking the bacteria, and phagocytosis by white blood cells adds to their destruction. Perhaps the person was given an antibiotic to supplement the body's defensive measures. In the culture tube, nutrients have become scarce, waste products have accumulated, and factors such as oxygen and water are in short supply.

If these conditions continue, the external environment will exert its limiting powers on the population and the **decline phase** (or **exponential death phase**) will

charge, the dye is attracted to the cytoplasm, where staining takes place. FIGURE 3.13 illustrates this principle.

The **negative stain technique** works in the opposite manner (Figure 3.13). Bacteria are mixed on a slide with an **acidic dye** such as nigrosin (a black stain) or Congo red (a red dye). The mixture is then smeared across the face of the slide and allowed to air-dry. Because the acidic dye carries a negative charge, it is repelled by the cytoplasm. The stain gathers around the negatively charged cells, and the microscopist observes clear or white cells on a colored background. Since this technique avoids chemical reactions and heat fixing, the cells appear less shriveled and less distorted and are closer to their natural condition.

Gram stain technique: a staining procedure that differentiates bacteria into two separate groups, Gram-positive and Gram-negative.

The **Gram stain technique** allows us to view stained cells while learning something about them. The technique is named for Christian Gram, the Danish physician who first suggested its use in 1884. It is a differential technique because it differen-

