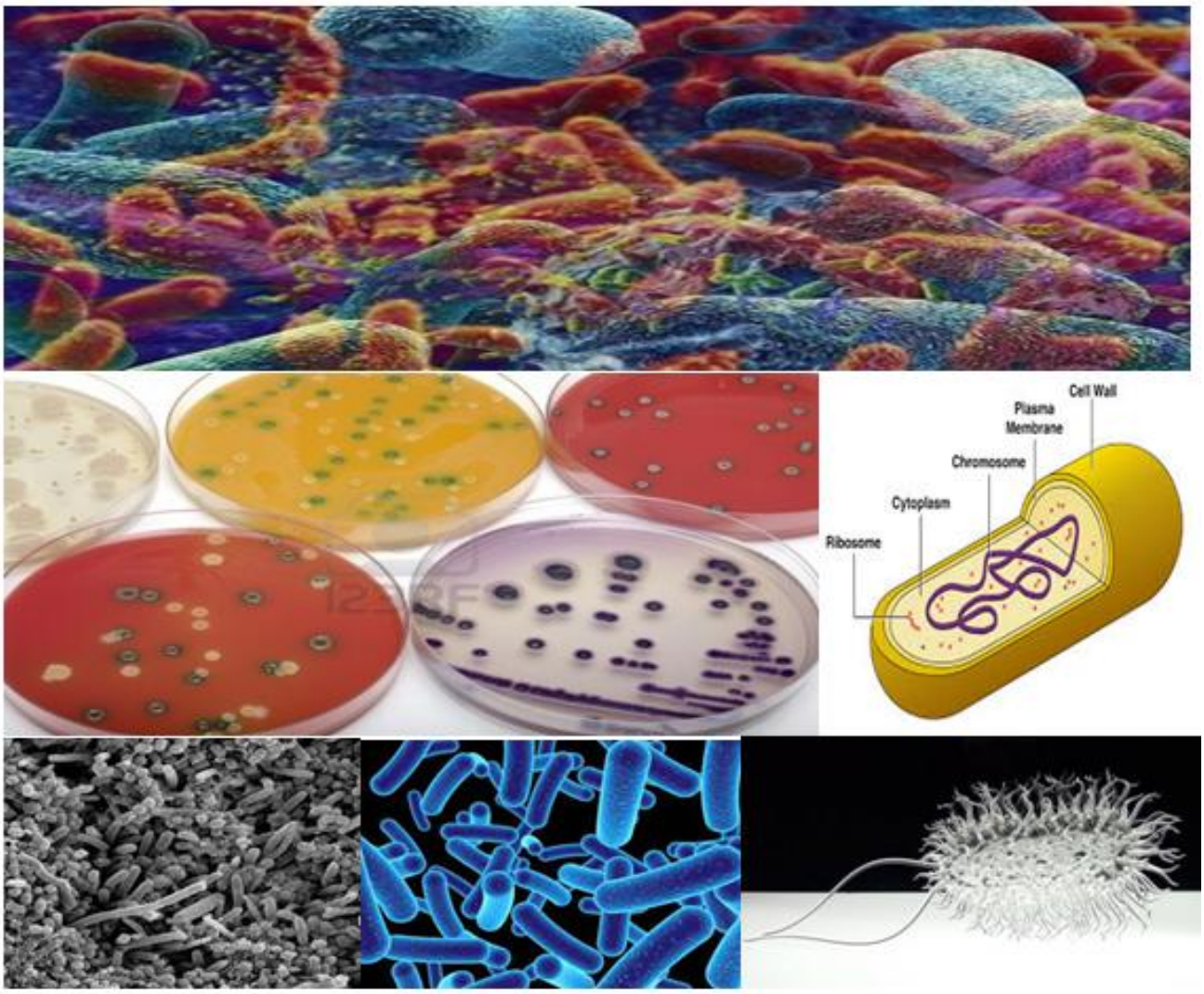
Karnataka State Open University
Mukthagangotri, Mysore-570006

M.Sc. Microbiology

First Semester



MORPHOLOGY AND ULTRASTRUCTURE OF BACTERIA (PROKARYOTES)

COURSE – MB 1.2 **BLOCK – 1, 2, 3 and 4**

Karnataka State Open University
Mukthagangotri, Mysore-570006

M.Sc. Microbiology

Course – MB 1.2

Morphology and Ultrastructure of Bacteria (Prokaryotes)

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WELCOME

MSc in Microbiology

Education has witnessed a rapid and tremendous transformation globally boosting a worldwide demand for online and distance education. Globalization, modern technologies, knowledge explosion, and increased international competition have only fuelled the growing demand for distance mode of education delivery.

Science is defined as the pursuit and application of knowledge and understanding of the natural and social world following a systematic methodology based on evidence. It is a vast field concerning almost everything that our eyes can see or cannot see. Biology is a natural science concerned with the study of life and living organisms. Biology has many sub-categories of which one important subject is Microbiology.

Microbiology is the — 'scientific study of the microorganisms'. It essentially deals with the elaborated investigation of 'microscopic organisms' termed as microbes, that are composed of single cell. Microbiology has grown leaps and bounds widening its horizons and opening new frontiers of knowledge.

There are many learners, both young and old, who could not afford to join the conventional microbiology degree course due to personal and professional responsibilities. This distance education mode of the M.Sc. in Microbiology is specially tailored to cater to those category of students who may not afford to attend full time classes like the employed persons, those who may not have secured admission in regular University/college, those who may have discontinued studied but interested to improve career opportunities and most importantly for those who want to gain knowledge in Microbiology.

Successful students rely on their proficiency to learn and monitor their own learning. In this context, this course has a well-structured set of self learning material customized to learner's capacity and aptitude. This essentially is a self-study course along with required coaching through contact classes. However, the course is modulated to assess the pupils progress through checks involving direct dialogue between the instructors and learners. Laboratory and field work component are designed at regular stages which will add to the experience of the learner.

The scope of microbiology is immense and multifaceted with applications in various fields like agriculture, industry, diary, medicine, forensics, pharmaceutical, clinical, environment, nanotechnology etc. A career in Microbiology holds tremendous scope and a bright future. Most lucrative and best job opportunities await microbiologists. After completion of the course these postgraduates have huge opportunities in various research and development laboratories of hospitals, research organisations, pharmaceutical, food, beverage and chemical industries labs, research institutes, industries, teaching filed etc.,

Come and join us in this comprehensive course and explore what microbiology holds for you in future!

Course – MB 1.2

Morphology and Ultrastructure of Bacteria (Prokaryotes)

Course Introduction

This is a complete, easy-to-use, and highly illustrated study material which provides functional and effective experiences that enable readers to learn, understand, and appreciate microbiology.

This block presents 16 units each of which have been chosen, designed, and arranged to help readers study and familiarize with fundamental aspects of the subject. Each unit provides up to date information stressing the basic principles and complete descriptions of fundamentals. Emphasis is laid on self study exercises, illustrations and diagrams, a valuable reference sources which help learners.

Block MB 1.2 A – This block familiarizes the reader with diversity in size, shape and arrangement of bacterial cells, describes the most common bacterial shapes and variations within these groups. It also gives an insight as how to recognize bacteria based on their size, shape and arrangement of cells. It helps you to understand the structure and function of gram positive and gram negative bacterial cell wall. The details of the differences between gram positive cell wall differs from gram negative cell wall are also covered. The lipopolysacchardies and their functions are discussed. In addition, a general account of archaea bacteria and their adaptation to extreme environment, morphological features of archaea bacteria, classification of archaea, detailed structure and functions of archaeal cell wall, metabolism, nutrition, reproduction, genetics, and applications of archaea are also dealt with.

Block MB 1.2 B – In this block a comprehensive account of structures and function of cell wall and capsule in bacteria is presented. The details of the bacterial cell structure particularly the surface layers and appendages are summarized. A detailed knowledge of the structure and function of the bacterial cell wall, differences between the gram positive and gram negative bacterial cell wall and structure and function of the bacterial cell capsule/slime layer is given. Composition and function of bacterial cell membrane, Fluid-mosaic model of the bacterial

cell membrane and functions of the bacterial cell membrane are discussed. An unit on bacterial nuclear martial provides you with information on the structure and composition of bacterial nuclear material, bacterial genome, bacterial chromosome, bacterial plasmids and their importance. The structure and function of bacterial ribosomes as sites of mRNA translation and protein synthesis, subunits of ribosomes, role of bacterial ribosomes during protein synthesis, process of ribosome genesis and ribosomal assembly mechanisms are also elaborately reviewed.

Block MB 1.2 C – In this block you will study about the **s**tructure and function of bacterial flagella and pili, different types and arrangement of flagella, structure and function of the bacterial flagella and pili and bacterial sex pili and their role in conjugation. A detailed account on vacuoles in bacteria is also covered. The block introduces the reader to different types of inclusion bodies found in bacterial cells like metachromatic granules, poly-β-hydroxybutyrate (PHB) granules, polyglucan granules, sulfur globules, gas vacuoles. A detailed account of poly-β-hydroxybutyrate granules and thier funciton is dealt separately.

Block MB 1.2 D – This block covers topics including miscellaneous types of incusion bodies like cyanophycin granules, carboxysomes, magnetosomes, phycobilisomes, crystals and paracrystalline arrays, chlorosomes and their specific functions. This block gives an understanding of the diversity of pigments produced by bacterial colonies, factors that influence pigmentation in bacteria, applications of bacterial pigments in various fields. This block highlights the fine structure and hydro-dynamics of bacterial flagella including functioning of the bacterial flagella during bacterial motility, flagellar assembly, hydrodynamics of bacterial flagellar movement and functions of the bacterial flagella. A separate unit on bacterial spores and cysts covers endospores structure and function, process of endospore development, specialized kinds of spores and their functions, different kinds of bacterial cysts and their functions

COURSE – MB 1.2

MORPHOLOGY AND ULTRASTRUCUTRE OF BACTERIA (PROKARYOTES)

BLOCK MB 1.2 A

UNIT 1

SIZE, SHAPE AND ARRANGEMENT OF BACTERIAL CELLS

STRUCTURE

1.1. OBJECTIVES

After reading this unit we will learn

- The diversity in shape and size of bacterial cells
- The most common bacterial shapes and variations within these groups.
- Different arrangements of bacterial cells and their colony morphology
- How to recognize bacteria based on their size, shape and arrangement of cells.

1.2. INTRODUCTION

The structure or morphology of bacterial cells includes the shape, size and morphological arrangement. Each bacterial cell/colony has a characteristic morphology i.e., the size, shape and arrangement of bacterial cells which is genetically determined. Bacteria come in a wide and exciting range of sizes and shapes. Bacteria range in size from approximately from about 0.1 to about 600 µm (Fig. 1).

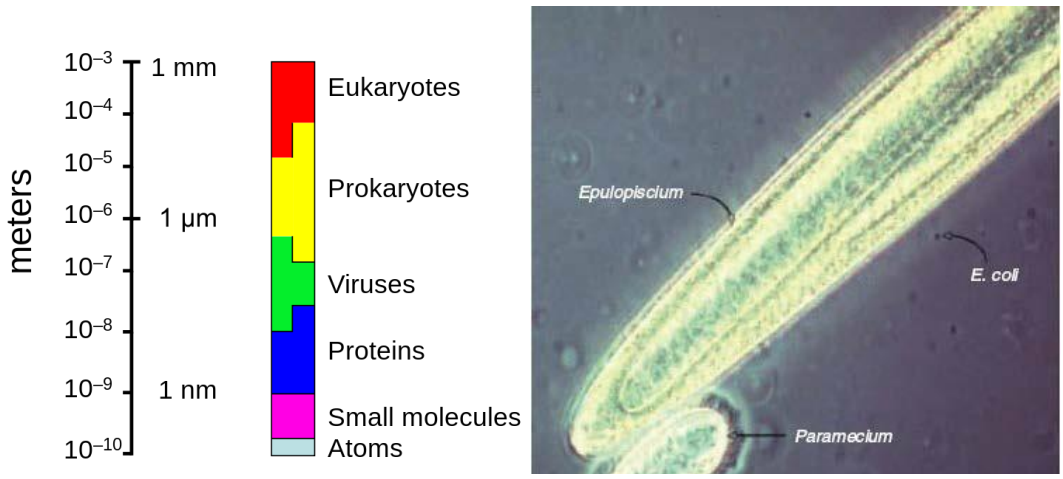


Figure 1: Size of prokaryotes (bacteria) in relation to other organisms and biomolecules. The relative sizes of *Escherichia coli*, *Paramoecium* and *Epulopiscium fishelsonii*

The most common bacterial shapes are rods (bacilli), spheres (cocci) and spiral (twisted). Apart from these common types, there are also bacteria which are square, rectangular, cube, star; net shaped etc., bacterial also exhibit wide variation in their arrangement of cells. They may be found in pairs, groups, clusters, chains,

masses etc., the shape and arrangement of the bacteria is also related to their age and external environment.

1.3. SIZE OF BACTERIA

The unit of measurement for bacterial cells is Micron or micrometer, μm ($1\mu m=10^{-3}mm$). Bacteria range in size from approximately from about 0.1 to about 600 μm. Most bacterial cells are 0.2 to 2.0 nm in diameter and 2 to 8 mm in length. Some bacteria are as small as the largest virus and some are so big that they can be viewed by naked eyes. Though bacterial cells are small in size, they have large surface to volume ratio, and hence all the cellular parts are close to the surface and can be quickly reached by nutrients. *Mycoplasma*-100 to 200 nm in diameter, *Escherichia coli*- 1.1 to 1.5 μm m wide by 2.0 to 6.0 μm m long; some spirochetes occasionally reach 500 μm m in length, cyanobacterium *Oscillatoria* is about 7 μm m in diameter Recently a huge bacterium has been discovered in the intestine of the brown surgeonfish, *Acanthurus nigrofuscus*. *Epulopiscium fishelsoni* grows as large as 600 μm by 80 μm, a little smaller than a printed hyphen.

1.4. SHAPE AND ARRANGEMENT OF BACTERIA

Like the variation in size, bacterial also show large variation in their shape and arrangement of cells (Table 1). As a result of binary fission the freshly formed bacteria take up a characteristic shape which varies largely among different species and serves as a tool for bacterial identification. Though bacteria assume several shapes the two most common shapes are the spheres (cocci) and rods (bacilli). Within each of these groups are hundreds of unique variations. Rods may be long, short, thick, and thin, have rounded or pointed ends, thicker at one end than the other etc. Cocci may be large, small, or oval shaped to various degrees. Spiral shaped bacteria may be fat, thin, loose spirals or very tight spirals. Bacteria may exist mainly as single cells or as common grouping such as chains (e.g. *Streptococci*) or clusters of cells (e.g. *Staphylococci*), uneven clusters, pairs, tetrads, octads, masses embedded within a capsule. There are square bacteria, star-shaped bacteria, stalked

bacteria, budding bacteria that grow in net-like arrangements and many other morphologies.

Coccus (plural, cocci): these cells are circular or spherical - E.g., *Staphylococcus epidermidis*

Streptococcus: cocci stringed together/arranged in chains - E.g., *Streptococcus pyogenes*

Staphylococcus: cocci arranged in cluster or bunch - E.g., Staphylococcus *aureus*

Diplococcus: two cocci arranged in pair - E.g., e.g. *Neisseria meningitidis*

Tetrad: a group of four spherical bacterial cells (square shape) - E.g., *Micrococcus* species

Sarcina (plural, sarcinae): a group of eight spherical bacterial cells (cube shape) - E.g., *Sarcina ventriculi*.

Bacillus (plural, bacilli): rod shaped bacterial cell - E.g., *Bacillus* species

Diplobacillus: two rod shaped bacteria arranged in a pair. E.g., *Diplobacillus liquefaciens*

Streptobacillus: rod shaped bacteria arranged in short/long chains - E.g., *Bacillus subtilus*

Coccobacillus: Intermediate shape between coccus and bacillus. Oval rods. - E.g., *Brucella abortus*

Coryneform bacillus: A bacterium with irregularly rod-shaped cells arranged at angles to form V- and L-shaped arrangements. E.g., *Coryneform diphtheriae*

Spirillum (plural, spirilla): spiral or helical shaped bacteria - E.g., *Borrelia*

Vibrio: curved or comma-shaped bacteria. - E.g., *Vibrio cholera*

Spirochaete: these are flexible spiral bacteria A bacterium with flexible, spiral-shaped cells. Spirochetes often appear helical or corkscrew-shaped with tapered ends - E.g., *Treponema pallidum*

Star-shaped – These are bacteria which are star shaped- E.g., *Stella*

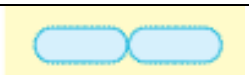
Rectangular - These are bacteria which are rectangular shaped - E.g., *Haloarcula*, a genus of halophilic archaea

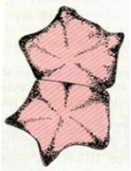
Pleomorphic bacteria: bacteria which have several shapes and also can change shape - E.g., *Corynebacterium*, *Rhizobium*

Monomorphic bacteria: Maintain a single shape. Most bacteria are monomorphic, due to age and environment their shape may be changed.

Table 1: Diversity in shape and arrangement of bacterial cells

Category	Shape	Example	Illustration
Coccus (plural, cocci)	These cells are circular or spherical	*Staphylococcus epidermidis*	
Streptococcus	Cocci stringed together/arranged in chains -	*Streptococcus pyogenes*	
Staphylococcus	Cocci arranged in cluster or bunch -	*Staphylococcus aureus*	
Diplococcus	Two cocci arranged in pair	*Neisseria meningitidis*	

Tetrad	A group of four spherical bacterial cells (square shape)	*Micrococcus* species	
Sarcina (plural, sarcinae)	A group of eight spherical bacterial cells (cube shape)	*Sarcina ventriculi*	
Bacillus (plural, bacilli)	Rod shaped bacterial cell	*Bacillus* species	
Diplobacillus	Two rod shaped bacteria arranged in a pair	*Diplobacillus liquefaciens*	
Streptobacillus	Rod shaped bacteria arranged in short/long chains	*Bacillus subtilus*	
Coccobacillus	Intermediate shape between coccus and bacillus. Oval rods	*Brucella abortus*	
Coryneform bacillus	A bacterium with irregularly rod-shaped cells arranged at angles to form V- and L-shaped arrangements	*Coryneform diphtheriae*	
Spirillum (plural, spirilla)	Spiral or helical shaped bacteria	*Borrelia*	
Vibrio	Curved or comma-shaped bacteria	*Vibrio cholera*	
Spirochaete	These are flexible spiral bacteria A bacterium with flexible, spiral-shaped cells. Spirochetes often appear helical or corkscrew-shaped with tapered ends	*Treponema pallidum*	

Star-shaped	These are star shaped bacteria	*Stella*	
Rectangular	These are rectangular shaped bacteria	*Haloarcula*, a genus of halophilic archaea	
Pleomorphic bacteria	bacteria which have several shapes and also can change shape	*Corynebacterium*, *Rhizobium*	
Monomorphic bacteria	Maintain a single shape	Most bacteria are monomorphic, due to age and environment their shape may be changed	

1.5. SUMMARY

- Bacteria come in a wide and exciting range of sizes and shapes. Bacteria range in size from approximately from about 0.1 to about 600 µm.
- The most common bacterial shapes are rods (bacilli), spheres (cocci) and spiral (twisted).
- Apart from these common types, there are also bacteria which are square, rectangular, cube, star, net shaped etc., bacterial also exhibit wide variation in their arrangement of cells.
- They may be found in pairs, groups, clusters, chains, masses etc., the shape and arrangement of the bacteria is also related to their age and external environment.

- Recently a huge bacterium has been discovered in the intestine of the brown surgeonfish, *Acanthurus nigrofuscus*. *Epulopiscium fishelsoni* grows as large as 600 µm by 80 µm, a little smaller than a printed hyphen.
- Like the variation in size, bacterial also show large variation in their shape and arrangement of cells. As a result of binary fission the freshly formed bacteria take up a characteristic shape which varies largely among different species and serves as a tool for bacterial identification.
- Coccus: spherical bacteria- Streptococcus: arranged in chains - *Streptococcus pyogenes;* Staphylococcus: arranged in cluster - *Staphylococcus aureus;* Diplococcus: arranged in pair - e.g. *Neisseria meningitides;* Tetrad: four spherical bacterial - *Micrococcus* species; Sarcina: a group of eight spherical bacterial cells- (cube shape)- *Sarcina ventriculi*.

- Bacillus: rod shaped bacteria- Diplobacillus: bacilli arranged in a pair; Streptobacillus: bacilli arranged chains - *Bacillus subtilus;* Coccobacillus- Intermediate shape between coccus and bacillus. Oval rods. - *Brucella abortus*
- Coryneform bacillus: A bacterium with irregularly rod-shaped cells arranged at angles to form V- and L-shaped arrangements- *Coryneform diphtheriae*.
- Spirillum: spiral bacteria – *Borrelia;*
- Vibrio-curved bacteria. - *Vibrio cholera;* Spirochaete: flexible spiral bacteria - *Treponema pallidum*
- Star-shaped – e.g., *Stella;* Rectangular - *Haloarcula*,
- Pleomorphic: bacteria which can change shape - *Corynebacterium*, *Rhizobium;* Monomorphic: bacteria that Maintain a single shape.

