MORPHOLOGY AND CLASSIFICATION OF BACTERIA

Book · October 2016

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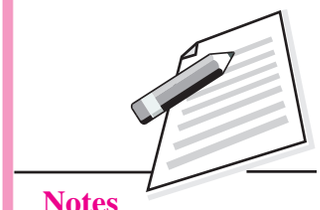

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MORPHOLOGY AND CLASSIFICATION OF BACTERIA

1.1 INTRODUCTION

Microorganisms are a heterogeneous group of several distinct classes of living beings. Based on the difference in cellular organization and biochemistry, the kingdom protista has been divided into two groups namely prokaryotes and eukaryotes. Bacteria and blue-green algae are prokaryotes, while fungi, other algae, slime moulds and protozoa are eukaryotes. Bacteria are prokaryotic microorganisms that do not contain chlorophyll. They are unicellular and do not show true branching, except in higher bacteria like actinomycetales.

OBJECTIVES

After reading this lesson, you will be able to:

- describe the structure of Prokaryotic and Eukaryotic cell
- explain the size of bacteria
- classify bacteria based on the shape and arrangements
- describe the structure of bacterial cell wall
- describe the phases of Growth curve
- explain the factors affecting the growth of bacteria

1.2 PROKARYOTES

The prokaryotic cells have the following characteristics such as

- No organelles, all the action takes place in the cytosol or cytoplasmic membrane

- Most bacteria possess peptidoglycan, a unique polymer that makes its synthesis a good target for antibiotics
- Protein synthesis takes place in the cytosol with structurally different ribosome's

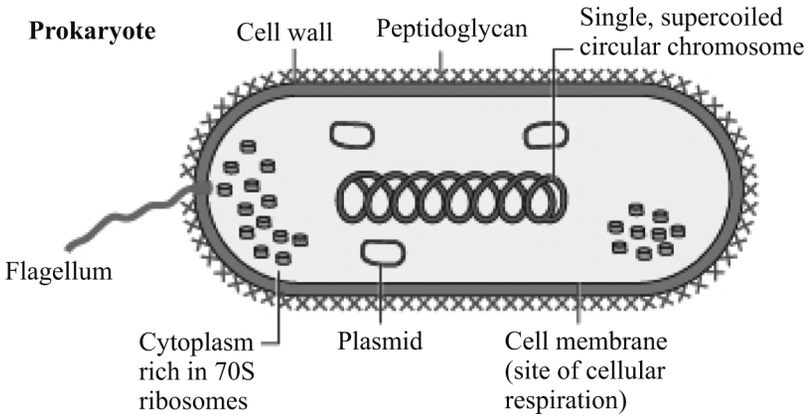


Fig. 1.1: Prokaryote Cell

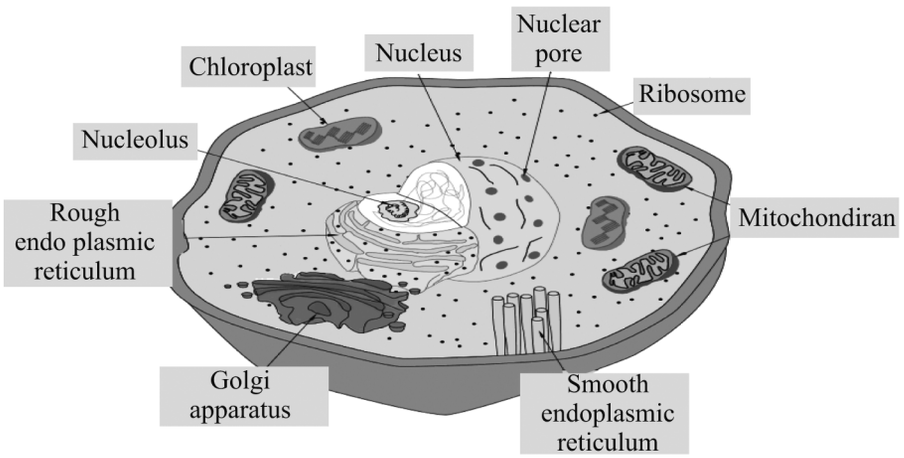
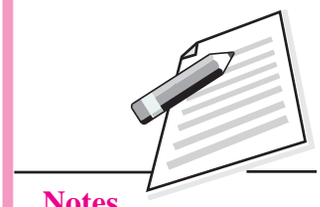