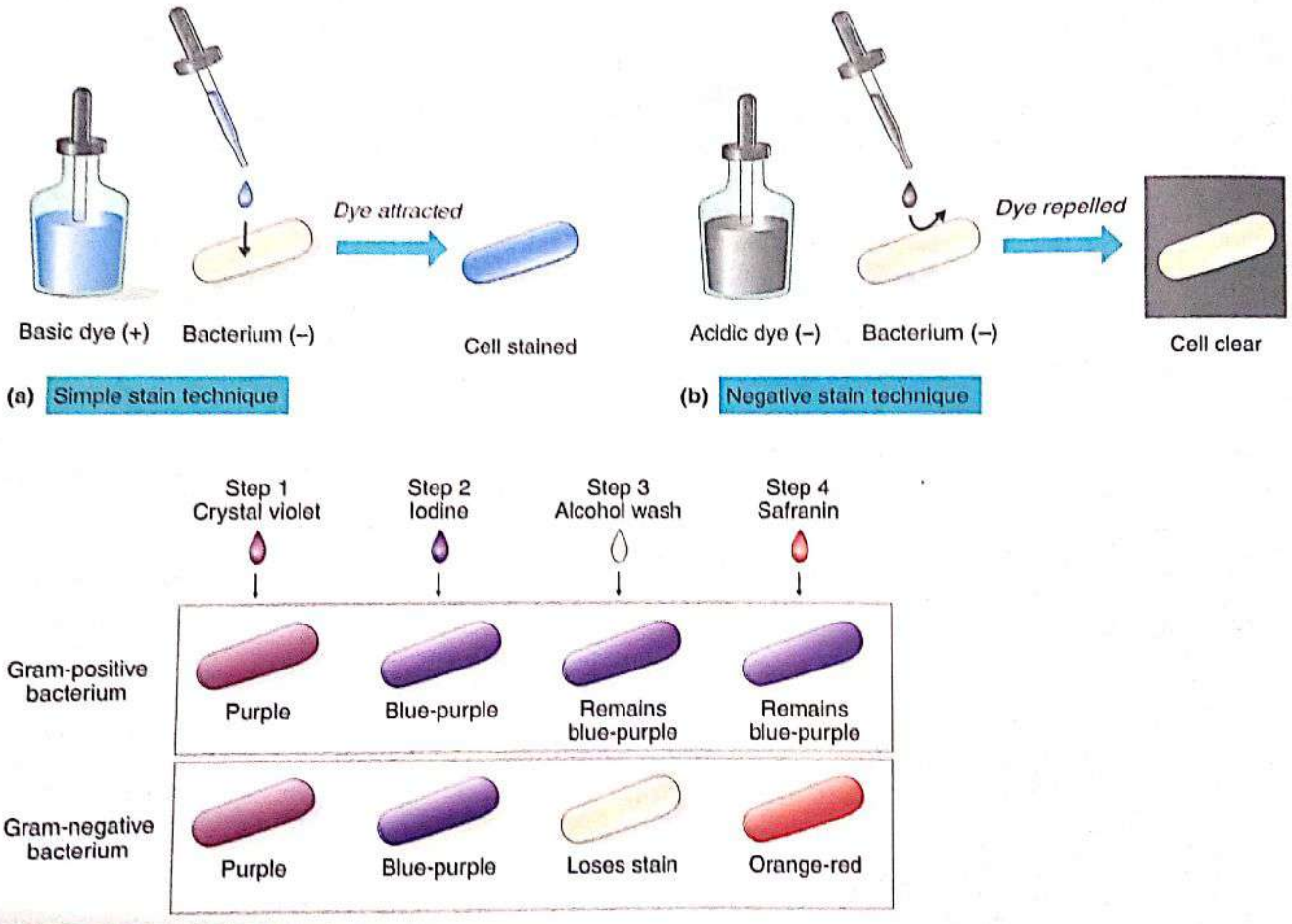

FIGURE 3.13

Important Staining Reactions in Microbiology

(a) In the simple stain technique, the positive-charged stain is attracted to the negative-charged bacteria, and staining takes place.
(b) With the negative stain technique, negative-charged dye is repelled by the bacteria, and the cells remain clear on a dark background.
(c) The Gram stain technique is a differential procedure. All bacteria stain with the crystal violet and iodine, but only Gram-negative bacteria lose the color when alcohol is applied. Subsequently, these bacteria stain with the safranin dye. Gram-positive bacteria remain blue-purple.

MicroFocus 3.5

"A.O. MEANS WHAT?"

In modern bacteriology laboratories, the crystal violet solution used for Gram staining is prepared by mixing solid dye particles with ammonium oxalate. This procedure has not changed since 1929, when a graduate student named Thomas Hucker introduced it. How this "Hucker modification" came about is part of the folklore of microbiology.

Hucker was studying bacteriology at Yale University. Early in 1929, his advisor suggested that he contact several hospital and university laboratories to see how they were performing the Gram stain technique. Hucker was to report his findings in a paper presentation at an upcoming scientific meeting in Philadelphia. He dutifully sent out a series of letters and learned that the standard procedures were being used at all laboratories—all, that is, except Dartmouth's.

The reply from Dartmouth College piqued his interest. At the time, the usual procedure was to dissolve crystal violet in aniline oil. But Dartmouth bacteriologists apparently were using ammonium oxalate. Hucker tried ammonium oxalate and found that the stain improved with age and gave clearer results. He prepared his paper for the Philadelphia meeting and sent a draft to Dartmouth's biology department with a note of thanks. Soon thereafter he received a phone call from Dartmouth—they had never heard of ammonium oxalate for Gram staining. Hucker was perplexed.

In the days that followed, Hucker learned that a chemist had intercepted his survey letter and sent the reply. In writing out the method for crystal violet preparation, the chemist had read "A.O." on the bottle of stain and assumed that it meant the dye was dissolved in ammonium oxalate. Aniline oil simply did not occur to him. Moreover, he had not bothered to check with the biology department because it was inventory time and other things were on his mind. Thus, a case of badly interpreted bacteriological shorthand led to the Hucker modification. Hucker became famous; the chemist remained anonymous.

tiates bacteria into two groups depending on the results. Certain bacteria are called Gram-positive bacteria; others are Gram-negative.

The first two steps of the technique are straightforward. Air-dried heat-fixed smears are stained with crystal violet (MicroFocus 3.5), then with a special Gram's iodine solution. All bacteria become blue-purple. Next the smear is rinsed with a decolorizer such as 95 percent alcohol or an alcohol-acetone mixture. At this point, certain bacteria lose their color and become transparent. These are the **Gram-negative bacteria.** Other bacteria retain the blue-purple stain. These are the **Gram-positive bacteria.** When safranin, a red dye, is applied to the slide, only the Gram-negative organisms accept the stain. Thus at the technique's conclusion, Gram-positive bacteria are blue-purple while Gram-negative organisms appear orange or red (Figure 3.13). By observing the color of the cells at the conclusion of the process, one may decide the group to which the bacteria belong.

Safranin:
a basic dye with a red color, used in Gram staining.

It is not totally clear why bacteria respond differently to the Gram stain technique. One theory suggests that crystal violet and iodine form a chemical complex in the bacterial cytoplasm. Since Gram-negative bacteria have a high lipid content in their cell walls, some microbiologists maintain that the alcohol dissolves the lipid and allows the crystal violet-iodine complex to leak out of the cytoplasm. Gram-positive bacteria, with less cell wall lipid, are less susceptible to the alcohol's effects. Another theory points to the heavy concentration of peptidoglycan in the cell wall of Gram-positive bacteria. Peptidoglycan, a complex carbohydrate, is thought to trap the crystal violet-iodine complex in its many cross-linkages. Gram-negative bacteria have considerably less peptidoglycan in their cell walls, hence they would trap less of the complex. Note that the words "positive" and "negative" are nothing more than convenient expressions and that electrical charges play a minimal role in Gram staining.

pep'ti-do-gli'kan
Peptidoglycan:
a complex carbohydrate present in the cell walls of bacteria.

Knowing whether an organism is Gram-positive or Gram-negative is important for several reasons. For instance, microbiologists use results from the Gram stain