1.5. CHECK YOUR PROGRESS:

1. Discuss the variation in size of bacterial cells
2. What are the basic shapes of bacterial cells?
3. What are monomorphic and pleomorphic bacteria. Give examples.

4. What are the different arrangements of cocci bacteria?
5. What are the different arrangements in Bacilli bacteria?
6. What are spirochetes? Give examples.
7. What are coryneform bacteria? Give examples.

1.7. KEYWORDS

Size and shape of bacteria, Cocci, Bacilli, Spirillum and Vibrio, arrangement of bacterial cells, monomorphic and pleomorphic bacteria.

1.8. FURTHER SUGGESTED READINGS

1. Veena. 2008. Microbiology. Sonali Publications.
2. Ravi Mantha. 2012. All about bacteria. Collins Publications.
3. Jerome J. Perry, James T. Staley, Stephen Lory. 2002. Microbial Life. Sinauer Associates.
4. Heritage. 2008. Introductory Microbiology. Cambridge University Press.

1.9. SOURCES

1. Alcamo. 2001. Fundamentals of Microbiology Sixth Edition. By, Edward Alcamo. Jones and Bartlett Publishers, London.
2. Purohit, S.S. 2008. Microbiology – Fundamentals and Application. Sixth Edition. Student Edition Publishers, Jodhpur.
3. Stanier, R.Y., Ingraham, J.L., Wheelis, M.L., and Painter, P.R. 2007. General Microbiology Fifth Edition. McMillan Publishers, London.
4. Purohit, S.S. 2006. Microbiology – Fundamentals and Application. Seventh Edition. Agrobios (India) Publishers, Jodhpur.

UNIT 2

STRUCTURE AND FUNCTION OF GRAM POSITIVE BACTERIAL CELL WALL

STRUCTURE

2.1. OBJECTIVES

After reading this section we will learn

- The structure of gram positive cell wall
- The function of gram positive cell wall
- How the gram positive cell wall differs from gram negative cell wall?
- The fine details and composition of gram positive cell wall

2.2. INTRODUCTION

The gram positive bacterial cell wall is relatively thick measuring 15-80 nanometres. Gram positive cell walls have only two layers, the cytoplasmic membrane and an outermost thick layer of peptidoglycan. The Gram-positive cell wall appears as dense layer typically composed of numerous rows of peptidoglycan, molecules of lipoteichoic acid, wall teichoic acid and surface proteins. The cell walls of gram positive bacteria are relatively thick and measure about 20-80 nm in thickness. The cell wall contains more than 50% peptidoglycan (Fig. 1).

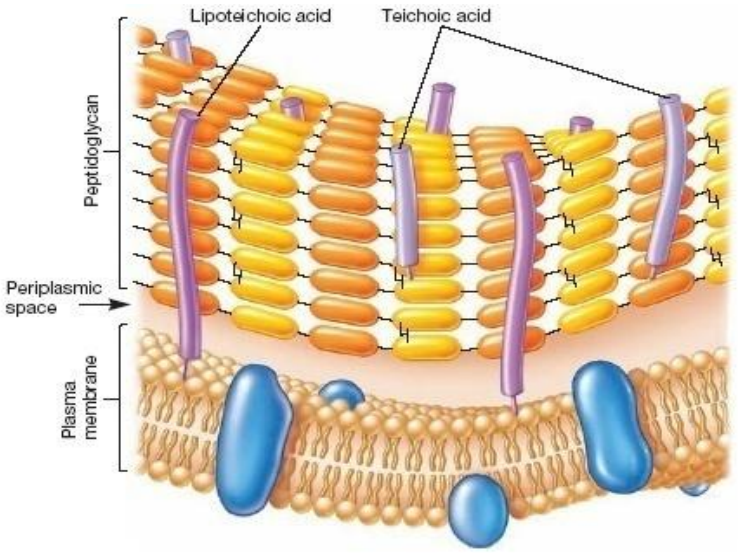


Figure 1: Structural details of a Gram positive bacterial cell wall

The presence of Teichoic acids is typical to gram positive cell walls. The lipid and lipoproteins content is less and accounts to 0-3%. There is no protein and lipopolysaccharide in the gram positive cell walls. Gram positive cell walls are highly sensitive to lyzozymes and some antibiotics. Gram-positive walls sometimes also contain other molecules like polysaccharides, such as the group-specific antigens of streptococci; proteins such as the M protein of group A *Streptococci*.

2.3. CYTOPLASMIC MEMBRANE

Cell membrane or the cytoplasmic membrane is the site of synthesis of DNA, cell wall polymers and membrane lipids. The cell membrane is selectively permeable and plays a role in transportation of solutes inside the cells. The bacterial cell membrane participates in the electron transport and oxidative phosphorylation. The cell wall also takes part in excretion of hydrolytic exoenzymes.

The cytoplasmic membrane is made up of a bilayer of phospholipids. Within his lipid bilayer several proteins are embedded which control what goes in and out of the cell. The proteins may be attached to any one side of the membrane, or to some other proteins of the membrane. Some proteins may occupy the entire span of the membrane on both the sides. Some proteins span the membrane, while others are attached to only one side or the other, or to other proteins embedded in the cytoplasmic membrane.

The cytoplasmic membrane is fluidic and delicate. The inner portion of this membrane is made up of lipid or fat molecules which make different molecules dissolved in water impermeable to enter, however, water molecules and move freely through this layer. One important characteristic of the cytoplasmic membrane is that protons (H+) are unable to cross the cytoplasmic membrane. The inner region of the membrane is hydrophobic. In the bilayered membrane, the lipid molecules form two layered facing each other. The phosphate groups are hydrophilic and positioned on the outer edges of the membrane. Many of the proteins involved in the transportation of molecules into or out of the cells are found embedded in the lipid bilayer. These

proteins act as sponge that absorb the nutrients and allow the cell to live in very dilute nutrient solutions.

2.4. PEPTIDOGLYCAN LAYER

The peptidoglycan layer constitutes 90% of the cell wall. Peptidoglycan layer has two major components, peptidoglycan and teichoic Acids. Additional carbohydrates and proteins are present depending on the species.

2.4.1. Peptidoglycan

Peptidoglycan layer is a linear glycan chain of alternating sugars, *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM). Each muramic acid residue bears a tetrapeptide of alternating l- and d-amino acids. Adjacent glycan chains are cross-linked into sheets by peptide bonds between the third amino acid of one tetrapeptide and the terminal d-alanine of another. The same cross-links between other tetrapeptides connect the sheets to form a three-dimensional, rigid matrix. The crosslinks may involve one third of the tetrapeptides and may be direct or may include a peptide bridge (Fig. 2). E.g., Pentaglycine bridge in *Staphylococcus aureus*.

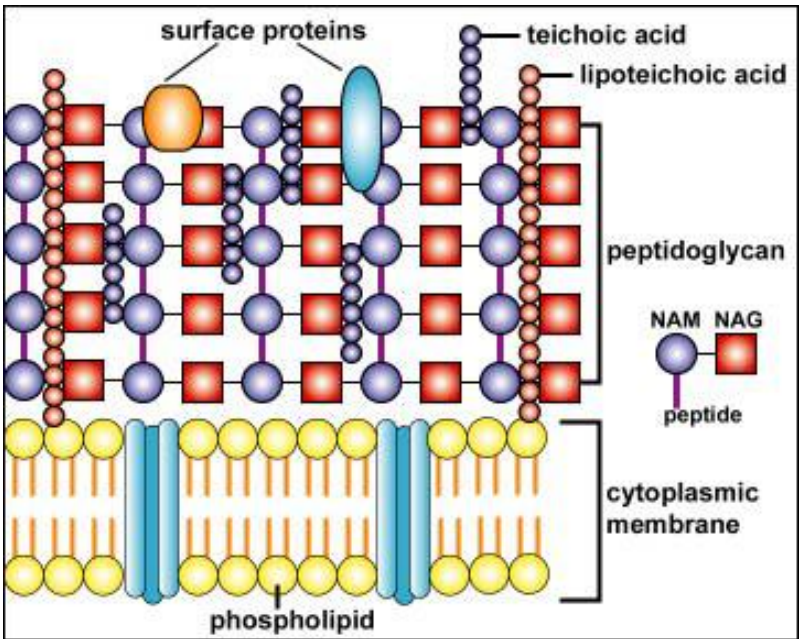


Figure 2: The details of the Peptidoglycan layer of Gram positive bacterial cell wall

Most enzymes fail to degrade peptidoglycan except lysozyme, hydrolytic enzymes found in tears and other secretions. Peptidoglycan layer confers osmotic resistance and shape to the cell. Penicillin destroys peptidoglycan sac resulting in cell lysis.

2.4.2. Teichoic acids

Gram positive cell wall has the characteristic Teichoic acids which are water soluble polymers of glycerol or ribitol linked by phosphate groups occurring in polymers up to 30 units long (Fig. 3).

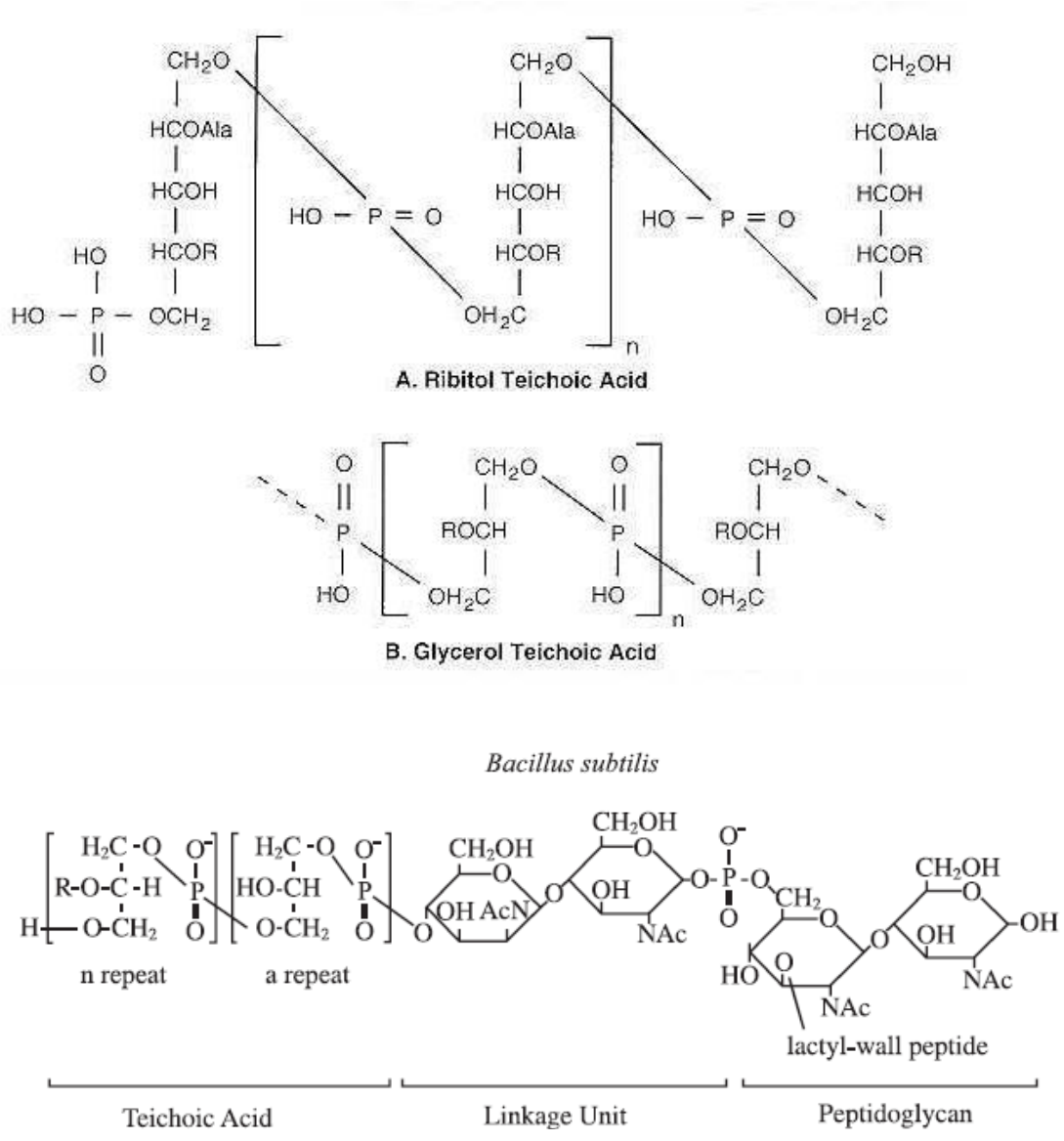


Figure 3: Types of teichoic acids and their linkage with peptidoglycan

Amino acids such as D-alanine are attached. Teichoic acid molecules run perpendicular to the peptidoglycan layer. Teichoic acids bind sugars and amino acids and tend to be covalently linked with the peptidoglycan of the cell wall as well as to plasma membrane lipids. The length, nature and location of the substituent vary from species to species and sometimes among strains within a species. Up to 50% of the wall may be teichoic acid, some of which is covalently linked to occasional NAM residues of the peptidoglycan.

There are two main types of teichoic acid, Ribitol teichoic acids and Glycerol teichoic acids. Further there are two different classes of Teichoic acids 1) Wall teichoic acids which are covalently linked the peptidoglycan and 2) Lipoteichoic acids which are attached to the cytoplasmic membrane. Teichoic acids comprising poly glycerol phosphate which are linked to the glycolipids in the underlying cell membrane are called lipoteichoic acid. Lipoteichoic acids have a fatty acid and are situated in the cytoplasmic membrane. Lipoteichoic acids are important in adhesion of the wall to the cell membrane.

2.4.3. Functions of Teichoic acids

- Teichoic acids are negatively charged and thus the cell wall is also negatively charged.
- Teichoic acids provide rigidity to the cell-wall by drawing cations such as magnesium and sodium.
- Lipoteichoic acids acts similar to the endotoxin of Gram-negative bacteria
- The main function of lipotechoic acids is to bind Mg++ and other divalent ions to the surface of the cell for use in synthetic process of bacterial cell.
- Teichoic acid acts as a chelating agent and facilitates attachment of the bacteriophages to surfaces. Teichoic acids facilitate adherence to tissue surfaces contributing to the pathogenicity E.g. *Streptococci*,
- Teichoic acids also assist in regulation of cell growth by limiting the ability of autolysins to break the β (1-4) bond between the N-acetyl glucosamine and the N-acetylmuramic acid.

- Teichoic acids are employed in serology for distinguishing strains gram-positive bacteria.

2.5. SUMMARY

The cell walls of gram positive bacteria are relatively think and measure about 20-80 nm in thickness. Gram positive cell walls have only two layers, the cytoplasmic membrane and an outermost thick layer of peptidoglycan. The Gram-positive cell wall appears as dense layer typically composed of numerous rows of peptidoglycan, molecules of lipoteichoic acid, wall teichoic acid and surface proteins.

The cytoplasmic membrane is made up of a bilayer of phospholipids. Within his lipid bilayer several proteins are embedded which control what goes in and out of the cell. The proteins may be attached to any one side of the membrane, or to some other proteins of the membrane. Some proteins may occupy the entire span of the membrane on both the sides.

The cytoplasmic membrane is fluidic and delicate. In the bilayered membrane, the lipid molecules form two layered facing each other. The phosphate groups are hydrophilic and positioned on the outer edges of the membrane. Many of the proteins involved in the transportation of molecules into or out of the cells are found embedded in the lipid bilayer. These proteins act as sponge that absorb the nutrients and allow the cell to live in very dilute nutrient solutions.

The peptidoglycan layer constitutes 90% of the cell wall. Peptidoglycan layer has two major components, peptidoglycan and teichoic Acids. Additional carbohydrates and proteins are present depending on the species.

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one tetrapeptide and the terminal d-alanine of another. The same cross-links between other tetrapeptides connect the sheets to form a three-dimensional, rigid matrix.

Gram positive cell wall has the characteristic Teichoic acids which are water soluble polymers of glycerol or ribitol linked by phosphate groups occurring in polymers up to 30 units long. Amino acids such as D-alanine are attached.

Teichoic acid molecules run perpendicular to the peptidoglycan layer. Teichoic acids bind sugars and amino acids and tend to be covalently linked with the peptidoglycan of the cell wall as well as to plasma membrane lipids.

There are two main types of teichoic acid, Ribitol teichoic acids and Glycerol teichoic acids. Further there are two different classes of Teichoic acids 1) Wall teichoic acids which are covalently linked the peptidoglycan and 2) Lipoteichoic acids which are attached to the cytoplasmic membrane.

Teichoic acids provide rigidity to the cell-wall by drawing cations such as magnesium and sodium. Lipoteichoic acids act similar to the endotoxin of Gram-negative bacteria. The main function of lipotechoic acids is to bind Mg++ and other divalent ions to the surface of the cell for use in synthetic process of bacterial cell.

Teichoic acid acts as a chelating agent and facilitates attachment of the bacteriophages to surfaces. Teichoic acids facilitate adherence to tissue surfaces contributing to the pathogenicity E.g. *Streptococci.*

Teichoic acids also assist in regulation of cell growth by limiting the ability of autolysins to break the β (1-4) bond between the N-acetyl glucosamine and the N-acetylmuramic acid.

Teichoic acids are employed in serology for distinguishing strains gram-positive bacteria.

2.6. CHECK YOUR PROGRESS

1. What are the general features of the gram positive bacterial cell wall?
2. What are the unique compositions in the gram positive bacterial cell wall?
3. Explain the structure of the gram positive bacterial cell wall.
4. What is the composition of peptidoglycan layer in gram positive bacterial cell wall?
5. How does the gram positive bacterial cell wall differ from gram negative bacterial cell wall?
6. What are Teichoic acids? What are their functions?
7. What are the functions of gram positive bacterial cell wall?

2.7. KEYWORDS

Gram positive bacterial cell wall, peptidoglycan, lipoteichoic acid, Cytoplasmic membrane, N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), Teichoic acids, serology.

2.8. FURTHER SUGGESTED READINGS

1. Aneja K.R., Jain P. and Aneja R. "*A Text Book of Basic and Applied Microbiology*" New Age International Pub. New Delhi (2008).
3. Ankit Gupta and Prafulla Songara. 2012. Smart Study Series in Microbiology. Elsevier Publishers.
4. Arora, D.R., and Arora B.B. 2012. Textbook of microbiology. Fourth edition. CBS Publishers.
5. Blackwell Science, Darralyn McCall, David Stock. 2001. 11th Hour: Introduction to Microbiology 1st Edition. Blackwell Science
6. Betsey Dexter Dyer. 2003. A field guide to bacteria. Comstock Publishing.
7. Gerhardt P.R., Murray G.E., Costlow R.N., Nester E.W.,Wood E.A., Kreig N.R., and Phillips G.B.(eds) "*Manual of Methods for General Bacteriology.* American Society for Microbiology, Washington D.C. (1981)
8. Hans G Schiegel. 2008. General Microbiology. 7th Edition. Cambridge University Press.

9. Jerome J. Perry, James T. Staley, Stephen Lory. 2002. Microbial Life. Sinauer Associates.
10. Heritage. 2008. Introductory Microbiology. Cambridge University Press.
11. Stanier R.L., Ingram J.L. and Wheelis M.L. "*General Microbiology*" Macmillan Press Ltd (2007)
12. Talaro K.P. and Talaro A. "*Foundations in Microbiology*" 6th edn. McGraw Hill (2006)
13. Tortora, G.J. 2008. Microbiology: An introduction. Ninth Edition. Pearson Publishers.
14. Truper H.G.and Kramer J."Principles of Characertization and Identifiction of Prokaryotes" in Stolp M.P. Truper H.G., Balows A and Schlegal H.G (eds) "*The Prokaryotes: A Handbook on Habitats, Isolation and Identification of Bacteria*" Springer-Verlag (1981)

2.9. SOURCES

1. Alcamo. 2001. Fundamentals of Microbiology Sixth Edition. By, Edward Alcamo. Jones and Bartlett Publishers, London.
2. Arthur L. Koch. 2007. The Bacteria: Their Origin, Structure, Function and Antibiosis. Springer.
3. James T. Drummond, David White, Clay Fuqua. 2011. The Physiology and Biochemistry of Prokaryotes 0004 Edition. Oxford University Press, USA
4. Pelczar M.J., Chan E.C.S. and Kreig N.R. "*Microbiology* – 5th edn., Tata McGraw-Hill Pub. Co. New Delhi (1986)
5. Purohit, S.S. 2008. Microbiology – Fundamentals and Application. Sixth Edition. Student Edition Publishers, Jodhpur.
6. Meena Kumari, S. 2006. Microbial Physiology. MJP Publishers, Chennai.
7. Ravi Mantha. 2012. All about bacteria. Collins Publications.
8. Stanier, R.Y., Ingraham, J.L., Wheelis, M.L., and Painter, P.R. 2007. General Microbiology Fifth Edition. McMillan Publishers, London.
9. Trivedi, P.C. 2006. Applied Microbiology. Agrobios (India) Publishers, Jodhpur.
10. Veena. 2008. Microbiology. Sonali Publications.