Fig. 1.2: Eukaryote Cell

Difference between Prokaryotic and Eukaryotic Cells

Character	Prokaryotes	Eukaryotes
Nucleus	Absent. No nuclear envelope	Present with nuclear envelope and nucleolus
Membrane-bound organelles	Absent	Present. Includes mitochondria, chloroplasts (plants), lysosomes
Chromosome (DNA)	Single coiled chromosome in cytoplasm 'nucleoid' region in association with 'histone-like' proteins	Multiple linear chromosomes with histone proteins



Cell wall	Eubacteria have a cell wall of peptidoglycan Archaea have cell walls of pseudomurein	No cell wall in animal cellsPlant cell walls = celluloseFungal cell walls = chitin
Mitotic division	Absent	Present
Ribosomes	70S. Free in cytoplasm	80S. Both free in cytoplasm and attached to rough E.R.70S in mitochondria and chloroplasts
Flagella	when present consist of protein flagellin	consist of 9+2 arrangement of microtubules
Cytoplasmic membrane lipids	Eubacteria= Fatty acids joined to glycerol by ester linkageArchaea= Hydrocarbons joined to glycerol by ether linkage	Fatty acids joined to glycerol by ester linkage
Mitochondria	Absent	Present
Lysosomes	Absent	Present
Golgi apparatus	Absent	Present
Endoplasmic Reticulum	Absent	Present

1.3 BACTERIA

The major characteristics of Bacteria are based on their size, shape and arrangements

1.3.1 Size

The unit of measurement used in bacteriology is the micron (micrometer)

1 micron (µ) or micrometer (µm)	– one thousandth of a millimeter
1 millimicron (mµ) or nanometer (nm)	– one thousandth of a micron or one millionth of a millimeter
1 Angstrom unit (Å)	– one tenth of a nanometer

The limit of resolution with the unaided eye is about 200 microns. Bacteria are smaller which can be visualized only under magnification. Bacteria of medical importance generally measure 0.2 – 1.5 µm in diameter and about 3-5 µm in length.

Notes

1.3.2 Microscopy

The morphological study of bacteria requires the use of microscopes. Microscopy has come a long way since Leeuwenhoek first observed bacteria using hand-ground lenses.

The types of microscope are

(i) Light or optical microscope

(ii) Phase contrast microscope

(iii) Dark field/ Dark ground microscope

(iv) Electron microscope

Light or optical microscope

They are of two types namely Simple and Compund Microscope

- Simple Microscope consists of a single lens. A hand lens is an example of a simple Microscope.
- Compound Microscope consists of two or more lenses in series. The image formed by the first lens is further magnified by another lens.

Bacteria may be examined under the compound microscope, either in the living state or after fixation and staining. Examination of wet films or hanging drops indicates the shape, arrangements, motility and approximately size of the cells. But due to lack of contrast details cannot be appreciated.

Phase contrast microscope

This imposes the contrast and makes evident the structure within the cells that differ in thickness or refractive index. The difference in the refractive index between bacteria cells and the surrounding medium makes them clearly visible. Retardation, by a fraction of a wavelength, of the rays of light that pass through the object, compared to the rays passing through the surrounding medium, produces phase difference between the two types of rays.

Dark field / Dark ground microscope

Another method of improving the contrast is the dark field microscope in which reflected light is used instead of the transmitted light used in the ordinal microscope. The contrast gives an illusion of increased resolution, so that very slender organisms such as spirochete, not visible under ordinary illumination, can be clearly seen under the dark field microscope.

Electron Microscope

Beams of electron are used instead of beam of light, used in light microscope. The object which is held in the path of beam scatters the electrons and produces an image which is focused on a fluorescent viewing screen. Gas molecules scatter electron, therefore it is necessary to examine the object in a vacuum.

INTEXT QUESTIONS 1.1

Match the following

	Microscopes		Properties:
1.	Light microscope	(a)	reflected light
2.	Phase contrast microscope	(b)	electron beam
3.	Dark field microscope	(c)	light beam
4.	Electron microscope	(d)	refractive index

1.3.3 Stained Preparations

Live bacteria do not show the structural detail under the light microscope due to lack of contrast. Hence staining techniques are used to produce colour contrast. Routine methods of staining of bacteria involve dying and fixing smears – procedures that kill them. Bacteria have an affinity to basic dyes due to acidic nature of their protoplasm. The commonly used staining techniques are

Simple Stains

Dyes such as methylene blue or basic fuchsin are used for simple staining. They provide colour contrast, but impart the same colour to all bacteria.