UNIT 3

STRUCTURE AND FUNCTION OF GRAM NEGATIVE BACTERIAL CELL WALL

STRUCTURE

3.1. OBJECTIVES

After reading this section we will learn

- The structure of gram negative bacterial cell wall
- The function of gram negative bacterial cell wall
- How the gram positive cell wall differs from gram negative cell wall?
- The fine details and composition of gram negative bacterial cell wall
- Lipopolysacchardies and their functions.

3.2. INTRODUCTION

The structure of the cell wall greatly differs among the gram positive and the gram negative bacteria. In Gram negative cell wall is relatively thin and measures around 10 nm. The cell wall is mainly composed of the polymer Peptidoglycan. A bacterium is either gram negative or gram positive based on the amount and location of peptidoglycan. The Gram-negative cell wall is composed of a thin, inner layer of peptidoglycan and an outer membrane. The peptidoglycan content is less and constitutes about 10-20%. There are no teichoic acids in gram negative cell wall. Outer membrane acts as a coarse sieve, has only minor control over transport into and out of the cell. The outer membrane contains Porin proteins, Adhesion proteins and Lipopolysaccharide layer. Outer membrane is involved in passive diffusion of small hydrophilic molecules through the outer membrane. The outer membrane also plays a role in antibiotic resistance. Lipid and lipoprotein content in gram negative bacterial cell wall is about 58% and lipopolysaccharide content is about 13%. Gram negative bacteria have a periplasmic space which lies between the outer membrane and the plasma membrane.

3.3. STRUCTURE OF GRAM NEGATIVE CELL WALLS

Cell walls of gram negative bacteria are relatively thinner, less compact and chemically more complex. Generally, a Gram negative cell wall contains three layers, the inner membrane (cytoplsamic membrane), a thin peptidoglycan layer and the outer membrane, which is unique to Gram negative bacteria (Fig. 1)

Gram negative cell walls consist of three components:

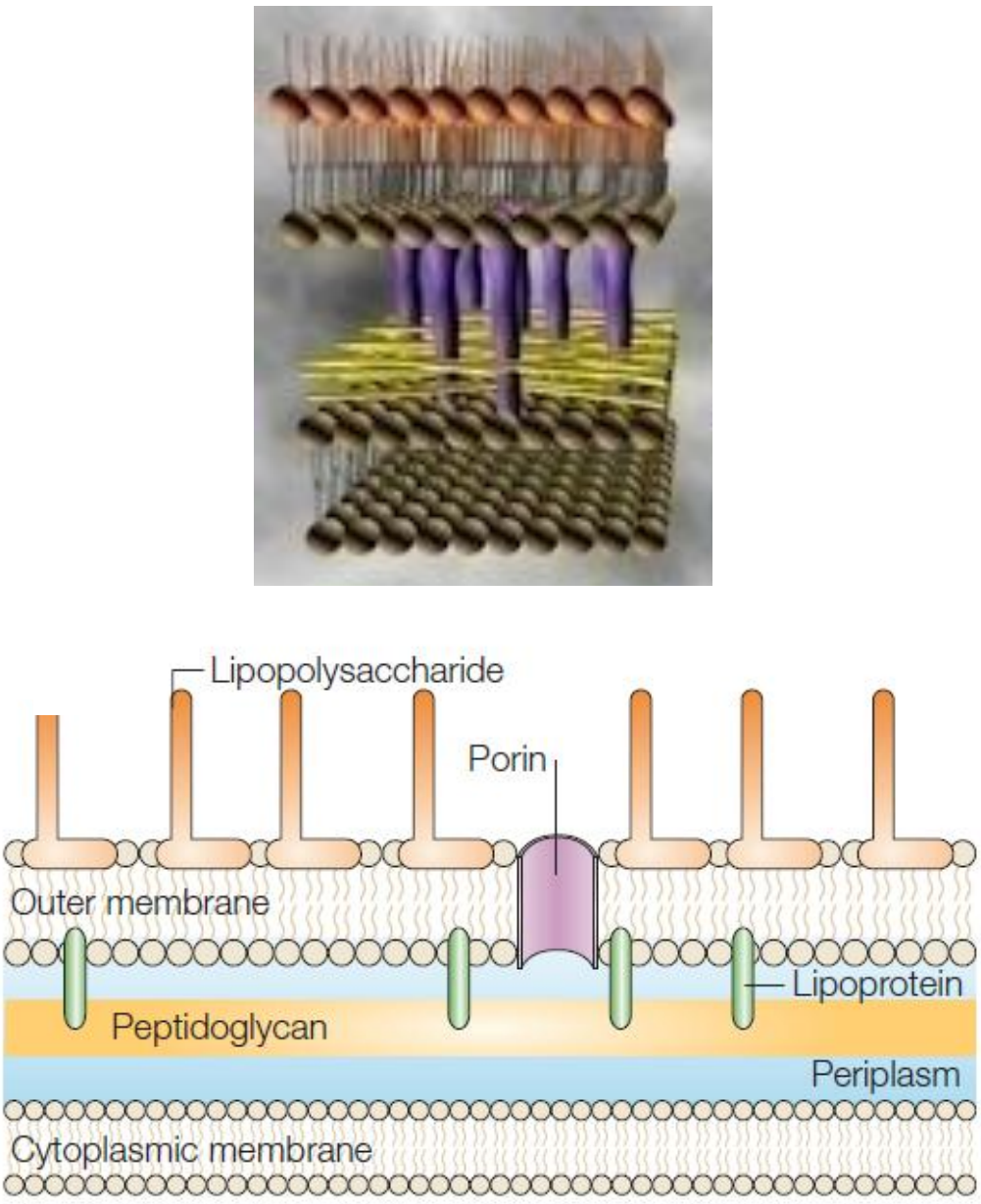

Figure 1: Structural details of the Gram negative bacterial cell wall

3.3.1. Cytoplasmic membrane

Cell membrane or the cytoplasmic membrane is the site of synthesis of DNA, cell wall polymers and membrane lipids. The cell membrane is selectively permeable and plays a role in transportation of solutes inside the cells. The bacterial cell membrane participates in the electron transport and oxidative phosphorylation. The cell wall also takes part in excretion of hydrolytic exoenzymes.

The cytoplasmic membrane is made up of a bilayer of phospholipids. Within his lipid bilayer several proteins are embedded which control what goes in and out of the cell. The proteins may be attached to any one side of the membrane, or to some other proteins of the membrane. Some proteins may occupy the entire span of the membrane on both the sides.

The cytoplasmic membrane is fluidic and delicate. The inner portion of this membrane is made up of lipid or fat molecules which make different molecules dissolved in water impermeable to enter, however, water molecules and move freely through this layer. One important characteristic of the cytoplasmic membrane is that protons (H^+) are unable to cross the cytoplasmic membrane. The inner region of the membrane is hydrophobic. In the bilayered membrane, the lipid molecules form two layered facing each other. The phosphate groups are hydrophilic and positioned on the outer edges of the membrane. Many of the proteins involved in the transportation of molecules into or out of the cells are found embedded in the lipid bilayer. These proteins act as sponge that absorb the nutrients and allow the cell to live in very dilute nutrient solutions.

3.3.2. Peptidogylcan layer/Molecule net

Found immediately outside of the cytoplasmic membrane comprising of a thin layer of sugar molecules. This layer covers the entire cytoplasmic membrane. This layer is called the Peptidoglycan and is responsible for the rigidity defining the shape of the cells. In the peptidoglycan polymer, molecules of *N*-acetylglucosamine

(gluNAc) alternate with molecules of *N*-acetylmuramic acid (murNAc). These molecules are cross-linked by tetrapeptides, chains of four amino acids (Fig. 2). The peptidoglycan layer is thinner and is located between the plasma membrane and the outer membrane. Peptidoglycan layer is a network formed by disaccharides interconnected by polypeptides. It is also called as 'murein' layer. The peptidoglycan layer constitutes only 5-20% of the total cell wall. The space between the cell wall and the plasma membrane is called the periplasm or the periplasmic space. Periplasm controls movement of molecules in and out of the cell. Periplasm contains a variety of hydrolytic enzymes required for the breakdown of large macromolecules for metabolism. These enzymes typically include proteases, phosphatases, lipases, nucleases, and carbohydrate-degrading enzymes. Periplasm of pathogenic gram negative bacteria contains virulence factors such as collagenases, hyaluronidases, proteases, and beta-lactamase. Components of the chemotaxis system, sugar transport systems, and binding proteins necessary for the uptake of different metabolites are also found in the periplasm. There are no teichoic or lipoteichoic acids in the Gram negative cell wall.

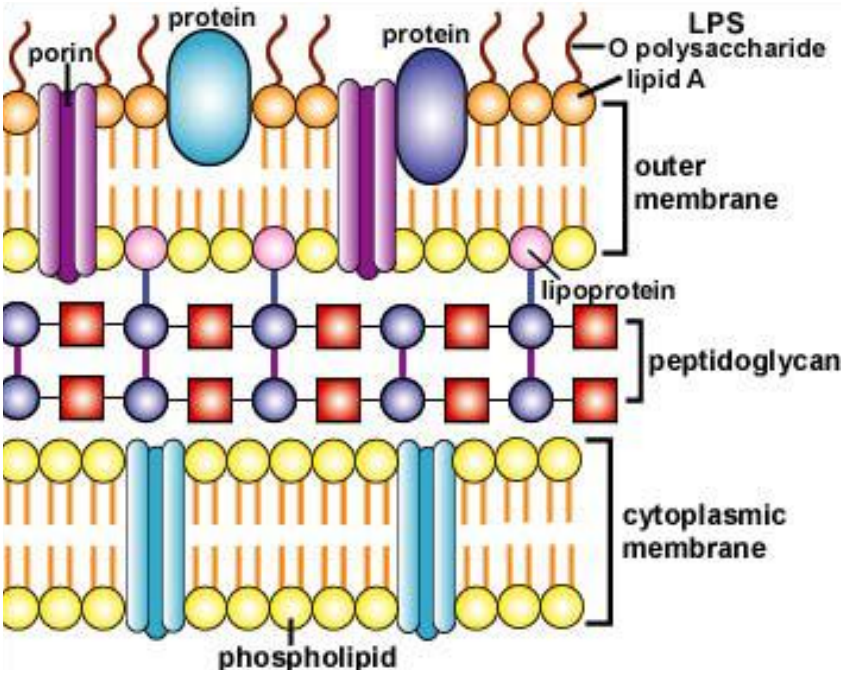

Figure 2: Fine structure of the peptidoglycan and LPS layer of the Gram negative bacterial cell wall

3.3.3. Outer membrane

The peptidoglycan layer is covered by an outer membrane that contains various proteins as well as lipopolysaccharides (LPS). It is the outermost layer made of lipid bilayer and is asymmetric. The outer membrane surrounds the bacteria like a stiff canvas bag. This layer is selectively permeable. The outer membrane consists of phospholipids, Lipopolysaccharides, Lipoproteins and surface proteins. This bilayered structure has an outer leaflet and an inner leaflet. The outer leaflet is composed of Lipopolysaccharides. LPS are amphipathic in nature i.e., they are having both hydrophobic and hydrophilic ends. The inner leaflet is made up of phospholipids (Fig. 3).

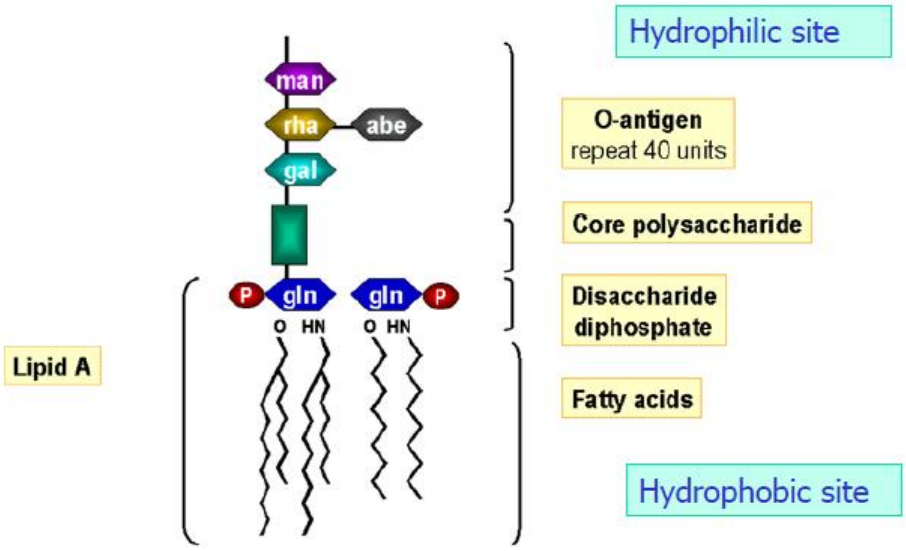

Figure 3: Strucutre of the Lipopolysacchardie

3.4. LIPOPOLYSACCHARIDES (LPS)

It is a large complex molecule containing both lipids and carbohydrates.

LPS consists of three parts: Lipid A, Core polysaccharide and O-side chain.

Lipid A: consists of two glucosamine sugar derivatives each attached to three fatty acids and phosphate. The lipid A residue is buried within the outer membrane and the remaining components are projected from the cell surface

Core Polysaccharide: The core polysaccharide consists of 10 sugar residues, most of which have an unusual structure, and are attached to the lipid A moiety.

O-Side Chain: The LPS structure ends with a terminal O side chain. The O antigen is a short polysaccharide chain that varies in composition and generally contains a number of unusual sugar residues

3.4. SURFACE PROTEINS

Porin proteins (trimeric protein pores) exist in the outer membrane and act as channels for low MW water soluble substances, phage receptors (Fig. 4). Note that in gram-positive bacteria, molecules as large as 105 daltons can pass through the cell wall. The permeable nature of the outer membrane is attributed to porin proteins. Three porin molecules group together to form narrow channels which allow only molecules smaller than about 600 to 700 daltons to pass through. The outer membrane is a barrier for large or hydrophobic antibiotics and proteins such as 1ysozyme. Large molecules such as vitamin B_{12} are therefore transported across the outer membrane via different carriers. The outer membrane also prevents the loss of constituents like periplasmic enzymes.

There are three types of surface porin proteins:

Braun lipoprotein: Bacterial lipoproteins having a lipid-modified cysteine at the N-terminus are important components of the cell envelope and responsible for various cellular activities.

They anchor the outer membrane to peptidoglycan (murein) sheet.

Omp C and Omp F porins: Proteins that form pores or channels through outer membrane (Gram-negative bacteria) for passage of useful molecules (nutrients) but not harmful substances from the environment.

Omp A protein: Provides receptor for some viruses and bacteriocins; stabilizes mating cells during conjugation.

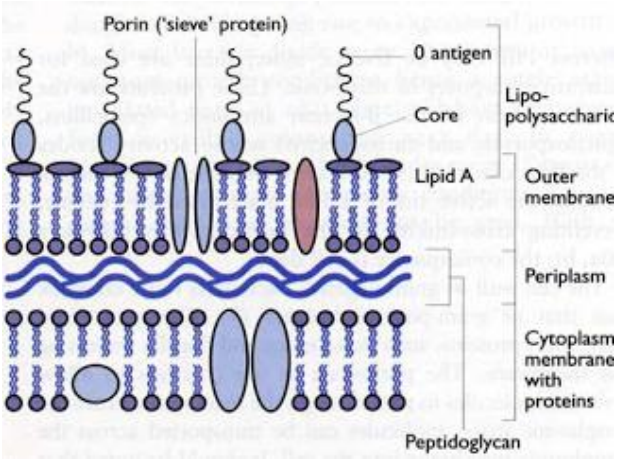

Figure 4: Gram negative bacterial cell wall with Porin proteins and their strucutre

Functions of the outer membrane:

The outer membrane provides protection from digestive system of the host (important for *Enterobacteriaceae* organisms).

LPS are also referred to as endotoxin which stimulates the host immunity. LPS acts as a signal that activates B cells and induces macrophage and other cells to release interleukin-I and interleukin-6, tumor necrosis factor, and other factors.

This outer layer is highly toxic to humans and most of the gram negative bacterial infections are caused by this LPS layer. LPS causes fever and can cause shock when released into the blood stream. E.g., The Shwartzman reaction (disseminated intravascular coagulation). *Neisseria meningitidis* releases large amounts lipooligosaccharide (LOS), resulting in fever and symptoms.

Protein content is higher in the outer membrane compared to the cytoplasmic membrane; however, the variety of proteins is limited. Most of these proteins traverse the entire lipid bilayer and are thus transmembrane proteins.

The outer membrane also has structural proteins and receptor molecules for bacteriophages and other ligands.

The outer membrane is linked to the cytoplasmic membrane at adhesion sites and is attached to the peptidoglycan layer by lipoprotein. The lipoprotein is covalently

attached to the peptidoglycan and is anchored in the outer membrane. The adhesion sites provide a membranous route for the delivery of newly synthesized outer membrane components to the outer membrane.

Divalent cation (Mg^{+2} and Ca^{+2}) linkages between phosphates on LPS molecules and hydrophobic interactions between the LPS and proteins hold together the outer membrane. These tough and firm membranes can be disrupted by antibiotics (e.g., polymyxin) or by the removal of Mg and Ca ions (chelation with ethylenediaminetetraacetic acid [FDTA]), thus allowing large, hydrophobic molecules into the cells.

LPS employed in identification of gram negative bacteria.

3.6. PERIPLASMIC SPACE

Gram negative bacteria have a periplasmic space which lies between the outer membrane and the plasma membrane. This periplasm is very small or nonexistent in gram-positive. The periplasm contains water, nutrients, and substances secreted by the cell, such as hydrolytic (digestive) enzymes and proteins such as alkaline phosphatase and beta-lactamase. There is a lot of activity in this area which has many soluble proteins that take part in transport, signaling in chemotaxis, and other processes.

Periplasmic enzymes are of several classes like the hydrolytic enzymes which are involved in breakdown of complex substances into simple substances. E.g., Phosphatases, Proteases etc., Periplasmic binding proteins are **i**nvolved in transport or substances. Periplasmic binding proteins for ions, amino acids, vitamins etc. there are also some biosynthetic enzymes which are involved in murine synthesis. E.g. Transglycosylases, Transpeptidases, Carboxypeptidases. The periplasm also has enzymes for fimbrial synthesis and assembly. It also has detoxifying or antibiotic degrading enzymes. E.g., Beta-lactamase or pencillinase, Aminoglycoside posphorylating enzymes (similar to lysosomes).

3.7. SUMMARY

Cell walls of gram negative bacteria are relatively thinner, less compact and chemically more complex. Generally, a Gram negative cell wall contains three layers, the inner membrane (cytoplsamic membrane), a thin peptidoglycan layer and the outer membrane

The cytoplasmic membrane is made up of a bilayer of phospholipids. Within this lipid bilayer several proteins are embedded which control what goes in and out of the cell. The proteins may be attached to any one side of the membrane, or to some other proteins of the membrane. Some proteins may occupy the entire span of the membrane on both the sides. . These proteins act as sponge that absorb the nutrients and allow the cell to live in very dilute nutrient solutions.

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Periplasmic binding proteins are involved in transport or substances. Periplasmic binding proteins for ions, amino acids, vitamins etc. there are also some biosynthetic enzymes which are involved in murine synthesis, fimbrial synthesis and assembly and antibiotic degrading enzymes.

3.8. CHECK YOUR PROGRESS

1. Explain the structure of the Gram negative bacterial cell wall.
2. What are the functions of the Gram negative bacterial cell wall?
3. What are the unique features of the Gram negative bacterial cell wall?
4. Explain the structure of peptidoglycan layer of Gram negative bacterial cell wall.
5. How do you distinguish between Gram negative and Gram positive bacterial cell wall?
6. What is Lipopolysaccharide layer? What are its functions?
7. What are the different parts of LPS?
8. What are porin proteins? Explain their function.
9. What are the functions of the outer membrane?
10. What is periplasmic space?

3.9. KEY WORDS

Gram negative bacterial cell wall, Peptidoglycan layer, cytoplasmic membrane, outer membrane, murein layer, lipopolysaccharides, Lipid A, Core polysaccharide and O-side chain, porin proteins, periplsamic space.

3.10. FURTHER SUGGESTED READING

1. Aneja K.R., Jain P. and Aneja R. "*A Text Book of Basic and Applied Microbiology*" New Age International Pub. New Delhi (2008).
2. Ankit Gupta and Prafulla Songara. 2012. Smart Study Series in Microbiology. Elsevier Publishers.
3. Arora, D.R., and Arora B.B. 2012. Textbook of microbiology. Fourth edition. CBS Publishers.
4. Blackwell Science, Darralyn McCall, David Stock. 2001. 11th Hour: Introduction to Microbiology 1st Edition. Blackwell Science
5. Betsey Dexter Dyer. 2003. A field guide to bacteria. Comstock Publishing.
6. Gerhardt P.R., Murray G.E., Costlow R.N., Nester E.W.,Wood E.A., Kreig N.R., and Phillips G.B.(eds) "*Manual of Methods for General Bacteriology*. American Society for Microbiology, Washington D.C. (1981)
7. Hans G Schiegel. 2008. General Microbiology. 7th Edition. Cambridge University Press.
8. Jerome J. Perry, James T. Staley, Stephen Lory. 2002. Microbial Life. Sinauer Associates.
9. Heritage. 2008. Introductory Microbiology. Cambridge University Press.
10. Stanier R.L., Ingram J.L. and Wheelis M.L. "*General Microbiology*" Macmillan Press Ltd (2007)
11. Talaro K.P. and Talaro A. "*Foundations in Microbiology*" 6th edn. McGraw Hill (2006)
12. Tortora, G.J. 2008. Microbiology: An introduction. Ninth Edition. Pearson Publishers.
13. Truper H.G.and Kramer J."Principles of Characertization and Identifiction of Prokaryotes" in Stolp M.P. Truper H.G., Balows A and Schlegal H.G (eds) "*The Prokaryotes: A Handbook on Habitats, Isolation and Identification of Bacteria*" Springer-Verlag (1981)

3.11. SOURCES

1. Alcamo. 2001. Fundamentals of Microbiology Sixth Edition. By, Edward Alcamo. Jones and Bartlett Publishers, London.
2. Arthur L. Koch. 2007. The Bacteria: Their Origin, Structure, Function and Antibiosis. Springer.
3. James T. Drummond, David White, Clay Fuqua. 2011. The Physiology and Biochemistry of Prokaryotes 0004 Edition. Oxford University Press, USA
4. Pelczar M.J., Chan E.C.S. and Kreig N.R. "*Microbiology* – 5th edn., Tata McGraw-Hill Pub. Co. New Delhi (1986)
5. Purohit, S.S. 2008. Microbiology – Fundamentals and Application. Sixth Edition. Student Edition Publishers, Jodhpur.
6. Meena Kumari, S. 2006. Microbial Physiology. MJP Publishers, Chennai.
7. Ravi Mantha. 2012. All about bacteria. Collins Publications.
8. Stanier, R.Y., Ingraham, J.L., Wheelis, M.L., and Painter, P.R. 2007. General Microbiology Fifth Edition. McMillan Publishers, London.
9. Trivedi, P.C. 2006. Applied Microbiology. Agrobios (India) Publishers, Jodhpur.
10. Veena. 2008. Microbiology. Sonali Publications.

UNIT 4

STRUCTURE AND FUNCTION OF ARCHAE BACTERIA

STRUCTURE

4.1. OBJECTIVES

After reading this section we will be able to learn about

- A general account of archaea bacteria and their adaptation to extreme environment
- The morphological features of archaea bacteria, classification of archaea
- The characteristic differences between bacteria an darchaea
- The detailed structure and functions of archaeal cell wall
- Metabolism, nutrition, reproduction and genetics of archaea
- Applications of archaea

4.2. INTRODUCTION

Archaea *is* one of the three domains of Life. Archaea are numerous and ubiquitous in nature. Most of them are extremophiles surviving in extreme conditions like very high temperatures, high salt concentration, extreme pH, extreme nutrient concentration, and extreme pressure. Some of them also exist in extreme cold conditions also. They are usually found in like hot springs, salt lakes, acidic mud pits, and submarine volcanic habitats. Many researchers suggest that ancestors of today's Archaea species might represent the first organisms that inhabited earth.

4.3. MORPHOLOGY

Archaea are tiny, their size ranges from 0.1 micrometers (μm) to over 15 μm in diameter. They show great diversity in their shape or form (Fig. 1):

Coccus- spherical- round or lobed and lumpy.

Bacillus- rod shaped - short bar-shaped rods to long slender hair-like.

Oddball- triangular shape, square shape

Other morphologies in the Crenarchaeota

Irregular lobed cells- *Sulfolobus*,

Needle-like filaments- *Thermofilum*,

Rectangular rods -*Thermoproteus* and *Pyrobaculum*.

Flat, square- *Haloquadratum walsbyi*

In *Thermoplasma* and *Ferroplasma* the lack of a cell wall means that the cells have irregular shapes, and can resemble amoebae.

Some species form aggregates or filaments of cells up to 200 μm long. These organisms can be prominent in biofilms. Notably, aggregates of *Thermococcus coalescens* cells fuse together in culture, forming single giant cells. The genus *Pyrodictium* produce an elaborate multicell colony involving arrays of long, thin hollow tubes called *cannulae* that stick out from the cells' surfaces and connect them into a dense bush-like agglomeration. The function of these cannulae is not settled, but they may allow communication or nutrient exchange with neighbours.

Multi-species colonies exist, such as the "string-of-pearls" community that was discovered in 2001 in a German swamp. Round whitish colonies of a novel Euryarchaeota species are spaced along thin filaments that can range up to 15 centimetres (5.9 in) long; these filaments are made of a particular bacteria species.

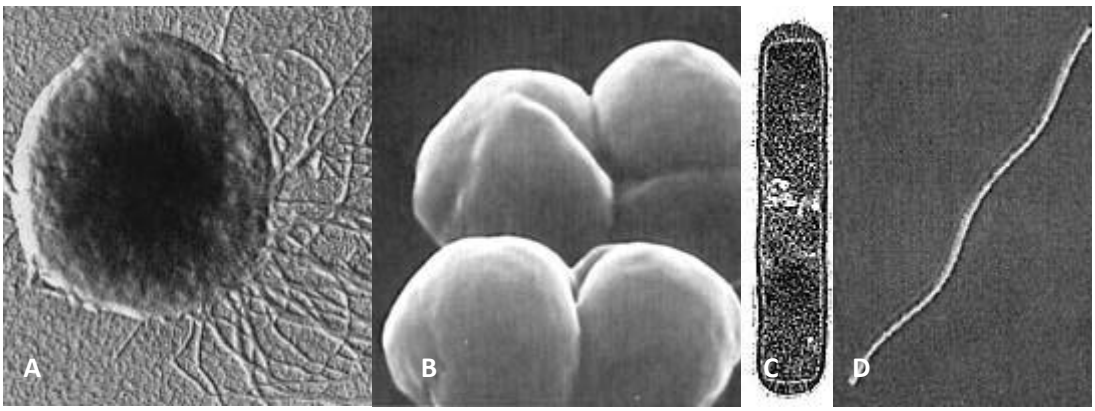

Figure 1: Diversity in Archaea A) *Methanococcus janaschii*, B) *Methanosarcina barkeri*, C) *Methanothermus fervidus*, D) *Methanobacterium thermoautotrophicum*.

4.4. CLASSIFICATION

The domain Archaea is classified into two major phyla the Euryarchaeota and Crenarchaeota.

4.4.1. Euryarchaeota

These are groups of extremophiles with varying physiologies. Methanogens – this group produce methane and are found in environments without oxygen. These bacteria release more than 2 billion tons of methane every year. Most of this is produced by archaeal species that thrive in stomach (rumen) of cows.

Extreme halophiles (salt loving) are archaea that require oxygen and high concentration of salts (NaCl) to survive and reproduce. Some of them are found in water having pH more than 11. They have distinct pink pigmentation.

Hyperthermophiles are archaea that survive only in temperatures as high as 100^0C or more.

4.4.2. Crenarchaeota

These are hyperthermophiles growing at temperatures over 80^0C and usually found in hot sulphur springs. Though the temperatures are 75-80^0C, these springs are highly acidic (pH 2-3). They are also found in volcanic vents. Many species are found inhabiting deep seas and polar seas.

In addition, two new phyla Nanoarchaeota and Korarchaeotasome have been tentatively proposed. *Nanoarchaeum equitans* belongs to Nanoarchaeota which shows features of both of the main phyla, but is more related to the Crenarchaeota. Korarchaeotacomprises some of the smallest microbes like Archaeal Richmond Mine acidophilic nanoorganisms (ARMAN), and unrelated to the main phyla.

Species concept in Archaea is controversial since they reproduce asexually. There is a high rate of horizontal gene transfer in Archaea.

Archaea differ from bacteria in terms of genetic, biochemical, and structural features (Table 1).

Differenes between bacteria and archaea

Table 1: Differences between various characteristics of bacteria and archaea

Character	Bacteria	Archaea
Histone proteins	Absent	presnt
Peptidoglycan cell wall	Presnet	absent
Ribosome senstiivty to diptheria toxin	No	Yes
First amino acid in a protein	Formylmethionine	Methionine
Chlorophyll based photosyntheis	Yes (Cyanobacteria)	No
Survive in temp above 100C	No	Yes

4.5. STRUCTURE

Structurally, archaea are most similar to Gram-positive bacteria. Most have a single outer membrane and cell wall. They lack internal membranes and periplasmic space. The exception to this general rule is *Ignicoccus*, which possess a particularly large periplasm that contains membrane-bound vesicles and is enclosed by an outer membrane.

The three primary structures of an archaeal cell are the cell wall, cell membrane and the cytoplasm.

4.5.1. Cell Wall

Most archaea (except *Thermoplasma* and *Ferroplasma*) have a cell wall. Cell wall maintains the shape and chemical equilibrium of the cell.

Archael species vary in the type of wall they possess. Archaeal cell walls do not contain peptidoglycan. However, Methanobacteriales possess pseudopeptidoglycan

like structure which does not have D-amino acids and N-acetylmuramic acid. Other archaeal cells have walls made of polysaccharide, protein, or both.

The outside of the cell is the S-layer made up of surface-layer proteins. This layer consists of hexagonal patterns of proteins or glycoproteins that form a crystalline network which is 5 nm to 25 nm thick. This layer is highly rigid and confers both structural and chemical protection.

4.5.2. Cell membrane

The overall arrangement of the cell membrane is similar to that found in Bacteria and Eukarya. The Archaea can alter the thickness of their membrane by including or removing pentacyclic rings in the structure

Archaeal cells have an outer cell membrane which acts as a barrier between the cell and its environment. Archaea lack internal membranes.

The cell membrane of Archaea is structurally and chemically distinct from other living forms. The characteristic differences are as follows:

(a) Chirality of glycerol: Phsopholipid is the basic building block of the cell membrane. Phospholipid is a glycerol molecule with phosphate added to one end and two side chains on the other end (Fig. 2). In the membrane, glycerol and phosphate end extend out from the surface sandwiching the long side chains in between. This arrangement acts as an effective chemical barrier and maintains cell equilibrium.

The glycerol found in archaeal phospholipids is a stereoisomer of the glycerol. Bacteria and eukaryotes membranes contain D-glycerol, archaeal membranes have L-glycerol. Chemical components of the cell have to be built by enzymes build the chemical components of the cell and the chirality of the molecule is determined by the shape of those enzymes. This suggests that archaea use entirely different enzymes for synthesizing phospholipids than do bacteria and eukaryotes.

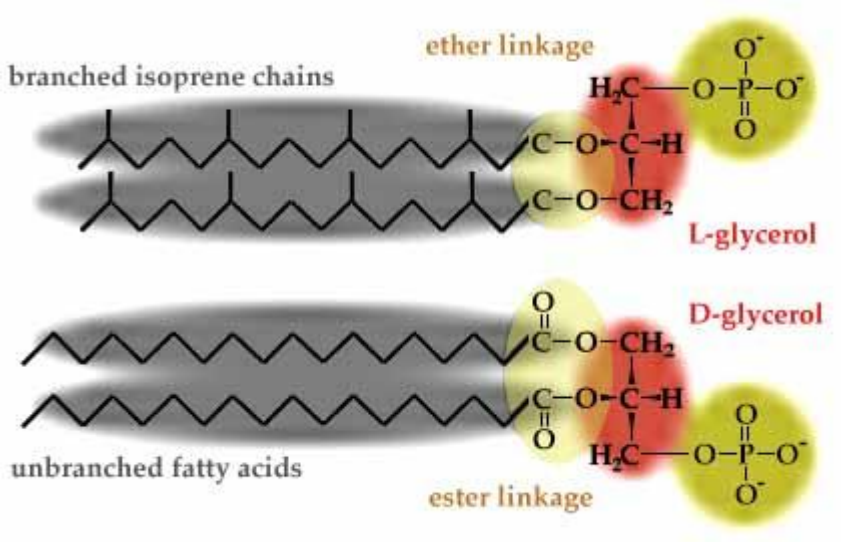

Figure 2: glycerol molecule with phosphate added to one end and two side chains on the other end

(b) Ether linkage: Majority of bacteria and eukaryotes use ester linkage to bind side chains to glycerol, whereas archaea use glycerol-ether linkage to bind lipid side chain to glycerol. Such side chains possess two oxygen atoms attached to one end, wherein one oxygen atom forms linkage with glycerol, while the other protrudes to the side during bonding. However, in archaea, ether linkage binding to the side chains does not have the additional protruding oxygen atom. Therefore, chemical properties archaeal phospholipid differs from membrane lipids of other organisms. Ether bonds are chemically more resistant than ester bonds. This stability might help archaea to survive extreme temperatures and very acidic or alkaline environments.

(c) Isoprenoid chains: bacteria and eukaryotes phospholipid side chains have **fatty acids** of 16 to 18 carbon atoms. But, in Archaea instead of fatty acids, they have chains of **isoprene** with of 20 carbon atoms. Isoprene is the simplest terpene.

(d) Branching of side chains: the side chains of archaeal membranes are chemically and structurally distinct from other organisms. Since the side chains contain isoprene, there are side branches arising from the main branch. These side branches are lacking in fatty acids of bacteria and eukaryotes. This special feature allows archaea to from transmembrane phospholipids by joining the isoprene side chains. That the two side chains of a single phospholipid can join together, or they

can be joined to side chains of another phospholipid on the *other side* of the membrane. Another distinctive feature of the side branches is their ability to form carbon rings. One of the side branches coils around and bonds with another atom to form a five carbon ring. These rings provide structural stability to the membranes.

In some archaea the lipid bilayer is replaced by a monolayer. In effect, the archaea fuse the tails of two independent phospholipid molecules into a single molecule with two polar heads (a bolaamphiphile); this fusion may make their membranes more rigid and better able to resist harsh environments. For example, the lipids in *Ferroplasma* are of this type, which is thought to aid this organism's survival in its highly acidic habitat.

4.6. CYTOPLASM

Cytoplasm is present within the outer membrane. Cytoplasm contains the DNA. Many archaeal cells contain plasmids (Fig. 3). Plasmids are stable, circular, extrachromosomal DNA molecules with 5 to 100 genes. A single cell can have one or more plasmids which contain same or different genes on them. Plasmids replicate independently and are transferred between cells during recombination. Though plasmids are not essential for cellular functions, they offer survival advantages. Some plasmids have genes for disease causing toxins and antibiotic resistance.

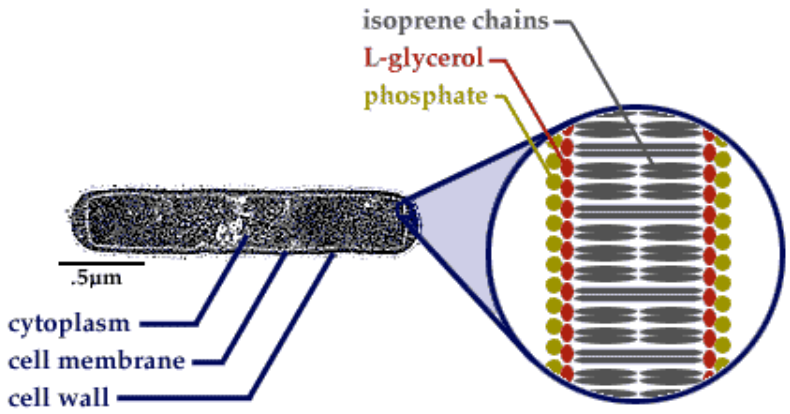

Figure 3: Archaea bacteria showing different structures of the cell

4.7. APPENDAGES

Archaea possess a number of different types of surface structures like archaeal flagella and pili, cannulae and hami, and bindosome.

4.7.1. Flagella

Archaeal flagella are widely distributed in both *Crenarchaeota* and *Euryarchaeota* . Archaea may possess one or more flagella and some may be without flagella. Flagella are hair like structures attached to the outer membrane of the cell wall and are locomotory in function.

Archaea flagella have long stalks that driven by rotatory motors at the base powered by proton gradient across the membrane. Archaeal flagella are different in composition and development compared to bacterial flagella. The bacterial flagellum evolved from type III secretion system while archaeal are evolved from bacterial type IV pili. Bacterial flagella develop by assembling subunits moving up the central pore to the tip of the flagella, but archaeal flagella are synthesized by adding subunits at the base.

4.8. METABOLISM

Based on the source of energy used Archaea are classified into nutritional groups, depending on energy and carbon sources (Table 2). Some archaea obtain energy from inorganic compounds such as sulfur or ammonia (they are lithotrophs). These include nitrifiers, methanogens and anaerobic methane oxidisers. Other groups of archaea use sunlight as a source of energy (they are phototrophs). However, oxygen–generating photosynthesis does not occur in any of these organisms.

Certain methanogens survive in anaerobic environments such as swamps. Methanogenesis involves a range of coenzymes that are unique to these archaea,

such as coenzyme M and methanofuran. Alternatively alcohols, acetic acid or formic acid are also used as electron acceptors by methanogens which inhabit the gut.

Biogas producing acetotrophs like *acetotrophic* archaea which belong to the order Methanosarcinales breakdown Acetic acid into methane and carbon dioxide.

Autotrophs that use CO_2 in the atmosphere as a source of carbon to fix carbon.

Archaea do not carry out photosynthesis. Their source is energy is highly diverse, Nitrosopumilales oxidze ammonia; *Sulfolobus* oxidize hydrogen sulfide/sulfur.

Phototrophic archaea use light to produce chemical energy in the form of ATP. E.g., Halobacteria, light-activated ion pumps like bacteriorhodopsin and halorhodopsin generate ion gradients by pumping ions out of the cell across the plasma membrane

Table 2: Nutritional types in archaeal metabolism

Nutritional type	**Source of energy**	**Source of carbon**	**Examples**
Phototrophs	Sunlight	Organic compounds	*Halobacteria*
Lithotrophs	Inorganic compounds	Organic compounds or carbon fixation	*Ferroglobus*, *Methanobacteria* or *Pyrolobus*
Organotrophs	Organic compounds	Organic compounds or carbon fixation	*Pyrococcus*, *Sulfolobus* or *Methanosarcinales*

4.9. GENETICS

Archaea are genetically distinct from bacteria and eukaryotes. Up to 15% of the coded proteins of genome are unique to archaea, although their functions remain

unknown. The remaining proteins are mostly from Euryarchaea that take part in methanogenesis.

Archaea have a single circular chromosome in varying sizes. *Methanosarcina acetivorans has* 5,751,492 base pair genome and *Nanoarchaeum equitans with* 490,885 base-pair genome. Smaller independent pieces of DNA, called *plasmids*, are also found in archaea which are transferred between cells by a process similar to bacterial conjugation.

Archaea can be infected by double-stranded DNA viruses- thermophilics, particularly the orders Sulfolobales and Thermoproteales

Two groups of single-stranded DNA viruses that infect archaea -Halorubrum pleomorphic virus 1 ("Pleolipoviridae") infecting halophilic archaea and Aeropyrum coil-shaped virus ("Spiraviridae") infecting a hyperthermophilic (optimal growth at 90–95 °C) host.

Some of the common proteins of archaea, bacteria and eukaryotes are the ones involved in transcription, translation, and nucleotide metabolism.

There are also protein networks which assist the cells for attachment to form groups. Transfer RNA or tRNA decodes DNA messages to form proteins. tRNAs of Archea are characteristic and differ from other life forms in several features. Archaeal tRNA resembles more of eukaryotic than bacteria, indicating that Archaea have more features of eukaryotes. Similarly, archaeal ribosomes, like eukaryote ribosomes, are not sensitive to chemical inhibitors. These characteristics imply a close connection between Archaea and eukaryotes.

RNA polymerase and ribosomes of archaea are similar to those in eukaryotes and thus Transcription and translation process of archea are more like that of eukaryotes. Archaea have one type of RNA polymerase which functions like the eukaryotic RNA polymerase II. However, other Archaean transcription factors are more related to bacteria. Most Archaean genes lack introns and therforePost-transcriptional modification is simpler.

4.10. REPRODUCTION

Archaea reproduce asexually by binary or multiple fission, fragmentation, or budding. All have the same genetic material due to lack of meiosis., cell divides after two daughter chromosomes separate and Cell cycle controls cell division. Cell cycle has similarities with both bacterial and eukaryotic cell cycle. DNA polymerases cause chromosome replication from many points of origin.

Archaea does not form spores. However, some species of Haloarchaea undergo phenotypic switching and grow into thick-walled structures resistant to osmotic shock and allow the archaea to survive in water at low salt concentrations, but these are not reproductive structures

4.11. HABITATS

Archaea are present in a wide range of habitats and roughly make up 20% of earths biomass. Extremophiles survive temperatures above 100^0C often found in geysers, black smokers and oil wells. They are also found in cold habitats and highly saline, acidic or alkaline conditions. However, there are also archaea which are found in mild conditions like oceans, soils, marshland and sewage.

Extremophiles are broadly classified as follows:

Halophiles, - found in extreme saline conditions like salt lakes with salinity more than 20–25%. E.g. Halobacterium

Thermophiles- survive at temperatures above 45 °C, in places such as hot springs; *hyperthermophilic* archaea grow optimally at temperatures greater than 80 °C, *Methanopyrus kandleri* Strain 116 grows at 122 °C

Alkaliphiles, - occur in alkaline conditions -

Acidophiles – occur only in acidic conditions - *Picrophilus torridus*, which grows at pH 0, which is equivalent to thriving in 1.2 molar sulfuric acid.

Archaea are also present at low temperatures as well - archaea found in cold oceanic environments such as polar seas.