Negative Staining

Bacteria are mixed with dyes such as Indian ink or nigrosin that provide a uniformly coloured background against which the unstained bacteria stand out in contrast. Very slender bacteria like spirochetes that cannot be demonstrated by simple staining methods can be viewed by negative staining.

Impregnation Methods

Cells and structures too thin to be seen under ordinary microscope may be rendered visible if they are impregnated with silver on the surface. These are used for demonstration of spirochetes and bacterial flagella.

Notes

Differential Stains

These stains impart different colours to different bacteria or bacterial structures, the two most widely used differential stains are the Gram stain and Acid fast stain. The gram stain was devised by histologist Christian Gram as a method of staining bacteria in tissues.

Gram positive cells are simpler chemical structure with a acidic protoplasm. It has a thick peptidoglycan layer. Teichoic acids are intertwined among the peptidoglycan and the teichoic acids are the major surface antigen determinants

Gram negative cells are more complex, they are rich in lipids. The membrane is bilayered as phospholipids, proteins and lipopolysaccharide. Lipopoly-saccharides (LPS) are also known as endotoxin. Gram negative cells have a peptidoglycan layer which is thin and formed by just one or two molecules. No Teichoic acids are found in the cell wall of Gram negative bacteria. The Outer membrane has Lipopolysaccharide channels with porins which transfer the solutes across. Lipoprotein cross link outer membrane and peptidoglycan layer

Gram reaction may be related to the permeability of the bacterial cell wall and cytoplasmic membrane to the dye-iodine complex, the Gram-negative, but not the Gram-positive cells, permitting the outflow of the complex during decolourisation. Gram staining is an essential procedure used in the identification of bacteria and is frequently the only method required for studying their morphology.

The acid fast stain was discovered by Ehrlich, who found that after staining with aniline dyes, tubercle bacilli resist decolourisation with acids. The method as modified by Ziehl and Neelsen, is in common use now.

INTEXT QUESTIONS 1.2

Match the following:

1. Simple stain	(a) Silver
2. Negative stain	(b) acids
3. Impregnation method	(c) iodine complex
4. Acid fast stain	(d) Methylene blue
5. Gram stain	(e) Indian ink

1.4 SHAPE OF THE BACTERIA

Depending on their shape, bacteria are classified into several varieties

1. Cocci (from kokkos meaning berry) are spherical or oval cells

2. Bacilli (from baculus meaning rod) are rod shaped cells
3. Vibrios are comma shaped curved rods and derive their name from their characteristics vibratory motility.
4. Spirilla are rigid spiral forms.
5. Spirochetes (from speira meaning coil and chaite meaning hair) are flexuous spiral forms
6. Actinomycetes are branching filamentous bacteria, so called because of a fancied resemblance to the radiating rays of the sun when seen in tissue lesions (from actis meaning ray and mykes meaning fungus)
7. Mycoplasmas are bacteria that are cell wall deficient and hence do not possess a stable morphology. They occur as round or oval bodies and as interlacing filaments.

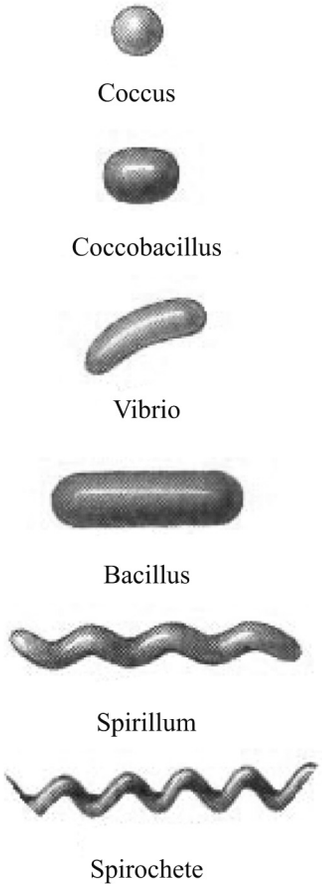


Fig. 1.3: Shapes of bacteria.

INTEXT QUESTION 1.3

Match the following:

1. Bacilli	(a) coma
2. Cocci	(b) flexous spiral form
3. Vibrio	(c) rigid spiral form
4. Sprillum	(d) rod shaped
5. Spirochetes	(e) spherical shaped

Bacteria sometime show characteristic cellular arrangement or grouping. According to the plane of cellular division, cocci may be arranged in pairs (diplococci), chains (streptococci), groups of four (tetrads) or eight (sarcina), or grape like clusters (staphylococci).