Vast numbers of archaea are also found in the sediments that cover the sea floor, with these organisms making up the majority of living cells at depths over 1 meter below the ocean bottom.

4.12. ROLE OF ARCHAEA IN CHEMICAL CYCLING

Carbon, nitrogen and sulfur are recycled by Archaea.

Nitrogen cycle: Archaea carry out many steps in the nitrogen cycle. Nitrogen removal by nitrate-based respiration and denitrification, and nitrogen addition by nitrate assimilation and nitrogen fixation are carried out by Archaea. They are also involved in oxidation of ammonia in oceans and soils. Archaea produce nitrites which are oxidized to nitrites by different microbes.

Sulfur cycle: Archaea that grow by oxidizing sulfur compounds and release sulfur from rocks. E.g. *Sulfolobus*. They produce sulfuric acid as a bi-product, thus causing acid mine drainage in abandoned mines

Carbon cycle: Methanogenic archaea act as decomposers and are important in the decay of organic matter by anaerobic microbes in places such as sediments, marshes and sewage treatment plants. Methanogens are the primary source of atmospheric methane and therefore are a main cause of greenhouse effect and global warming.

4.13. INTERACTIONS WITH OTHER ORGANISMS

Mutual or commensal relationship with other organisms are reported. Methanogenic archaea have a symbiotic relationship with termites. Archaeal Richmond Mine Acidophilic Nanoorganisms (ARMAN) occasionally connects with other archaeal cells in acid mine drainage biofilms.

Archaea are not pathogenic or parasitic. However, there are recent reports of archaea pathogenic to humans. E.g., some methanogens infect mouth; *Nanoarchaeum equitans* is parasitic on other acheaea *Ignicoccus hospitalis*.

4.13.1. Mutualism

Mutualism exists between protozoas and methanoges in the guts of animals that digest cellulose, such as ruminants and termites. Protozoa break down plant cellulose to obtain energy by releasing hydrogen as a waste product, but high levels of hydrogen reduce energy production. Methanogens convert hydrogen to methane, protozoa benefit from more energy.

Archaea live inside the anaerobic protozoa *Plagiopyla frontata and consume excess* hydrogen produced in their hydrogenosomes.

Cenarchaeum symbiosum a marine archaea lives as endosymbiont in the sponge *Axinella mexicana*.

4.13.2. Commensalism

Commensalism also exists in Archaea, methanogen *Methanobrevibacter smithii* found in human gut. In termites and in humans, these methanogens interact with other microbes helping digestion.

Archaea are also reported to be associated with corals and in plant rhizospheres.

4.14. APPLICATIONS

- Extremophile archaea, resistant to heat or extremes of acidity and alkalinity, are a source of enzymes that function under these harsh conditions. E.g. Thermostable DNA polymerases produced from *Pyrococcus furiosus*,

- Amylases, galactosidases and pullulanases isolated from *Pyrococcus* that function at over 100 °C are used in food processing industries for the production of low lactose milk and whey.
- Thermophilic archaea produce Enzymes stable in organic solvents which are used in synthesis of organic compounds by green chemistry technology. Their stability is exploited for structural biology studies.
- Methanogenic archaea are employed in sewage treatment. They are produce biogas by anaerobic digestion.
- Acidophilic archaea are mineral processing for extraction of metals like gold, cobalt and copper from ores.
- Several antibiotics called archaeocins are produced from Archaea. E.g. *Haloarchaea* and *Sulfolobus*.
- They are also employed as selectable markers archaeal molecular biology studies

4.15. SUMMARY

Archaea are numerous and ubiquitous in nature. Most of them are extremophiles surviving in extreme conditions like very high temperatures, high salt concentration, extreme pH, extreme nutrient concentration, and extreme pressure. Some of them also exist in extreme cold conditions also. They are usually found in like hot springs, salt lakes, acidic mud pits, and submarine volcanic habitats.

Archaea are tiny, their size ranges from 0.1 micrometers (μm) to over 15 μm in diameter. They show great diversity in their shape or form spherical, rod shaped, triangular, rectangular, square, irregular lobed, needle like, flat shaped etc.,

The domain Archaea is classified into two major phyla the Euryarchaeota and Crenarchaeota. *Euryarchaeota:* these are groups of extremophiles with varying physiologies. Includes Methanogens, Extreme halophiles (salt loving) and Hyperthermophiles.

Crenarchaeota: these are hyperthermophiles growing at temperatures over 80^0C and usually found in hot sulphur springs.

Differenes between bacteria and archaea

Character	Bacteria	Archaea
Histone proteins	Absent	presnt
Peptidoglycan cell wall	Presnet	absent
Ribosome senstiivty to diptheria toxin	No	Yes
First amino acid in a protein	Formylmethionine	Methionine
Chlorophyll based photosyntheis	Yes (Cyanobacteria)	No
Survive in temp above 100C	No	Yes

Structure

The three primary structures of an archaeal cell are the cell wall, cell membrane and the cytoplasm.

Cell wall

Cell wall maintains the shape and chemical equilibrium of the cell. Archaeal cells have walls made of polysaccharide, protein, or both. Archaeal cell walls do not contain peptidoglycan. The outside of the cell is the S-layer made up of surface-layer proteins. This layer is highly rigid and confers both structural and chemical protection.

Cell membrane

Archaeal cells have an outer cell membrane which acts as a barrier between the cell and its environment. Archaea lack internal membranes.

The cell membrane of Archaea is structurally and chemically distinct from other living forms. The characteristic differences are as follows:

(1) Chirality of glycerol: Phsopholipid is the basic building block of the cell membrane. Phospholipid is a glycerol molecule with phosphate added to one end and two side chains on the other end. In the membrane, glycerol and phosphate end extend out from the surface sandwiching the long side chains in between. This arrangement acts as an effective chemical barrier and maintains cell equilibrium. The glycerol found in archaeal phospholipids is a stereoisomer of the glycerol. Bacteria and eukaryotes membranes contain D-glycerol; archaeal membranes have L-glycerol.

(2) Ether linkage: Majority of bacteria and eukaryotes use ester linkage to bind side chains to glycerol, whereas archaea use glycerol-ether linkage to bind lipid side chain to glycerol. Such side chains possess two oxygen atoms attached to one end, wherein one oxygen atom forms linkage with glycerol, while the other protrudes to the side during bonding. However, in archaea, ether linkage binding to the side chains does not have the additional protruding oxygen atom.

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(4) Branching of side chains: the side chains of archaeal membranes are chemically and structurally distinct from other organisms. Since the side chains contain isoprene, there are side branches arising from the main branch. These side branches are lacking in fatty acids of bacteria and eukaryotes. This special feature allows archaea to from transmembrance phospholipids by joining the isoprene side chains. That the two side chains of a single phospholipid can join together, or they can be joined to side chains of another phospholipid on the *other side* of the membrane. Another distinctive feature of the side branches is their ability to form carbon rings. One of the side branches coils around and bonds with another atom to form a five carbon ring. These rings provide structural stability to the membranes.

Cytoplasm

Cytoplasm is present within the outer membrane. Cytoplasm contains the DNA. Many archaeal cells contain plasmids. Though plasmids are not essential for cellular functions, they offer survival advantages. Some plasmids have genes for disease causing toxins and antibiotic resistance.

Surface structures

Archaea possess a number of different types of surface structures like archaeal flagella and pili, cannulae and hami, and bindosome.

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Role of Archaea in chemical cycling

Carbon, nitrogen and sulfur are recycled by Archaea.

Interactions with other organisms

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Commensalism

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Applications

- Extremophile archaea, resistant to heat or extremes of acidity and alkalinity, are a source of enzymes that function under these harsh conditions. E.g. Thermostable DNA polymerases produced from *Pyrococcus furiosus*,
- Amylases, galactosidases and pullulanases isolated from *Pyrococcus* that function at over 100 °C are used in food processing industries for the production of low lactose milk and whey.
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- Several antibiotics called archaeocins are produced from Archaea. E.g. *Haloarchaea* and *Sulfolobus*.
- They are also employed as selectable markers archaeal molecular biology studies

4.16. CHECK YOUR PROGRESS

1. Write a general account on archaea
2. What are archaea bacteria and what are their structural modifications to survive in extreme conditions
3. What are the differences between archaea and bacteria?
4. What are the important features of archaeal cell wall?
5. Write an essay about morphology of archaea
6. Write a note on archaea classification
7. Write in detail the structure of archaeal cell membrane
8. What are the locomotory structures in archaea?
9. What are the nutritional types in archaea?
10. What are the different habitat adaptations in archaea?
11. Write an account of Role of Archaea in chemical cycling
12. What are the applications of archaea?

4.17. KEY WORDS

Archaea, Euryarchaeota, Crenarchaeota, methanogens, extremophiles, halophiles, alkaliphiles, acidophiles, hyperthermophiles, chirality of glycerol, ether linkage, isoprenoid chains, archaeal flagella, pili, chemical cycling, thermostable DNA polymerases, archaeocins, selectable markers.

4.18. FURTHER SUGGESTED READING

1. Alcamo. 2001. Fundamentals of Microbiology Sixth Edition. By, Edward Alcamo. Jones and Bartlett Publishers, London.
2. Ankit Gupta and Prafulla Songara. 2012. Smart Study Series in Microbiology. Elsevier Publishers.
3. Purohit, S.S. 2008. Microbiology – Fundamentals and Application. Sixth Edition. Student Edition Publishers, Jodhpur.
4. Sivakumaar, P.K. 2010. An introduction to industrial microbiology. S. Chand Publishers.
5. Tortora, G.J. 2008. Microbiology: An introduction. Ninth Edition. Pearson Publishers.
6. Trivedi, P.C. 2006. Applied Microbiology. Agrobios (India) Publishers, Jodhpur.

4.19. SOURCES

1. Brock, T. D., Madigan, M. T., Martinko, J. M. and Parker. J. 1994. Biology of Microorganisms, 7th ed. New Jersey: Prentice Hall.
2. Cell structure and function in the bacteria and archaea. Chapter 4. Jones and Bartlett publishers, L.L.C.
3. John L. Howland. 2000. The Surprising Archaea. New York and Oxford: Oxford University Press.
4. Kates, M., Kushner, D. J., and Matheson, A. T. (eds.) 1993. The Biochemistry of Archaea (Archaebacteria) Amsterdam: Elsevier Science Publishers.
5. Paul B. Eckburg, Paul W. Lepp and David A. Relman. 2003. Archaea and their potential role in human disease. Infection and Immunity 71: 591-596.
6. Sandy Y. M. Ng, Behnam Zolghadr, Arnold J. M. Driessen, Sonja-Verena Albers and Ken F. Jarrell. 2008. Cell surface structures of archaea. Journal of Bacteriology 190: 6039-6047.

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UNIT 5

STRUCTURE AND FUNCTION OF CELL WALL AND CAPSULE IN BACTERIA

STRUCTURE

5.1. OBJECTIVES

After reading this section we will be able to learn about

- The details of the bacterial cell structure particularly the surface layers and appendages
- The structure and function of the bacterial cell wall
- The differences between the gram positive and gram negative bacterial cell wall
- The structure and function of the bacterial cell capsule/slime layer

5.2. INTRODUCTION

Bacterial cell is made up of three essential structures, the cytoplasm, the surface layers and the appendages. The structures found external to the cell wall include capsule, flagella, pili and fimbriae. Capsule or Glycocalyces are gelatinous, sticky substance surrounding the outside of most prokaryotic cells. These are composed of polysaccharides, polypeptides, or both. Capsule is involved in bacterial adhesion.

Flagella are small semi-rigid whips that are free at one end and attached to a cell at the other which help in motility of the bacteria. The terms pili and fimbriae are often used interchangeably.

Pili are short, hair-like structures on the surfaces of bacterial cells. Pili are found mostly in male cells. Unlike flagella pili grow from the inside of the cell outward, and not from the tip of the fibre. Pili also serve as attachment structures. The cell structure greatly varies in Gram positive and Gram negative bacteria (Fig. 1)

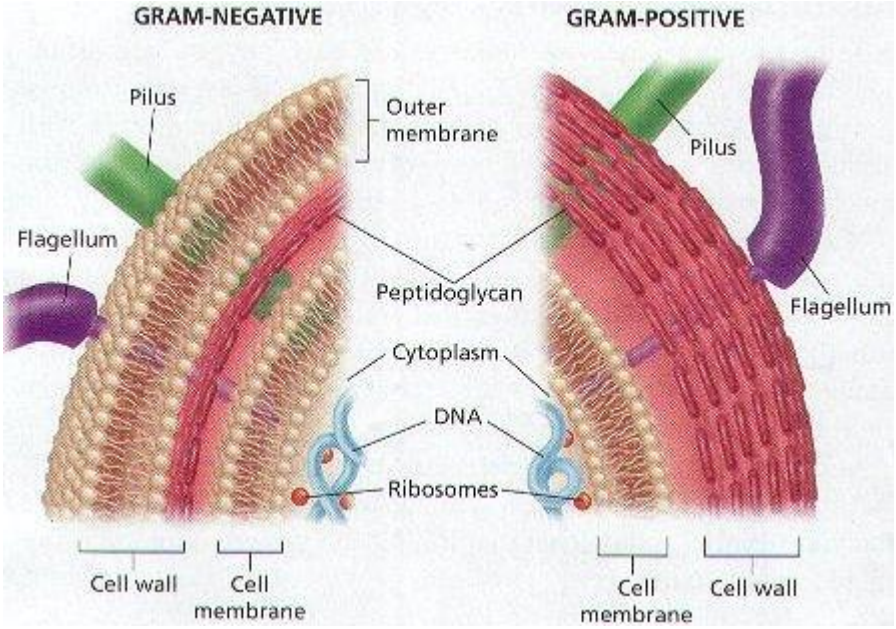

Figure 1: The details of cell surface structures in Gram positive and Gram negative bacterial cell

5.3. THE SURFACE LAYERS:

It is made up of the capsule, cell wall and the cell membranes.

5.3.1. Capsule

The bacterial cell envelope is surrounded by certain special structures called capsules or made up of polysaccharides or proteins (Fig. 2). If these structures are loosely adherent and nonuniform in density or thickness, the material is referred to as a slime layer. The capsule and slime layers are also called the glycocalyx.

Glycocalyces or capsules are gelatinous, sticky substance surrounding the outside of most bacterial cells. These are composed of polysaccharides, polypeptides, or both. The term glycocalyx can be used to describe extracellular structures including the capsule and S-layer.

Based on the strength of bonding between cell wall and glycocalyx it can be further classified as a Capsule or a Slime layer. Capsule has a defined layer with an outer edge but slime layer is not well defined. In some cases the polymers are tightly

integrated with the cell called capsule while in others they are loosely associated and are called a slime layer.

These are primarily made up of polysaccharides but sometimes may also contain polypeptides (polyglutamic acid, e.g., *Bacillus anthracis*,) which are excreted by the cell under certain environments. These capsules are not essential for cell growth or viability but are necessary for survival in host cells.

Capsule and slime production is genetically determined, however, it is affected by nutrient availability. Little or no capsule/slime is produced when nutrients are deprived, but under nutrient rich conditions thick layers may be produced.

Glycocalyx is composed of 90% of water and protect bacteria from desiccation or dehydration or drying up by acting as osmotic barrier and defensive buffer and may contain antigenic sites. Capsulated bacteria are more resistant to phagocytosis (antiphagocytic). Pseudomonas and Streptococcus have slime layer. Streptococcus mutants via capsule/slime attaches to cell surface protein of pellicle of teeth and produce lactic acid that causes decalcification of teeth.

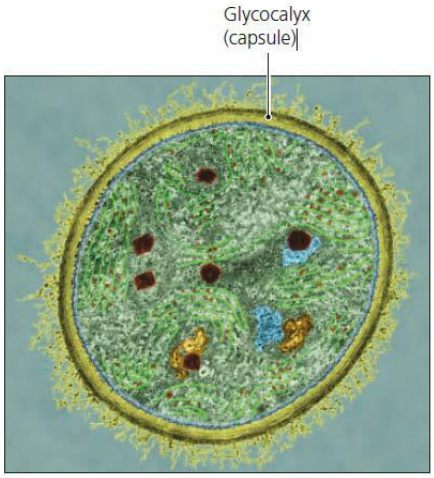

Figure 2: A bacterial cell showing the capsule layer

Capsule and slime layers help in adhesion to host cells for invasion or to a solid surface, and to initiate and stabilize biofilm formation.

5.3.2. Glycocalyx: Capsule and S-Layers

S-layer is a surface protein layer found in many different bacteria and in some archaea where it serves as the cell wall. The s-layer is directly attached to the outer membrane, rather than the peptidoglycan (Fig. 3).

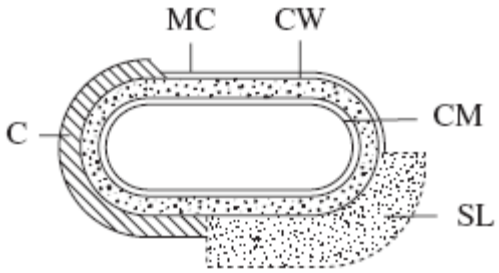

Figure 3: An illustration of the capsule and S-layer in bacterial cell
C, capsule; CM, cytoplasmic membrane; CW, cell wall; MC, micro-capsule; SL, slime layer.

5.3.3. A surface protein layer Glycoproteins

The S-layer is directly attached to the outer membrane, rather than the peptidoglycan. S-layer is a surface protein layer found in many different bacteria and in some archaea where it serves as the cell wall. It is somewhat looser structures, more easily deformed layer. All S-layers are made up of a two-dimensional array of proteins and have a crystalline appearance, the symmetry of which differs between species. In certain bacteria the slime layer that surrounds the outermost components of cell walls are made up of glycoproteins of high molecular weight. In addition to forming these s-layers, glycoproteins also function as bacterial flagella. These are made up of bundles of glycoproteins protruding from the cell's surface. Their rotation provides propulsion.

5.4. FUNCTIONS OF SLIME LAYER AND CAPSULE

Slime layer as an adhesion is involved in attachment of bacteria (including pathogens) to other cells or environmental surfaces in order to colonize and form biofilms. It may contribute to virulence by protecting the bacterium against complement attack and phagocytosis. The S-layer may protect bacteria from harmful

enzymes or changes in pH. 4. It protects bacteria from desiccation. Slime layers can also be used as a food reserve for the cell.
It also allows dental caries to attach to teeth forming dental plaques.

Capsule also involved in disease, attachment. Capsules provide a protective function to bacterial cells. Prevents ingestion by phagocytes (WBC) allowing bacteria to evade destruction; Virulence – associated with pathogen ability to cause disease; Increased virulence may occur in pathogen if they have structures that allow them to overcome host immune defences such as having a capsule; prevents cell drying.

Capsule/slime layers

- Act as a site of attachment of bacteria (part of biofilms)
- Prevents the cells from drying and desiccation
- Protection from phagocytosis
- stores for cellular waste products
- extra sources of nutrients under extreme conditions
- It prevents damage due to white blood cells.
- It is toxic and breaks the host's defence system and causes disease development
- It is poorly antigenic and a major virulence factor (e.g. Streptococcus pneumonia)
- It acts as a barrier to hydrophobic molecules like detergents and aids in attachment. E.g., the dextran and levan capsules of Streptococcus mutans, help to attach to the tooth enamel

5.5. CELL WALL

Cell wall is the structure exterior to cytoplasm. It is situated in the outermost surface and is about 15-30 nm thick and constitutes 10-25% of the total cell dry weight. It includes the cytoplasmic membrane and one or two other layers in most prokaryotes.

Without a cell wall, the cell will burst because the number of particles (solutes) tends to equalize on both sides of the cell (osmosis). Since there are more particles inside the cell, water tends to flow into the cell pressurizing the membrane. Most bacteria have a cell wall that maintains cell shape and protects against osmotic lysis. It is relatively porous and is not considered to be a permeability barrier for small substrates.

The structure of the cell wall greatly differs among the gram positive and the gram negative bacteria (Fig. 4 and 5) (Table 1). The cell wall is mainly composed of the polymer Peptidoglycan. The molecule Peptidoglycan is exclusively found in bacterial cell walls which impart rigidity and shape to the bacterial cell. This layer surrounds plasma membrane and acts as a protective coat. Peptidoglycan is a polymer made up of many interlocked chains of monomers.

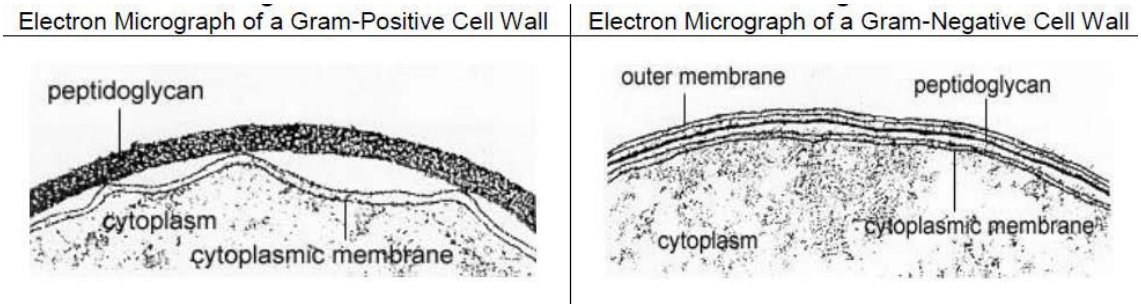

Figure 4: Electron micrographs showing the differenes in Gram postiive and Gram negative bacterial cell wall

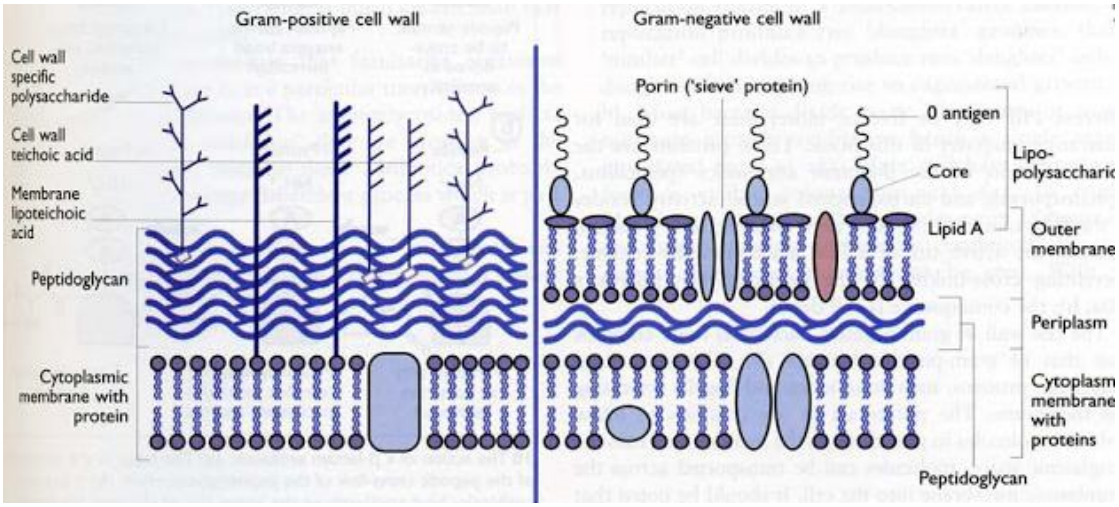

Figure 5: Detailed structural differences between the Gram positive cell wall and Gram negative cell wall in bacteria

N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) which are glucose derivatives and form the backbone of peptidoglycan. The NAG and NAM chains are linked by interpeptide bridges. A bacterium is either gram-negative or gram –positive based on the amount and location of peptidoglycan. There are a set of identical tetrapeptide side chains attached to the N-acetyl-muramic acid which differs in the composition and binding modes in gram positive and gram negative bacteria.

Table 1: Differences between cell wall of Gram positive and Gram negative bacteria

Characteristics	Gram positive	Gram negative
Wall thickness	Thick 20-80 nm	Thin 10 nm
Layers	One layer	Two layers
Peptidoglycan (murein)	More than 50%	10-20%
Teichoic acids in wall	Present	Absent
Lipid and lipoprotein content	0-3%	58%
Protein content	Nil	9%
Lipopolysaccharide content	Nil	13%
Sensitivity to lysozyme and penicillin	High	Low

5.5.1. Peptidoglycan and Antibiotics

Gram positive bacteria due to the absence of the membrane outside the peptidoglycan layer are highly susceptible to antibiotics. Antibiotics like Penicillins and Cephalosporins act on the interpeptides of peptidoglycan layer, but the LPS membrane prevents their access to peptidoglycan layer in gram negative bacterial hence these are resistant to antibiotics.

5.5.2. Functions of bacterial cell wall

The bacterial cell wall serves in many important cell functions like:

1. The characteristic shape of the bacterial cell is maintained by the cell wall. The flexibility of the phospholipid membrane is countered by the rigid cell wall thus prevents the cell from becoming spherical/changing shape.
2. Cell walls also have an essential role in cell division
3. The effects of osmotic pressure on the cell are countered by the cell wall.
4. The cell wall acts as a site of attachment for bacteriophages.
5. Cell wall acts as a firm support for structures like flagella, fimbriae, and pili
6. Cell walls act as important sites of major antigenic determinants of the cell surface。
7. Cell walls are also responsible for development of resistance of Antibiotics

5.5.3. Bacteria without cell walls

Wallless bacteria are formed when bacterial cell walls come in contact with lytic enzymes (lysozyme) or certain antibiotics that prevent biosynthesis of peptidoglycan. These interactions make the bacterial cells non-viable. Such non-viable wallless bacteria that cannot replicate are termed spheroplasts. Usually these treatments generate non-viable organisms. Such wallless bacteria fail to replicate. Such bacteria which have an outer membrane called spheroplasts and which do not have an outer membrane are called protoplasts. Sometimes, wallless bacteria also replicate under the influence of some treatments called as L-forms.

5.6. SUMMARY

Bacterial cell is made up of three essential structures, the cytoplasm, the surface layers and the appendages. The structures found external to the cell wall include capsule, flagella, pili and fimbriae.

Capsule and Slime layer: capsules are gelatinous, sticky substance surrounding the outside of most bacterial cells. These are composed of polysaccharides, polypeptides, or both. The term glycocalyx can be used to describe extracellular structures including the capsule and Slime layer. Based on the strength of bonding between cell wall and glycocalyx it can be further classified as a Capsule or a Slime layer. Capsule has a defined layer with an outer edge but slime layer is not well defined. In some cases the polymers are tightly integrated with the cell called capsule while in others they are loosely associated and are called a slime layer.

Capsule helps in attachment. Capsules provide a protective function to bacterial cells. Prevents ingestion by phagocytes (WBC) allowing bacteria to evade destruction, capsules are associated with pathogen ability to cause disease, capsules prevent cell drying.

Slime layer is involved in attachment of bacteria (including pathogens) to other cells or environmental surfaces in order to colonize and form biofilms. It may contribute to virulence by protecting the bacterium against complement attack and phagocytosis. The S-layer may protect bacteria from harmful enzymes or changes in pH. 4. It protects bacteria from desiccation. Slime layers can also be used as a food reserve for the cell.

Cell Wall: Cell wall is the structure exterior to cytoplasm. It is situated in the outermost surface and is about 15-30 nm thick and constitutes 10-25% of the total cell dry weight. It includes the cytoplasmic membrane and one or two other layers in most prokaryotes.

It is relatively porous and is not considered to be a permeability barrier for small substrates. The structure of the cell wall greatly differs among the gram positive and the gram negative bacteria. The cell wall is mainly composed of the polymer Peptidoglycan. The molecule Peptidoglycan is exclusively found in

bacterial cell walls which impart rigidity and shape to the bacterial cell. This layer surrounds plasma membrane and acts as a protective coat.

Peptidoglycan is a polymer made up of many interlocked chains of monomers. N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) which are glucose derivatives and form the backbone of peptidoglycan. The NAG and NAM chains are linked by interpeptide bridges. A bacterium is either gram-negative or gram –positive based on the amount and location of peptidoglycan. There are a set of identical tetrapeptide side chains attached to the N-acetyl-muramic acid which differs in the composition and binding modes in gram positive and gram negative bacteria.

Differences between cell wall of Gram positive and Gram negative bacteria

Characteristics	**Gram positive**	**Gram negative**
Wall thickness	Thick 20-80 nm	Thin 10 nm
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Teichoic acids in wall	Present	Absent
Lipid and lipoprotein content	0-3%	58%
Protein content	Nil	9%
Lipopolysaccharide content	Nil	13%
Sensitivity to lysozyme and penicillin	High	Low

The characteristic shape of the bacterial cell is maintained by the cell wall. The effects of osmotic pressure on the cell are countered by the cell wall. Cell walls also have an essential role in cell division

The cell wall acts as a site of attachment for bacteriophages, acts as a firm support for structures like flagella, fimbriae, and pili

Cell walls act as important sites of major antigenic determinants of the cell surface and are also responsible for development of resistance of Antibiotics

5.7. CHECK YOUR PROGRESS

1. Discuss the detailed structure of bacterial cell wall
2. Explain the structure and composition of slime layer/capsule
3. What are the functions of bacterial cell wall?
4. What are the differences between the gram positive and gram negative bacterial cell wall?

5.8. KEY WORDS

Bacterial surface layers, capsule, glycocalyx, slime layer.

5.9. FURTHER SUGGESTED READING

1. Alcamo. 2001. Fundamentals of Microbiology Sixth Edition. By, Edward Alcamo. Jones and Bartlett Publishers, London.
2. Aneja K.R., Jain P. and Aneja R. "*A Text Book of Basic and Applied Microbiology*" New Age International Pub. New Delhi (2008).
3. Gerhardt P.R., Murray G.E., Costlow R.N., Nester E.W.,Wood E.A., Kreig N.R., and Phillips G.B.(eds) "*Manual of Methods for General Bacteriology.* American Society for Microbiology, Washington D.C. (1981)
4. James T. Drummond, David White, Clay Fuqua. 2011. The Physiology and Biochemistry of Prokaryotes 0004 Edition. Oxford University Press, USA
5. Pelczar M.J., Chan E.C.S. and Kreig N.R. "*Microbiology* – 5th edn., Tata McGraw-Hill Pub. Co. New Delhi (1986)
6. Purohit, S.S. 2006. Microbiology – Fundamentals and Application. Seventh Edition. Agrobios (India) Publishers, Jodhpur.
7. Ravi Mantha. 2012. All about bacteria. Collins Publications.
8. Stanier, R.Y., Ingraham, J.L., Wheelis, M.L., and Painter, P.R. 2007. General Microbiology Fifth Edition. McMillan Publishers, London.
9. Talaro K.P. and Talaro A. Foundations in Microbiology 6th edn. McGraw Hill (2006).

10. Trivedi, P.C. 2006. Applied Microbiology. Agrobios (India) Publishers, Jodhpur.

5.10. SOURCES

1. Ammar et al .,2004. An attachment tip and pili-like structures in insect- and plantpathogenic spiroplasmas of the class Mollicutes. Arch Microbiol 181: 97-105.

2. B. H. Kim and G. M. Gadd. 2008. Bacterial Physiology and Metabolism. Cambridge University Press.

3. Bacteriology. BI 3206 Lecture notes.

4. Cabeen, M. T. and Jacobs-Wagner. C. 2005. Bacterial cell shape. Nature Reviews,Microbiolgy, pp.601-610.

5. J. W. Dale and S. F. Park. 2004. Molecular Genetics of Bacteria 4th Edition

6. Ken Jarrell. Editor. 2009. Pili and flagella. Caister Academic Press

7. Prescott,2006. Procaryotic cell structure and function, Chapter 3. pp.39-78.

8. Seelke, R.W. 2010. Microbiology Lecture Notes.

9. Todar, K. 2003. Structure and function of prokaryotic cells. University of Wisconsin-Madison Department of Bacteriology.

UNIT 6

COMPOSITION AND FUNCTION OF BACTERIAL CELL MEMBRANE

STRUCTURE

6.1. OBJECTIVES

After reading this section we will learn about

- Composition and structure of bacterial cell membrane
- The Fluid-mosaic model of the bacterial cell membrane
- The functions of the bacterial cell membrane

6.2. INTRODUCTION

The bacterial cytoplasmic membrane is composed of a phospholipid bilayer and proteins and encloses the contents of the bacterial cell. The cell membrane acts as a selective barrier separating the cell machinery from the exterior of cell wall and outside. The cell membrane is selectively permeable and plays a role in transportation of solutes inside the cells. The bacterial cell membrane participates in the electron transport and oxidative phosphorylation. The cell wall also takes part in excretion of hydrolytic exoenzymes. Cell membrane is the site of synthesis of DNA, cell wall polymers and membrane lipids.

6.3. STRUCTURE OF THE CELL MEMBRANE

The cell membrane is thin, elastic, selectively permeable membrane found internal to the cell wall. The cell membrane is composed of phospholipids (40%) and proteins (60%). It measures approximately 5-10 nm in thickness. The cytoplasmic membrane is made up of a bilayer of phospholipids (Fig. 1). Within his lipid bilayer several proteins are embedded which control what goes in and out of the cell. The proteins may be attached to any one side of the membrane, or to some other proteins of the membrane. Some proteins may occupy the entire span of the membrane on both the sides.

The cytoplasmic membrane is fluidic and delicate. The inner portion of this membrane is made up of lipid or fat molecules which make different molecules

dissolved in water impermeable to enter, however, water molecules move freely through this layer. One important characteristic of the cytoplasmic membrane is that protons (H+) are unable to cross the cytoplasmic membrane. The inner region of the membrane is hydrophobic. In the bilayered membrane, the lipid molecules form two layered facing each other. The phosphate groups are hydrophilic and positioned on the outer edges of the membrane. Many of the proteins involved in the transportation of molecules into or out of the cells are found embedded in the lipid bilayer. These proteins act as sponge that absorb the nutrients and allow the cell to live in very dilute nutrient solutions.

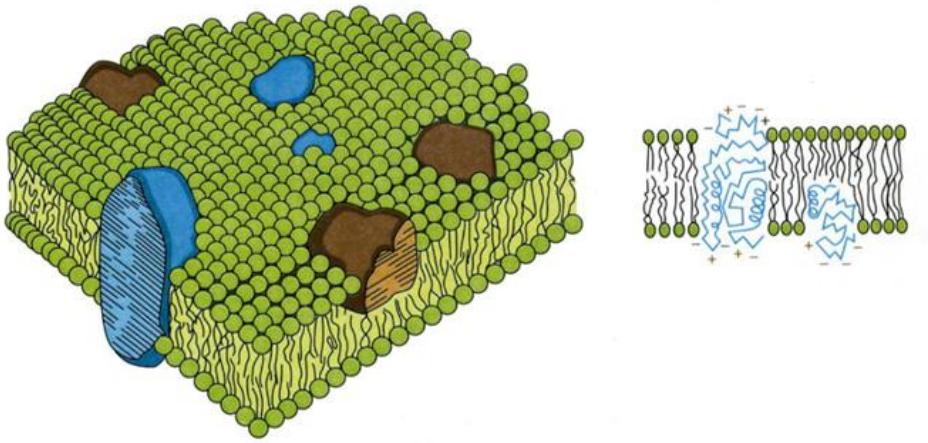

Figure 1: Structural illustration of the bacterial cell membrane

6.4. Fluid Mosaic Model of the Cell Membrane

In 1972, Jonathan Singer and Garth Nicolson proposed the fluid mosaic model of membrane structure. In this model, membranes are viewed as two-dimensional fluids in which proteins are inserted into lipid bilayers (Fig. 2). Cytoplasmic membranes are composed of a Phospholipid Bilayer with various protein molecules floating around within it. The 'Fluid' part represents how some parts of the membrane can move around freely, if they are not attached to other parts of the cell. The 'mosaic' part illustrates the 'patchwork' of proteins that is found in the Phospholipid Bilayer.

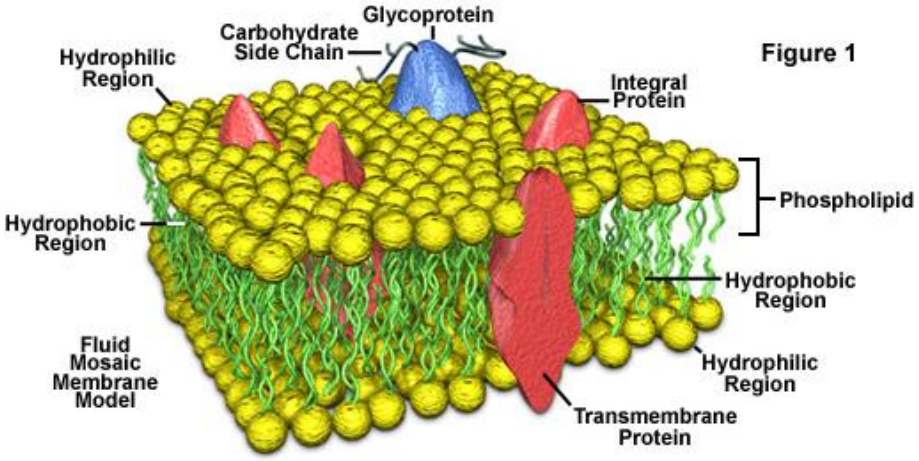

Figure 2: The Fluid-Mosaic model of the bacterial cell membrane

The membranes have two types of proteins, 'Intrinsic/integral' proteins which completely span the bilayer and 'Extrinsic/peripheral' proteins which are partly embedded in the bilayer (Fig. 3). Carbohydrate Polymers may attach to parts of the membrane, forming glycolipids when attach to phospholipid molecules and glycoproteins when they attach to proteins. Both glycolipids and glycoproteins can act as cell receptor sites. Hormones and drugs bind to them to instigate a response within the cell. They may also be involved in cell signalling in the immune system.

Some intrinsic proteins are channel proteins which are transport proteins that allow the movement of molecules that are normally too large or too hydrophilic to pass through the membrane by forming a tube-like structure that goes through the whole membrane. Other transport proteins are carrier proteins which use energy in the form of ATP to actively move substances across the membrane.

Enzymes and coenzymes may be attached to part of the membrane in order to carry out metabolic reactions. Mitochondria have infoldings of the membrane (called Cristae) containing enzymes which are partly responsible for aerobic respiration.

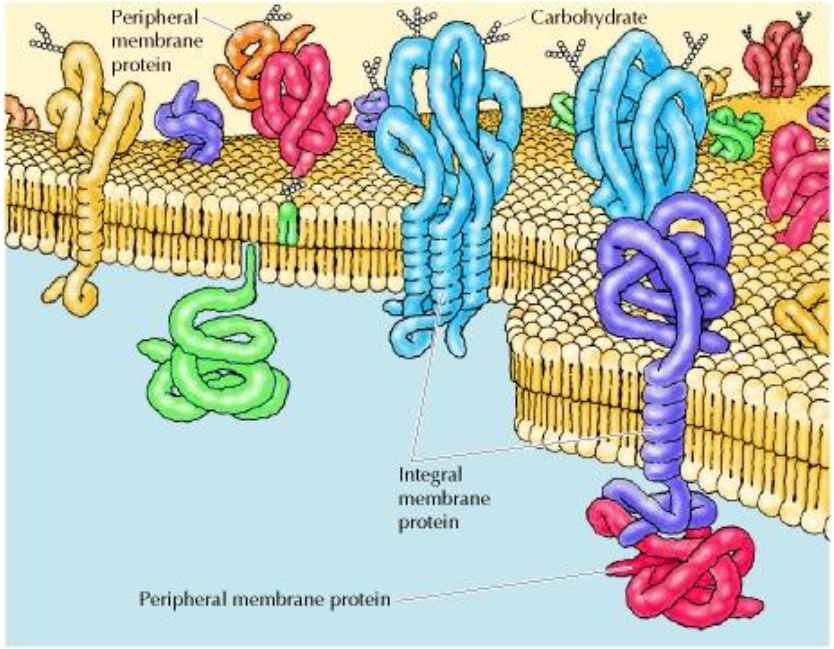

Figure 3: The structure and arrangement of integral and peripheral proteins in bacterial cell membrane

6.5. FUNCTIONS OF THE CELL MEMBRANE

1. The cell membrane is selectively permeable and controls passage of substances into and out of the cell. There are numerous proteins moving within or upon this layer that are primarily responsible for transport of ions, nutrients and waste across the membrane.
2. It controls the transportation of electrons and protons for cellular metabolism.
3. The cell membrane contains enzymes for synthesis and transport of cell wall substances and metabolism, it also secretes hydrolytic enzymes.
4. The cell membrane regulates cell division. It is also involved in protein assembly and secretion.
5. The cell membrane is the site for respiration (production of energy), synthesis of cell wall components, DNA synthesis, transport and secretion of molecules and acts as an osmotic barrier.
6. The cell membrane is also involved in energy transducing functions (ATP synthesis) by causing establishment of proton motive force.

7. The cell membrane is involved in chemotactic sensing and motility to taxis. It is also involved in locomotion by having a part of flagellar apparatus.
8. The cell membrane is involved in attachment, replication, segregation and formation of septum and thus cell division.
9. The cell membrane harvests light energy in photosynthetic prokaryotes.

6.6. SUMMARY

The cell membrane is thin, elastic, selectively permeable membrane found internal to the cell wall. The cytoplasmic membrane is made up of a bilayer of phospholipids. Within his lipid bilayer several proteins are embedded which control what goes in and out of the cell. The proteins may be attached to any one side of the membrane, or to some other proteins of the membrane. Some proteins may occupy the entire span of the membrane on both the sides.

The inner region of the membrane is hydrophobic. In the bilayered membrane, the lipid molecules form two layered facing each other. The phosphate groups are hydrophilic and positioned on the outer edges of the membrane. Many of the proteins involved in the transportation of molecules into or out of the cells are found embedded in the lipid bilayer.

In 1972, Jonathan Singer and Garth Nicolson proposed the fluid mosaic model of membrane structure. In this model, membranes are viewed as two-dimensional fluids in which proteins are inserted into lipid bilayers.

The membranes have two types of proteins, 'Intrinsic/integral' proteins which completely span the bilayer and 'Extrinsic/peripheral' proteins which are partly embedded in the bilayer.

Carbohydrate Polymers may attach to parts of the membrane, forming glycolipids when attach to phospholipid molecules and glycoproteins when they attach to proteins. Both glycolipids and glycoproteins can act as cell receptor sites.