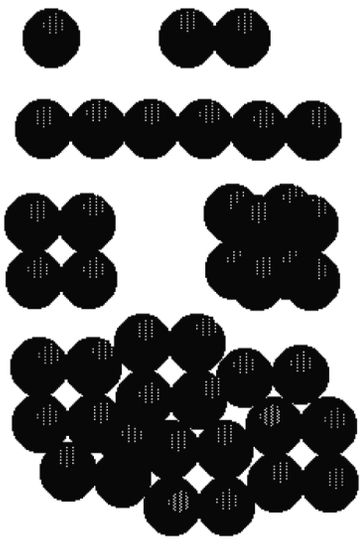


Fig. 1.4: Arrangement of Cocci.

INTEXT QUESTIONS 1.4

Match the following:

1. Diplococci	(a) groups of four
2. Streptococci	(b) groups of eight
3. Tetrads	(c) occurs in pairs
4. Sarcina	(d) grape like clusters
5. Staphylococci	(e) occurs in chains

1.5 BACTERIAL STRUCTURE

The outer layer or cell envelope consists of two components, a rigid cell wall and beneath it a cytoplasmic or plasma membrane. The cell envelope encloses the protoplasm, comprising the cytoplasm, cytoplasmic inclusions such as ribosomes and mesosomes, granules, vacuoles and the nuclear body.

Cell wall

Beneath the external structures is the cell wall. It is very rigid & gives shape to the cell. Its main function is to prevent the cell from expanding & eventually bursting due to water uptake. Cell Wall constitutes a significant portion of the dry weight of the cell and it is essential for bacterial growth & division. The cell wall cannot be seen by direct light microscopy and does not stain with simple