The cell membrane regulates cell division. The cell membrane contains enzymes for synthesis and transport of cell wall substances and metabolism, it also secretes hydrolytic enzymes. It is also involved in protein assembly and secretion.

The cell membrane is the site for respiration (production of energy), synthesis of cell wall components, DNA synthesis, transport and secretion of molecules and acts as an osmotic barrier.

The cell membrane is involved in chemotactic sensing and motility to taxis. It is also involved in locomotion by having a part of flagellar apparatus.

The cell membrane harvests light energy in photosynthetic prokaryotes

6.7. CHECK YOUR PROGRESS

1. Explain the composition of the bacterial cell membrane
2. Discuss in detail the fine structure of bacterial cell membrane
3. Explain the fluid-mosaic model of the bacterial cell membrane
4. What are intrinsic and extrinsic proteins?
5. What are the functions of the bacterial cell membrane?

6.8. KEYWORDS

Bacterial cytoplasmic membrane, phospholipid bilayer, Fluid Mosaic Model, intrinsic/integral proteins, extrinsic/peripheral' proteins, functions of the cell membrane.

6.9. FURTHER SUGGESTED READING

1. Alcamo. 2001. Fundamentals of Microbiology Sixth Edition. By, Edward Alcamo. Jones and Bartlett Publishers, London.
2. Aneja K.R., Jain P. and Aneja R. "*A Text Book of Basic and Applied Microbiology*" New Age International Pub. New Delhi (2008).
3. Gerhardt P.R., Murray G.E., Costlow R.N., Nester E.W.,Wood E.A., Kreig N.R., and Phillips G.B.(eds) "*Manual of Methods for General Bacteriology*. American Society for Microbiology, Washington D.C. (1981)
4. James T. Drummond, David White, Clay Fuqua. 2011. The Physiology and Biochemistry of Prokaryotes 0004 Edition. Oxford University Press, USA
5. Pelczar M.J., Chan E.C.S. and Kreig N.R. "*Microbiology* – 5th edn., Tata McGraw-Hill Pub. Co. New Delhi (1986)
6. Purohit, S.S. 2006. Microbiology – Fundamentals and Application. Seventh Edition. Agrobios (India) Publishers, Jodhpur.
7. Ravi Mantha. 2012. All about bacteria. Collins Publications.
8. Stanier, R.Y., Ingraham, J.L., Wheelis, M.L., and Painter, P.R. 2007. General Microbiology Fifth Edition. McMillan Publishers, London.
9. Talaro K.P. and Talaro A. Foundations in Microbiology 6th edn. McGraw Hill (2006).
10. Trivedi, P.C. 2006. Applied Microbiology. Agrobios (India) Publishers, Jodhpur.

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1. Ammar et al .,2004. An attachment tip and pili-like structures in insect- and plantpathogenic spiroplasmas of the class Mollicutes. Arch Microbiol 181: 97-105.
2. B. H. Kim and G. M. Gadd. 2008. Bacterial Physiology and Metabolism. Cambridge University Press.
3. Bacteriology. BI 3206 Lecture notes.

4. Cabeen, M. T. and Jacobs-Wagner. C. 2005. Bacterial cell shape. Nature Reviews, Microbiolgy, pp.601-610.

5. J. W. Dale and S. F. Park. 2004. Molecular Genetics of Bacteria 4th Edition

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7. Prescott,2006. Procaryotic cell structure and function, Chapter 3. pp.39-78.

8. Seelke, R.W. 2010. Microbiology Lecture Notes.

9. Todar, K. 2003. Structure and function of prokaryotic cells. University of Wisconsin-Madison Department of Bacteriology.

UNIT 7

BACTERIAL NUCLEAR MATERIAL

STRUCTURE

7.1. OBJECTIVES

After reading this unit we will be able to understand:

- The structure and composition of bacterial nuclear material
- The bacterial genome and bacterial chromosome
- Diversity in size and arrangement of bacterial chromosome
- Genome organization in different bacteria
- Bacterial plasmids and their importance
- Differences between a bacterial chromosome and a bacterial plasmid
- Plasmids transfer, plasmid replication and bacterial conjugation

7.2. INTRODUCTION

The cytoplasm of the bacterial cell contains the bacterial chromosome (nucleoid), ribosomes, and several hundred proteins and enzymes (Fig. 1). Here, the chromatin or genetic materials are concentrated. The bacterial cell lacks nuclear membrane and nucleolus. The nuclear material lacks any definite shape since it has no membrane. The bacterial chromosome is typically one large circular molecule of DNA, more or less free in the cytoplasm. The total DNA content of bacteria is referred to as the cell genome. The cell chromosome is the genetic control centre of the cell which determines all the properties and functions of the bacterium. During cell growth and division, the prokaryotic chromosome is replicated in to make an exact copy of the molecule for distribution to progeny cells.

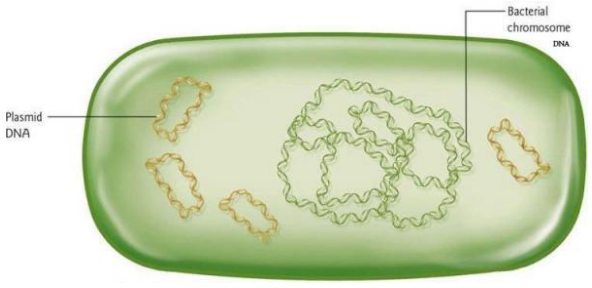

Figure 1: A bacterial cell with prokaryotic nucleoid/bacterial chromosome

7.3. THE BACTERIAL GENOME

The important feature of the bacterial cytoplasm is the genetic material/genome. The genetic material is located in the central region of the cell and it is called the nucleoid. The genome is a clump or coil of DNA that controls all the functions of the bacterial cell and produces the proteins that the bacterium needs to survive. This nucleoid does not have a membrane and the bacterial DNA is found free-floating. The bacterial DNA is 2-3% of cell weight but covers 10 or more % of volume of cell. In some bacteria the DNA is attached to the cell membrane. Bacterial DNA does not have histone protein and does not coil to form well defined chromosome during multiplication.

7.4. BACTERIAL CHROMOSOMES

The genetic material in bacteria occurs in the form of a molecule of double stranded DNA (Fig. 2 and 3). On a macroscopic scale, bacterial chromosomes are either circular or linear. Circular chromosomes are most common, at least among the best-studied bacteria. However, the causative agent of Lyme disease, *Borrelia burgdorphei*, has a 2-Mb linear chromosome plus 12 different linear plasmids.

Unlike the chromosomes of eukaryotes, the bacterial chromosomes do not have histones. The chromosomes occur in the form of dispersed fibrous areas called nucleotides or chromatin network.

The DNA is in the form of a double helix. It is permanently attached to a mesosome, an infolding of the plasma membrane. A small amount of protein, mainly in the form of an enzyme called RNA polymerase, may be found associated with the bacterial chromosome. The amount of DNA is much less and codes for fewer proteins compared to an eukaryotic cell. In the bacterium Escherichia coli the 1100 Mm long DNA is packed into a space of just 1 Mm due to intense coiling. It has about 2500 genes.

Most bacteria have a single chromosome with DNA that is about 2Mbp (mega base pairs) long (1Mbp 51000000 base pairs), but the DNA content of

different species varies from 0.58 to greater than 9 Mbp of DNA, and some bacteria have multiple chromosomes. For example, *Leptospira* has two chromosomes of4.4 and 4.6 Mbp and the largest bacterial genome yet analysed is that of *Myxococcus xanthus*, with 9.2 Mbp (9200000 bp). However, the best studied organism in nature is E. coli, which has a 4.6-Mbp chromosome with 4288 genes for proteins, seven operons for ribosomal ribonucleic acids (RNAs), and 86 genes for transfer RNAs. The *E. coli* chromosome contains numerous gene families (Fig. 4). By contrast, the smallest free living organism is *Mycoplasma genatalium* with a 0.58-bacteria have complete information for the synthesis of cell walls, cell membranes and critical enzymes of intermediary metabolism, plus the RNA molecules, ribosomal proteins and a clutch of enzymes (the replisome) to replicate DNA efficiently.

Other bacteria having multiple chromosomes include *Agrobacterium tumefaciens, Rhizobium, Brucella, Paracoccus denitrificans, Ochrobactrum anthropi, Leptospira interrogans, Burkholderia, Vibrio cholerae, Deinococcus radiodurans*, and many others from diverse groups of bacteria.

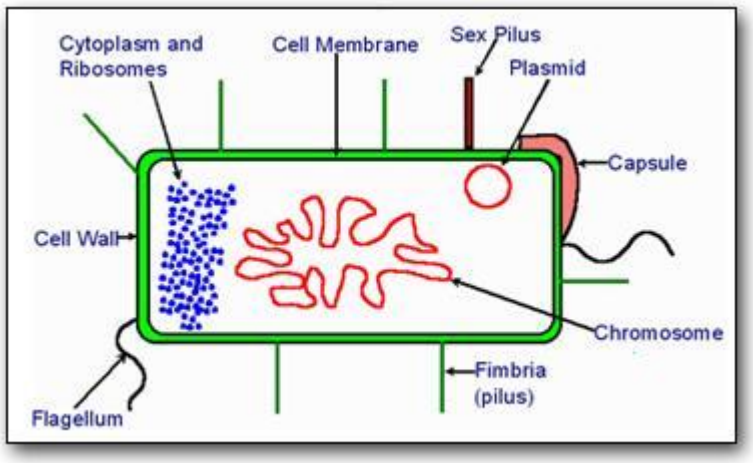

Figure 2: Diagrammatic representation of a Bacterial Chromosome

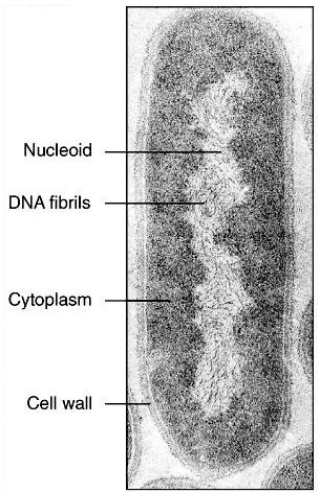

Figure 3: The fine structure of bacterial nuclear material

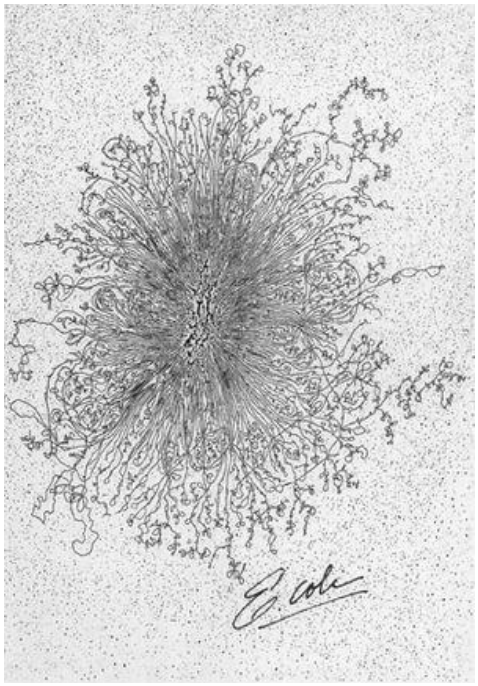

Figure 4: The bacterial chromosome as seen in *Escherichia coli* which is a supercoiled chromosome

Genome organization in different bacteria

Bacteria	Chromosome(s)	Plasmid(s)
Agrobacterium tumefaciens	One linear (2.1Mb) + one circular (3.0 Mb)	Two circular (450 + 200 Kb)
Bacillus thuringiensis	One circular (5.7 Mb)	Six (each > 50 Kb)
Borrella	One linear (0.91 Mb)	Multiple circular + linear (5-200 Kb)
Buchnera sp. Strain APS	One circular (640 Kb)	Two circular (< 7.8 Kb each)
Deinococcus radiodurans	Two circular (2.6 + 0.4 Mb)	Two circular (177 + 45 Kb)
Rhizobacterium meliloti	Two circular (3.4 + 1.7 Mb)	One circular megaplsamid (1400 Kb)
Xylella fastidiosa	One circular (2.7 Mb)	Two circular (51 + 13 Kb)

Chromatin is a term that refers not just to DNA but to the proteins attached to a chromosome. In the dimensions of B-form DNA, *E. coli* is a sphere that is 6 kbp long (8 nm) and 4 kbp wide (2 nm), so the 4.6-Mbp chromosome must be folded many times to fit within a cell. Negative supercoiling forces DNA into an interwound configuration. Interwound supercoiling is produced at the expense of ATP by the enzyme gyrase, and supercoiling produces two important consequences. First, the DNA molecule doubles back on itself so that length is halved relative to that of the linear form. Second, supercoiled branches are dynamic so that opposing DNA sites in the interwound network are constantly changing. A protein bound to DNA in one supercoiling domain interacts more than 100 times more frequently with other proteins in the same domain than it does with proteins bound to a different domain. When DNA is liberated from cells by breaking the peptidoglycan coat, chromosomes form bundled loops that represent domains. Such preparations (called nucleoids) behave as discrete bodies. Many reactions of the chromosome require the formation of intricate DNA–protein machines to replicate, transcribe or

recombine DNA at specific sequences. A group of 'chromosome-associated' proteins assists in the formation of these complexes by shaping DNA. These proteins are sometimes described as histone-like, although they share no structural similarity with the eukaryotic histones. Chromosome-associated proteins include HU, H-NS, integration host factor (IHF) and factor for inversion stimulation (FIS). HU is encoded by two genes, hupA and hupB, which are closely related to each other and to the genes encoding the two IHF subunits.

7.5. BACTERIAL PLASMIDS

Bacteria sometimes possess smaller extrachromosomal pieces of DNA called plasmids. Plasmids are small genetic structures found in many bacteria that contain strands of coiled DNA. These extra chromosomal strands of DNA do not carry genetic material that is essential the organism. They are independent and self replicating. Plasmid DNA is not used in reproduction.

Plasmids range in size from 1 kbp (Kilo base pair) (1000 bp) to 100 kbp, and these DNA molecules encode genetic systems for specialized functions (Fig. 5). Some plasmids make extracellular appendages that allow bacteria to infect and colonize sensitive eukaryotic hosts. Plasmids often carry genes that confer on bacteria the ability to survive in the presence of antibiotics such as tetracycline, kanamycin and penicillin. Many plasmids also contain genes that promote DNA transfer so that plasmid genes can move into other bacterial species. Plasmid transfer has caused the emergence of bacterial pathogens that are resistant to most of the useful antibiotics in medicine, with notable examples including multidrug-resistant strains of *Staphylococcus* and *Mycobacterium tuberculosis*. Plasmids are also used in biodegradation of a variety of toxic substances such as toluene and other organic hydrocarbons, herbicides, and pesticides.

Plasmids have variable properties F-Plasmids, R-Plasmids and Ti Plasmids respectively enhance fertility factors, resistance to antibiotics and tumor inducing properties. Similarly, virulent plasmids cause disease symptoms. These characters have great importance in genetic engineering and crop improvement.

Another type of plasmid called episome may be present. Episomes can integrate into the bacterial chromosomes in contrast to the plasmids.

The plasmids can be easily isolated from or introduced into the bacterial cells. They can be integrated with desired genes. Hence, plasmids are of immense use in genetic engineering.

Plasmids are capable of being stably inherited without being linked to the chromosome and can be transferred horizontally between cells. One of the most important aspects of bacterial plasmids is their carriage and spread of antibiotic resistance genes that ultimately have an impact on the treatment of diseases of animals and humans. They can also carry genes that code for a wide range of metabolic activities, enabling their host bacteria to degrade pollutant compounds, and produce antibacterial proteins (colicins). They can also harbour genes for virulence that help to increase pathogenicity of bacteria causing diseases such as plague, dysentery, anthrax and tetanus

Plasmids that can coexist within a bacterium are said to be compatible. Plasmids which cannot coexist are said to be incompatible and after a few generations one or other of these is lost from the cell. On a clinical level plasmids have been classified based upon their incompatibility grouping. Plasmids that encode their own transfer between bacteria are termed conjugative. Non-conjugative plasmids do not have these transfer genes but can be carried along by conjugative plasmids via a mobilisation site. This ability ensures that conjugative plasmids are highly promiscuous and can be found in a wide variety of bacteria (thereby having a broad host range; BHR).

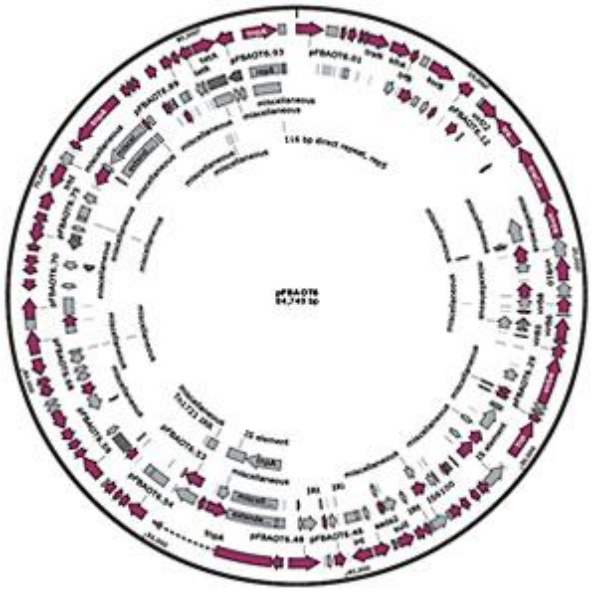

Figure 5: A plasmid map of pFBAOT6 (c) *Glenn Rhodes*

Differences between a bacterial chromosome and a bacterial plasmid

Sl. No.	Bacterial chromosome	Bacterial Plasmid
1.	They have several thousands of genes	They have very few genes
2.	They have a centromere and two sister chromatids	They do not have a centromere and chromatids
3.	They are covered with proteins	They are not covered with protein
4.	Their size is large expressed in mega units	They are smaller in size ranging from 1.0-1400 kb.
5.	They do not function as gene carriers	They are used as gene vectors to insert DNA into foreign hosts
6.	They carry genetic information	They are involved in bacterial conjugation and antibiotic production

7.6. PLASMIDS TRANSFER

Plasmids are transferred horizontally, among bacteria, with a process of conjugation, that is, by intercellular contact in which donor's DNA (a cell which gives a plasmid) is transferred to recipient (a cell which receives a plasmid) (Fig. 6). This process usually is regulated by genes carried by conjugative plasmids, which code creation of pili which are necessary for a contact between cells. The conjugation requires the presence of broad genetic region of plasmid DNA. Plasmids from the same incompatibility group have usually emphasized DNA homology and they code similar F-pili and conjugation systems. During conjugational transfer of DNA occurs in the following steps:

1. Initially a "nick" is formed and DNA transfer at the origin of transfer (oriT) begins.

2. The two plasmid DNA chains are segregated.

3. One of the DNA chains is transferred into recipient cell.

4. Complete plasmid DNA is synthesised in both the donor and the recipient cells.

5. Circular forms of plasmid replicons are formed.

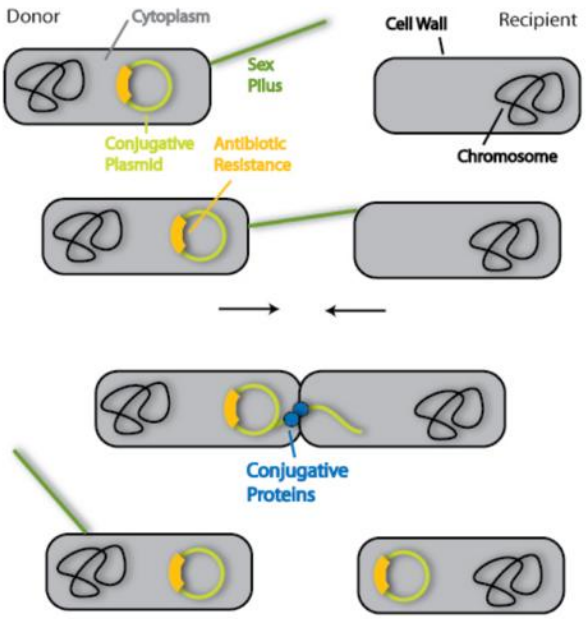

Figure 6: Diagrammatic representation of the conjugation process in bacteria

7.7. PLASMID REPLICATION PROCESS

This can be divided into three stages: initiation, elongation, and termination. F-pilus makes specific contact with potential recipient cell and its retraction leads to close connection between cell membranes of a donor and a recipient. A small canal, formed in this way, allows DNA transfer, or through the pilus itself, which is wide enough to enable passing of single chain DNA, or the fusion of cell membranes at the contact spot which leads to creation of transmembrane pore as a passage for DNA. Initiation is catalyzed most frequently by one or a few plasmid-encoded initiation proteins that recognize plasmid-specific DNA sequences and determine the point from which replication starts (the origin of replication). Transfer of plasmid-specific DNA starts at the specific spot on plasmid molecule recognized as origin of transfer, or oriT. This spot is asymmetric and it is oriented in such a way so genetic region, which controls the transfer, transfers at the end (Fig. 7).