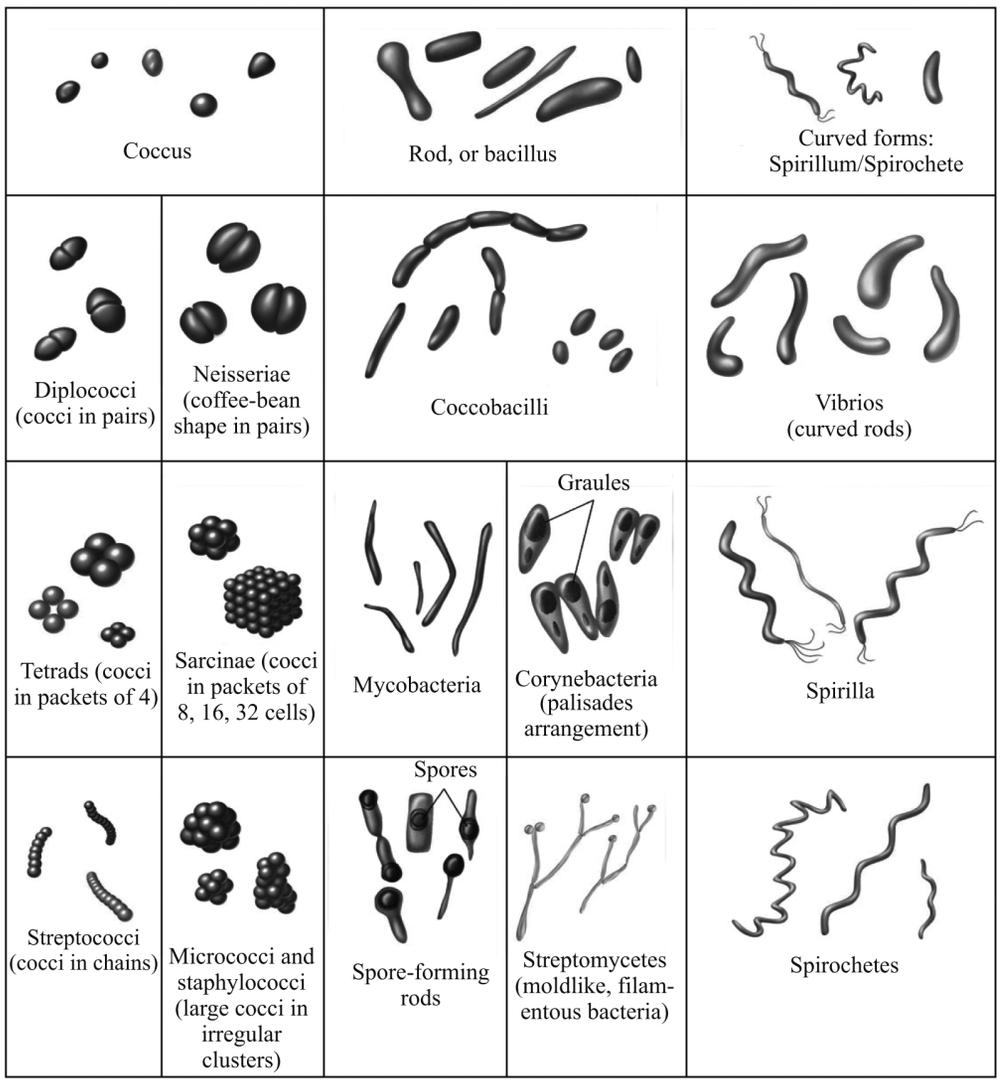


Fig. 1.5

stains. It may be demonstrated by microdissection, reaction with specific antibodies, mechanical rupture of the cell, differential staining procedures or by electron microscopy.

Chemically the cell wall is composed of peptidoglycan. Mucopeptide (peptidoglycan or murien) formed by N acetyl glucosamine & N acetyl muramic acid alternating in chains, cross linked by peptide chains. Embedded in it are polyalcohol called Teichoic acids. Some are linked to Lipids & called Lipoteichoic acid. Lipotechoic acid link peptidoglycan to cytoplasmic membrane and the peptidoglycan gives rigidity.

The functions of Teichoic acid are

- gives negative charge
- major antigenic determinant
- transport ions
- anchoring
- external permeability barrier

Characteristics	Gram Positive	Gram Negative
Thickness	Thicker	Thinner
Variety of amino acids	Few	Several
Lipids	Absent	Present
Teichoic acid	Present	absent

Outer Membrane

Outer membrane is found only in Gram-negative bacteria, it functions as an initial barrier to the environment and is composed of lipopolysaccharide (LPS) and phospholipids

Lipopolysaccharide (LPS)

The LPS present on the cell walls of Gram-negative bacteria account for their endotoxic activity and antigen specificity.

A bacterium is referred as a protoplast when it is without cell wall. Cell wall may be lost due to the action of lysozyme enzyme, which destroys peptidoglycan. This cell is easily lysed and it is metabolically active but unable to reproduce.

A bacterium with a damaged cell wall is referred as spheroplasts. It is caused by the action of toxic chemical or an antibiotic, they show a variety of forms and they are able to change into their normal form when the toxic agent is removed, i.e. when grown on a culture media

Cytoplasmic membrane

Cytoplasmic membrane is present immediately beneath the cell wall, found in both Gram positive & negative bacteria and it is a thin layer lining the inner surface of cell wall and separating it from cytoplasm. It acts as a semipermeable membrane controlling the flow of metabolites to and from the protoplasm.

Cytoplasm

The cytoplasm is a Colloidal system containing a variety of organic and inorganic solutes containing 80% Water and 20% Salts, Proteins. They are rich in ribosomes, DNA & fluid. DNA is circular and haploid. They are highly coiled with intermixed polyamines & support proteins. Plasmids are extra circular DNA.

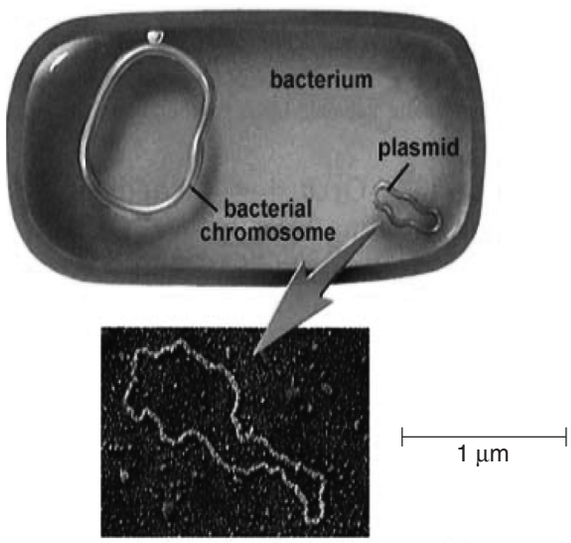


Fig. 1.6

Ribosomes

They are the centers of protein synthesis. They are slightly smaller than the ribosomes of eukaryotic cells

Mesosomes

They are vesicular, convoluted tubules formed by invagination of plasma membrane into the cytoplasm. They are principal sites of respiratory enzymes and help with cell reproduction

Cytoplasmic Inclusions

The Inclusion bodies are aggregates of polymers produced when there is excess of nutrients in the environment and they are the storage reserve for granules,

Notes

phosphates and other substances. Volutin granules are polymetaphosphates which are reserves of energy and phosphate for cell metabolism and they are also known as metachromatic granules.

Nucleus

The Nucleus is not distinct and has no nuclear membrane or nucleolus and the genetic material consist of DNA. The cytoplasmic carriers of genetic information are termed plasmids or episomes.

Capsule

Capsule is the outer most layer of the bacteria (extra cellular). It is a condensed well defined layer closely surrounding the cell. They are usually polysaccharide and if polysaccharide envelops the whole bacterium it is capsule and their production depends on growth conditions. They are secreted by the cell into the external environment and are highly impermeable. When it forms a loose mesh work of fibrils extending outward from the cell they are described as glycocalyx and when masses of polymer that formed appear to be totally detached from the cell and if the cells are seen entrapped in it are described as slime layer.

The Capsule protects against complement and is antiphagocytic. The Slime layer & glycocalyx helps in adherence of bacteria either to themselves forming colonial masses or to surfaces in their environment and they resists phagocytosis and desiccation of bacteria.

Flagella

Flagella are long hair like helical filaments extending from cytoplasmic membrane to exterior of the cell. Flagellin is highly antigenic and functions in cell motility. The location of the flagella depends on bacterial species as polar situated at one or both ends which swims in back and forth fashion and lateral at along the sides.

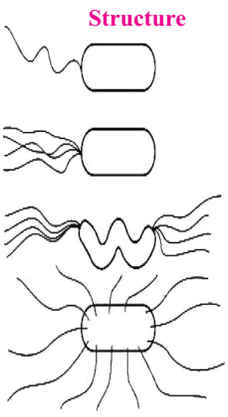
The parts of flagella are the filament, hook and the basal body. Filament is external to cell wall and is connected to the hook at cell surface, the hook & basal body are embedded in the cell envelope. Hook & filament is composed of protein subunits called as flagellin. Flagellin is synthesized within the cell and passes through the hollow centre of flagella. The arrangement of flagella may be described as

(i) Monotrichous – single flagella on one side

(ii) Lophotrichous – tuft of flagella on one side

(iii) Amphitrichous – single or tuft on both sides

(iv) Peritrichous – surrounded by lateral flagella



Structure	Flagella Type	Example
	Monotrichous	Vibrio cholerae
	Lophotrichous	Bartonella bacillifornis
	Amphitrichous	Spirillum serpens
	Peritrichous	Escherichia coli

Fig. 1.7: Flagella.

Various types of mobility is observed because of the presence of the flagella as Serpentine motility is seen with Salmonella, Darting motility with Vibrio and Tumbling motility with Listeria monocytogenes

Pili / Fimbriae

Hair-like proteinaceous structures that extend from the cell membrane to external environment are pili which are otherwise known as fimbriae. They are thinner, shorter and more numerous than flagella and they do not function in motility. The fimbriae is composed of a subunit called pilin.

There are two types pili namely Non-sex pili (Common pili) eg. fimbriae or type IV and the sex pili. The fimbriae are antigenic and mediate their adhesion which inhibits phagocytosis. The sex pili help in conjugation.

INTEXT QUESTIONS 1.5

Match the following:

1. Monotrichous	(a) single or tuft on both sides
2. Lophotrichous	(b) surrounded by lateral flagella
3. Amphitrichous	(c) single flagella on one side
4. Peritrichous	(d) tuft of flagella on one side

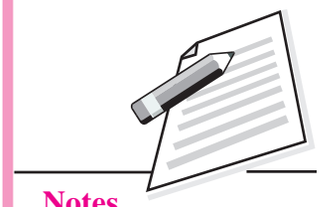
Spore

Some bacteria have the ability to form highly resistant resting stage called spores, which helps them to overcome adverse environmental conditions that are unfavorable for vegetative growth of cell. They are not a reproductive form and

Notes

Characteristics of Bacteria Cell Structures

Structure	Functions(s)	Predominant chemical composition
Flagella	Swimming movement	Protein
Pili		
Sex pilus	Stabilizes mating bacteria during DNA transfer by conjugation	Protein
Common pili or fimbriae	Attachment to surfaces; protection against phagotrophic engulfment	Protein
Capsules (includes "slime layers" and glycocalyx)	Attachment to surfaces; protection against phagocytic engulfment, occasionally killing or digestion; protection against desiccation	Usually polysaccharide; occasionally polypeptide
Cell wall		
Gram-positive bacteria	confers rigidity and shape on cells	Peptidoglycan (murein) complexed with teichoic acids
Gram-negative bacteria	confers rigidity and shape; outer membrane is permeability barrier; associated LPS and proteins have various functions	Peptidoglycan (murein) surrounded by phospholipid protein-lipopolysaccharide "outer membrane"
Plasma membrane	Permeability barrier; transport of solutes; energy generation; location of numerous enzyme systems	Phospholipid and protein
Ribosomes	Sites of translation (protein synthesis)	RNA and protein
Inclusions	Often reserves of nutrients; additional specialized functions	Highly variable; carbohydrate, lipid, protein or inorganic
Chromosome	Genetic material of cell	DNA
Plasmid	Extrachromosomal genetic material	DNA



not a storage granule. These spores are resistant to bactericidal agents and adverse physical conditions. Each spore can give rise to only one endospore which play a role in heat resistance. Spores consists of three layers namely core, cortex and spore coat

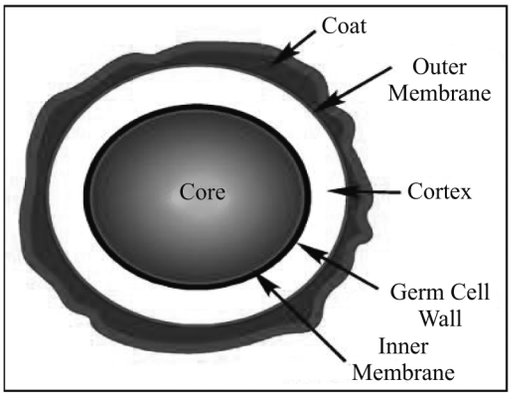


Fig. 1.8: Spere

1.6 GROWTH AND MULTIPLICATION OF BACTERIA

Bacteria divide by binary fission and cell divides to form two daughter cells. Nuclear division precedes cell division and therefore, in a growing population, many cells having two nuclear bodies can be seen. Bacterial growth may be considered as two levels, increase in the size of individual cells and increase in number of cells. Growth in numbers can be studied by bacterial counts that of total and viable counts. The total count gives the number of cells either living or not and the viable count measures the number of living cells that are capable of multiplication.

1.6.1 Bacterial Growth Curve

When bacteria is grown in a suitable liquid medium and incubated its growth follows a definite process. If bacterial counts are carried out at intervals after innoculation and plotted in relation to time, a growth curve is obtained. The curve shows the following phase

(i) Lag phase

Immediately following innoculation there is no appreciable increase in number, though there may be an increase in the size of the cells. This initial period is the time required for adaptation to the new environment and this lag phase varies with species, nature of culture medium and temperature.

(ii) Log or exponential phase

Following the lag phase, the cell starts dividing and their numbers increase exponentially with time.

(iii) Stationary phase

After a period of exponential growth, cell division stops due to depletion of nutrient and accumulation of toxic products. The viable count remains stationary as an equilibrium exists between the dying cells and the newly formed cells.

(iv) Phase of decline

This is the phase when the population decreased due to cell death.

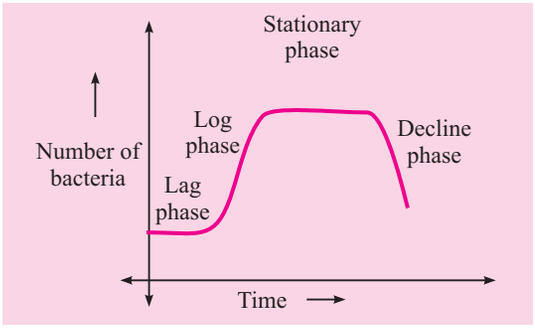


Fig. 1.8: The growth curve of bacteria showing different phases

The various stages of bacterial growth curve are associated with morphological and physiological alterations of the cells. The maximum cell size is obtained towards the end of the lag phase. In the log phase, cells are smaller and stained uniformily. In the stationary phase, cells are frequently gram variable and show irregular staining due to the presence of intracellular storage granules. Sporulation occurs at this stage. Also, many bacteria produce secondary metabolic products such as exotoxins and antibiotics. Involution forms are common in the phase of decline.

1.7 FACTORS THAT AFFECT THE GROWTH OF BACTERIA

Many factors affect the generation time of the organism like temperature, oxygen, carbon dioxide, light, pH, moisture, salt concentration.

Nutrition

The principal constituents of the cells are water, proteins, polysaccharides, lipids, nucleic acid and mucopeptides. For growth and multiplication of bacteria, the minimum nutritional requirement is water, a source of carbon, nitrogen and some inorganic salts.

Bacteria can be classified nutritionally, based on their energy requirement and on their ability to synthesise essential metabolites. Bacteria which derive their energy from sunlight are called phototrophs, those who obtain energy from chemical reactions are called chemotrophs. Bacteria which can synthesise all their organic compounds are called autotrophs and those that are unable to synthesise their own metabolites are heterotrophs.

Some bacteria require certain organic compounds in minute quantities. These are know as growth factors or bacterial vitamins. Growth factors are called essential when growth does not occur in their absence, or they are necessary for it.

Oxygen

Depending on the influence of oxygen on growth and viability, bacteria are divided into aerobes and anaerobes.

Aerobic bacteria require oxygen for growth. They may be obligate aerobes like cholera, vibrio, which will grow only in the presence of oxygen or facultative anaerobes which are ordinarily aerobic but can grow in the absence of oxygen.

Most bacterial of medical importance are facultative anaerobes. Anaerobic bacteria, such as clostridia, grow in the absence of oxygen and the obligate anaerobes may even die on exposure to oxygen. Microaerophilic bacteria are those that grow best in the presence of low oxygen tension.

Carbon Dioxide

All bacteria require small amounts of carbon dioxide for growth. This requirement is usually met by the carbon dioxide present in the atmosphere. Some bacteria like Brucella abortus require much higher levels of carbon dioxide.

Temperature

Bacteria vary in their requirement of temperature for growth. The temperature at which growth occurs best is known as the optimum temperature. Bacteria which grow best at temperatures of 25-40°C are called mesophilic. Psychrophilic bacteria are those that grow best at temperatures below 20°C. Another group of non pathogenic bacteria, thermophiles, grow best at high temperatures, 55-80°C.

The lowest temperature that kills a bacterium under standard conditions in a given time is known as thermal death point.

Notes

Moisture and Drying

Water is an essential ingredient of bacterial protoplasm and hence drying is lethal to cells. The effect of drying varies in different species.

Light

Bacteria except phototrophic species grow well in the dark. They are sensitive to ultraviolet light and other radiations. Cultures die if exposed to light.

H-ion concentration

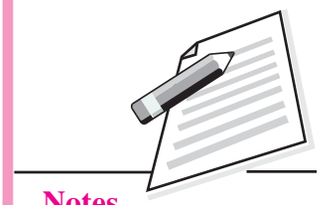
Bacteria are sensitive to variations in pH. Each species has a pH range, above or below which it cannot survive and an optimum pH at which it grows best. Majority of pathogenic bacteria grow best at neutral or slightly alkaline pH (7.2 – 7.6)

Osmotic Effect

Bacteria are more tolerant to osmotic variation than most other cells due to the mechanical strength of their cell wall. Sudden exposure to hypertonic solutions may cause osmotic withdrawal of water and shrinkage of protoplasm called plasmolysis.

WHAT YOU HAVE LEARNT

- Bacteria are prokaryotic microorganism that do not contain chlorophyll
- They are unicellular and do not exhibit true branching.
- The morphological study of bacteria requires the use of microscope like optical or light microscope, phase control microscope, dark/field microscope, electron microscope
- Staining techniques like simple stain, negative stain, impregnation stain, differential stains are used to exhibit structure of bacteria
- Bacteria are classified based on the shape as cocci, bacilli, vibrio, Spirilla. And based on arrangements they are classified as diplococci, streptococci, tetrads, sarcina, staphylococci
- Bacterial cell has cell wall, inner protoplasm and other components
- Bacterial growth phase has a lag phase, log phase, stationary phase and a decline phase



TERMINAL QUESTIONS

1. Describe the structure of cell wall
2. Classify bacteria based on shaped and arrangement with examples
3. Explain the factors affecting the growth of the bacteria
4. Describe growth curve

ANSWERS TO INTEXT QUESTIONS

1.1

1. (c) 2. (d) 3. (a) 4. (b)

1.2

1. (d) 2. (e) 3. (a) 4. (b) 5. (c)

1.3

1. (d) 2. (e) 3. (a) 4. (c) 5. (b)

1.4

1. (c) 2. (e) 3. (a) 4. (b) 5. (d)

1.5

1. (c) 2. (d) 3. (a) 4. (b)