For mobilization of non-conjugative plasmids, an interaction between theirs oriT sequences and products of mob genes is required as well as identification of mobilization system by conjugational system of plasmids. The enzyme placed in cytoplasm, DNA helicase I, which uncoils DNA chain. Uncoiling of DNA requires binding of 70 – 80 molecules protein's enzymes to single chain DNA region of two hundred nucleotides. Multimer created in this way, migrates as a stabile complex along the DNA chain using the energy from adenosine triphosphate (ATP) for uncoiling. The migration develops to 5'-3' direction, and for the rate of DNA helicase I uncoiling it is estimated to be around 1200 base pairs per second. Enzyme which transcripts unwinding, closed, circled DNA duplex into negative, winding form is named as DNA gyrase.

Transfer of a single chain plasmid-specific DNA is always associated with the synthesis and the missing chain replacement along with a DNA polymerase III enzymes activities. The creation of the transferred DNA circled form is based probably on endonuclease enzymes activities. Integration of plasmid-specific DNA occurs by binding of the 5' end of the one molecule to the 3' end of another and vice versa

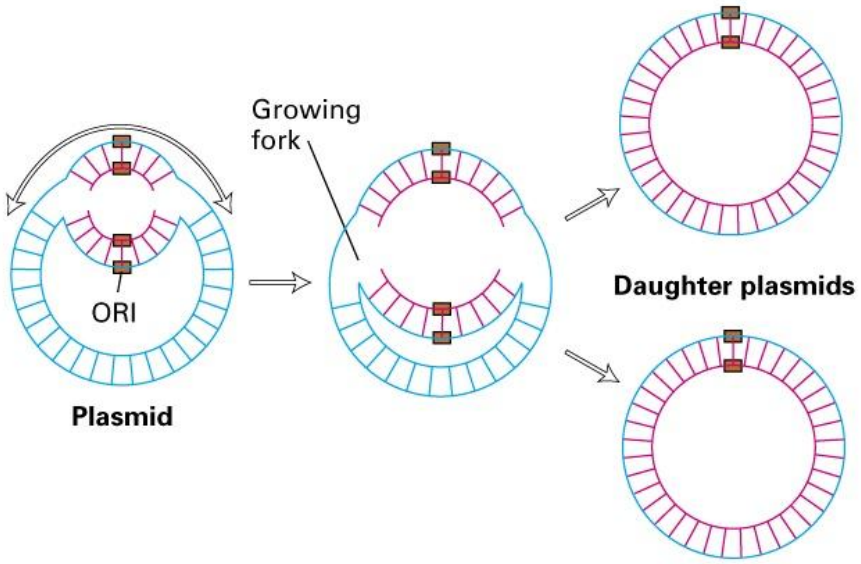

Figure 7: Diagrammatic representation of the plasmid replication process in bacteria

7.8. SUMMARY

In bacteria the genetic material is located in the central region of the cell and it is called the nuclioid. The genetic material in bacteria occurs in the form of a molecule of double stranded DNA.

This nucleoid does not have a membrane and the bacterial DNA is found free-floating. Bacterial DNA does not have histone protein and does not coil to form well defined chromosome.

The DNA is in the form of a double helix. It is permanently attached to a mesosome, an infolding of the plasma membrane. A small amount of protein, mainly in the form of an enzyme called RNA polymerase, may be found associated with the bacterial chromosome.

Most bacteria have a single chromosome with DNA that is about 2Mbp (mega base pairs) long (1Mbp 51000000 base pairs), but some bacteria have multiple chromosomes. For example, *Leptospira* has two chromosomes of4.4 and 4.6 Mbp.

Bacteria sometimes possess smaller extrachromosomal pieces of DNA called plasmids. Plasmids are small genetic structures found in many bacteria that contain strands of coiled DNA. Plasmids range in size from 1 kbp (Kilo base pair) (1000 bp) to 100 kbp.

These extra chromosomal strands of DNA do not carry genetic material that is essential the organism. They are independent and self replicating. Plasmid DNA is not used in reproduction.

Some plasmids make extracellular appendages that allow bacteria to infect and colonize sensitive eukaryotic hosts. Plasmids often carry genes that confer on bacteria the ability to survive in the presence of antibiotics such as tetracycline, kanamycin and penicillin.

Many plasmids also contain genes that promote DNA transfer so that plasmid genes can move into other bacterial species. Plasmid transfer has caused the emergence of bacterial pathogens that are resistant to most of the useful antibiotics in medicine, with notable examples including multidrug-resistant strains of Staphylococcus and Mycobacterium tuberculosis.

Plasmids are also used in biodegradation of a variety of toxic substances such as toluene and other organic hydrocarbons, herbicides, and pesticides.

Plasmids that can coexist within a bacterium are said to be compatible. Plasmids which cannot coexist are said to be incompatible and after a few generations one or other of these is lost from the cell. On a clinical level plasmids have been classified based upon their incompatibility grouping. Plasmids that encode their own transfer between bacteria are termed conjugative. Non-conjugative plasmids do not have these transfer genes but can be carried along by conjugative plasmids via a mobilisation site. This ability ensures that conjugative plasmids are highly promiscuous and can be found in a wide variety of bacteria.

Plasmids are transferred horizontally, among bacteria, with a process of conjugation, that is, by intercellular contact in which donor's DNA (a cell which

gives a plasmid) is transferred to recipient (a cell which receives a plasmid). This process usually is regulated by genes carried by conjugative plasmids, which code creation of pili which are necessary for a contact between cells. The conjugation requires the presence of broad genetic region of plasmid DNA. Plasmids from the same incompatibility group have usually emphasized DNA homology and they code similar F-pili and conjugation systems.

During conjugational transfer of DNA occurs in the following steps:

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2. The two plasmid DNA chains are segregated.
3. One of the DNA chains is transferred into recipient cell.
4. Complete plasmid DNA is synthesised in both the donor and the recipient cells
5. Circular forms of plasmid replicons are formed

7.9. CHECK YOUR PROGRESS

1. Discuss the structure and composition of bacterial nuclear material.
2. Explain in detail the bacterial genome.
3. What are bacterial chromosomes? Discuss their structure and composition.
4. Add a note on genome organization in different bacteria
5. What are bacterial plasmids?
6. What are the differences between a bacterial chromosome and a bacterial plasmid?
7. What is the role of plasmids in bacterial conjugation?
8. Explain the process of plasmids transfer
9. Discuss in detail the plasmid replication process

7.10. KEY WORDS

Bacterial chromosome, nucleoid, bacterial genome, bacterial chromosomes, bacterial plasmids, plasmids transfer, conjugation, plasmid replication.

7.11. FURTHER SUGGESTED READING

1. Alcamo. 2001. Fundamentals of Microbiology Sixth Edition. By, Edward Alcamo. Jones and Bartlett Publishers, London.
2. Aneja K.R., Jain P. and Aneja R. "*A Text Book of Basic and Applied Microbiology*" New Age International Pub. New Delhi (2008).
3. Gerhardt P.R., Murray G.E., Costlow R.N., Nester E.W.,Wood E.A., Kreig N.R., and Phillips G.B.(eds) "*Manual of Methods for General Bacteriology.* American Society for Microbiology, Washington D.C. (1981)
4. James T. Drummond, David White, Clay Fuqua. 2011. The Physiology and Biochemistry of Prokaryotes 0004 Edition. Oxford University Press, USA
5. Pelczar M.J., Chan E.C.S. and Kreig N.R. "*Microbiology* – 5th edn., Tata McGraw-Hill Pub. Co. New Delhi (1986)
6. Purohit, S.S. 2006. Microbiology – Fundamentals and Application. Seventh Edition. Agrobios (India) Publishers, Jodhpur.
7. Ravi Mantha. 2012. All about bacteria. Collins Publications.
8. Stanier, R.Y., Ingraham, J.L., Wheelis, M.L., and Painter, P.R. 2007. General Microbiology Fifth Edition. McMillan Publishers, London.
9. Talaro K.P. and Talaro A. Foundations in Microbiology 6th edn. McGraw Hill (2006).
10. Trivedi, P.C. 2006. Applied Microbiology. Agrobios (India) Publishers, Jodhpur.

7.12. SOURCES

1. Biljana Miljkovic-Selimovic, Tatjana Babic, Branislava Kocic, Predrag Stojanovic, Ljiljana Ristic and Marina Dinic. 2007. Bacterial Plasmids. Acta Medica Medianae 2007; 46(4):61-65. www.medfak.ni.ac.yu/amm

2. Bacterial Chromosome. Tutorvista.com
3. Dale, J. W., and Park, S. F. 2004. Molecular Genetics of Bacteria 4th Edition.

4. Kenneth Todar. 2009. Structure and Function of Bacterial Cells. Lectures in Microbiology by Kenneth Todar PhD University of Wisconsin-Madison Department of Bacteriology.

5. Kim, B. H., and Gadd, G. M. 2008. Bacterial Physiology and Metabolism. Cambridge University Press.
6. Patrick Higgins, N. 2001. Chromosome Structure. Encyclopedia of life sciences. Macmillan Publishers Ltd, Nature Publishing Group / www.els.net.
7. Prescott. 2006. Prokaryotic cell structure and function, Chapter 3. pp.39-78.
8. Todar, K. 2003. Structure and function of prokaryotic cells. University of Wisconsin-Madison Department of Bacteriology.

UNIT 8

BACTERIAL RIBOSOMES

STRUCTURE

8.1. OBJECTIVES

After reading this unit we will be able to learn

- The structure and function of bacterial ribosomes.
- Ribosomes as sites of mRNA translation and protein synthesis.
- The two subunits of ribosomes namely 30S (small subunit) and 50S (large subunit).
- Role of bacterial ribosomes during protein synthesis
- Process of ribosome genesis and ribosomal assembly mechanisms
- The structure and function of 30S subunit, 16S rRNA and 30S ribosomal proteins
- The structure and function 50S subunit, 23S and 5S rRNA and 50S ribosomal proteins.

8.2. INTRODUCTION

Bacterial ribosomes are cytoplasmic nucleotproteins composed of RNA and proteins which are the sites of mRNA translation and protein synthesis. They translate the information encoded in messenger RNA (mRNA) into a polypeptide. Ribosomes are more or less spherical and distributed throughout the cytoplasm which are many in number and usually measure 15-20nm in diameter and have a mass of about 2.5 MDa, with RNA accounting for 2/3 of the mass. Clusters of ribosomes are called polysomes which are held together by messenger RNA (mRNA).

8.3. STRUCTURE OF BACTERIAL RIBOSOMES

Bacterial ribosomes have two subunits named as 30S (small subunit) and 50S (large subunit) (Fig. 1). The "S" stands for Svedbergs, a unit used to measure how fast molecules move in a centrifuge. When united, the ribosome has a

sedimentation coefficient of 70S as opposed to 80S due to tertiary structure. The structure of the bacterial ribosomes was first deduced by electron microscopy which showed that they are made up of two subunits. James Lake in the 1970s further determined the shape of each subunit (50S and 30S) and how the subunits link together (70S). In 1970, E. Kaldschmidt and H.F. Whittmann obtained complete resolution of both subunits and other proteins by two-dimensional gel electrophoresis. Kaldschmidt and Whittmann also identified proteins S1-S21 for the 30S ribosomal subunit and proteins L1-L33 for the 50S subunit. Joachim Frank in 1995, studied their structure in detail using cryoelectron microscopy. Masayasu Nomura and others determined the locations of proteins in subunits.

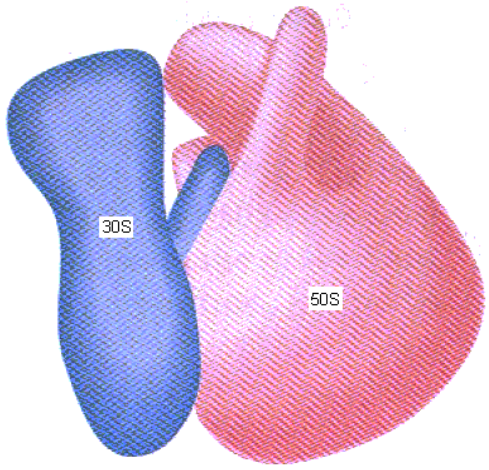

Figure 1: Structure of the bacterial ribosome showing the 30S and 50S subunits

During protein synthesis a ribosome moves along an mRNA molecule, reading the codon and attaching the correct amino acid (from the corresponding aminoacyl tRNA) to the growing protein chain (Fig. 2). When a stop codon is encountered, translation process stops ceases, and the mRNA and protein are released.

The two subunits assemble around a mRNA to be translated as such:

Figure 2: Diagrammatic representation of the movement of ribosome during mRNA synthesis

8.4. RIBOSOME GENESIS

Recent studies on the structures of a complete ribosome confirm that the ribosome is actually a ribozyme and the catalytic sites are located inside ribosomal RNA components. Information about how the ribosome assembles into a stable multi-component complex is still unclear. Ribosome genesis involves the following steps:

(1) Accurate ribosomal RNA and ribosomal protein folding

(2) Attaching and detaching of assembly factors

(3) Ribosomal protein modification and translation

(4) Binding of ribosomal proteins

(5) Processing, modification, and transcription of ribosomal RNA

8.5. RIBOSOMAL ASSEMBLY MECHANISMS

The metabolism and assembly of ribosomes is vital for the synthesis of other cellular components. Ribosomes are large and complex macromolecule and therefore require efficient steps during their assembly. The assembly of bacterial ribosomes proceeds through an alternating series of RNA conformational changes and protein-binding events. The assembly of these bacterial ribosomes occurs very

fast and these steps are simultaneously carried out with the transcription of the ribosomal RNAs. During this binding process the RNA folding process is stabilized by ribosome proteins.

Structure of ribosome is used to study principles of RNA, RNA protein recognition, protein folding, and the assembly of multi-component complexes. Ribosome assembly plays an important role in protein recognition. Ribosomes if not assembled properly may lead to various diseases.

8.6. THE 30S SUBUNIT

The 30S subunit comprises of a head with base and an extended arm like structure (Fig. 3). The 30S subunit plays vital role in mRNA decoding by providing the A, P, and E binding sites. It monitors the base-pairing between the codon on mRNA and the anticodon on tRNA. The 30S subunit is made up of 16SrRNA and 21 ribosomal proteins. The fine structure of 30S subunit is deduced using crystal diffraction and NMR methods.

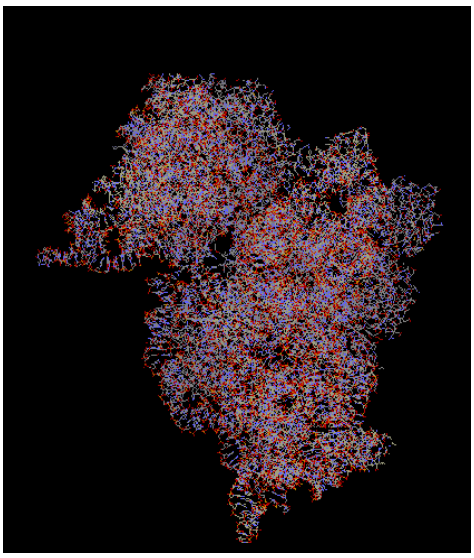

Figure 3: The structure of 30S subunit of the ribosome

8.6.1. 16S rRNA

Major bulk of 30S subunit is composed of 16S rRNA. 16S rRNA is vital for subunit association and translocational accuracy. 16S rRNA is comprised of 1542 bases and substrate binding A-, P-, and E- sites (Fig. 4). The P- site is located in the major groove in the upper portion of the rRNA and is occupied by the peptidyl-tRNA. Incoming aminoacyl-tRNA attaches to the A- site. This site is wide and deep giving it a lower affinity for tRNA for relocating to the P-site. Deacylated tRNAs while exiting occupy the E-site. This E-stie more associated with ribosomal proteins compared to the A- or P-site.

The primary structure of 16S rRNA is highly conserved. Its secondary structure is reminiscent if the clover shape of tRNA-small double helical regions are punctuated by short single stranded regions. The tertiary structure is the general shape of the whole subunit.

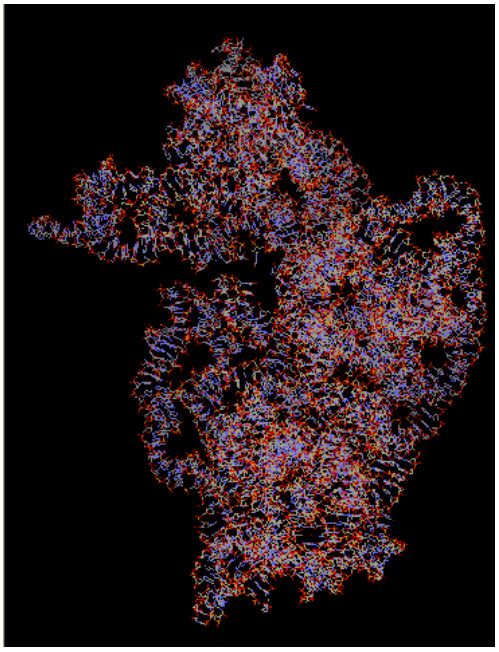

Figure 4: Structure of the 16S rRNA

The structure of the 16S rRNA has a 5' domain, central domain, 3' major domain, and a 3' minor domain. Major bulk of the 5’ domain is the 19 double helices. The 5’ domain traverses the ribosome diagonally. The central domain is an elongated, curved platform of nine helices, with the junction of helices 20, 21, and 22 at the center. The 3' major domain contains 15 helices which comprises the head. The 3' minor domain contains 2 helices and projects from the subunit to interact with the 50S subunit.

8.6.2. 30S RIBOSOMAL PROTEINS

The 21 ribosomal proteins of 30S are studied in *E. Coli* and are designated as S1 to S21. These proteins are categorised into three groups S1 and primary binding proteins, secondary binding proteins and late binding proteins (Fig. 5).

S1 and primary binding proteins: S4, S7, S8, S15, S17, S20 which independently bind to 16S rRNA.

Secondary binding proteins: S5, S6, S9, S12, S13, S16, S18, and S19 bind to the growing ribosome

Late binding proteins: S2, S3, S10, S11, S14, and S21.

Accurate tertiary folding of the RNA is initiated by binding of these proteins to helical junctions. Most of these proteins have one or more globular domains and contain lengthy extensions so as to reach all the RNA. The basic residues neutralize the repulsion charge of the RNA thus rendering additional stability. The structure is held together by electrostatic and hydrogen bonding interactions between the proteins.

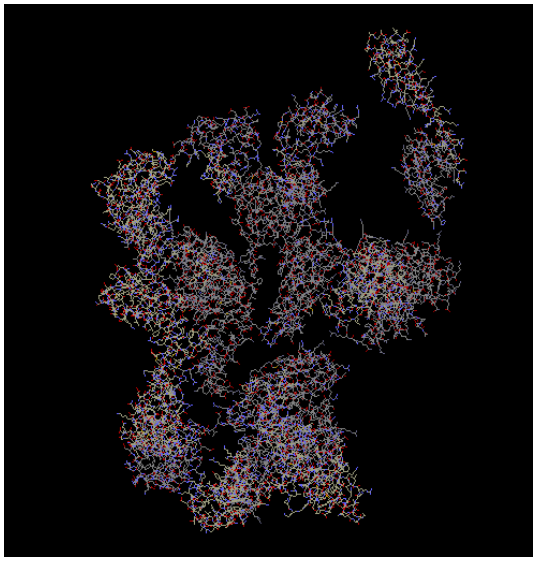

Figure 5: An image of the ribosomal proteins as located in the 30S subunit.

8.7. THE 50S SUBUNIT

This subunit is made up of two rRNA molecules the 23S and 5S rRNA. 23S rRNA has six interwoven domains that make-up most of the 50S subunit (Fig. 6). Portions of domains II, IV, V, and VI extend from the body of the 50S subunit. Some of these extensions form bridges with the 30S subunit. The most important feature of the 23S rRNA is the peptidyl transferase center, the site where peptide bonds are formed.

The 50S subunit functions in peptide bond formation through peptidyl transferase activity. GTP binding proteins can interact with this subunit helping in translational processes like initiation, elongation, and termination.

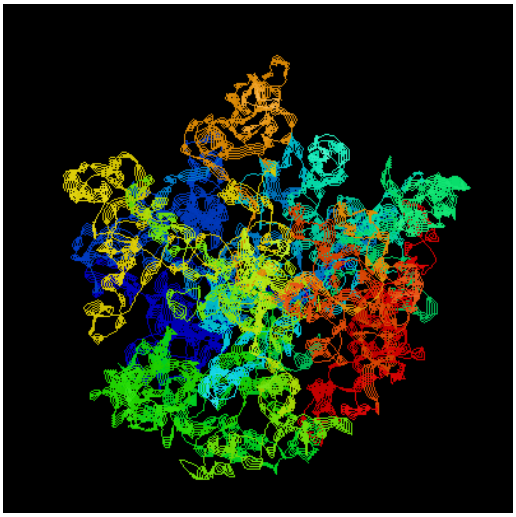

Figure 6: Structure of the 50S subunit of the ribosome

8.7.1. 23S rRNA

The proteins of this large subunit participate in stabilization of the three-dimensional rRNA structure (Fig. 7). Further, most of these proteins are involved in initiation, elongation, or termination events. These proteins aggregate near the periphery of the complex indicating their involvement in maintain the conformity of mRNA and tRNA binding in translation.

So far, 28 of the 34 proteins contained within the large 50S ribosomal subunit have been isolated and structurally identified.

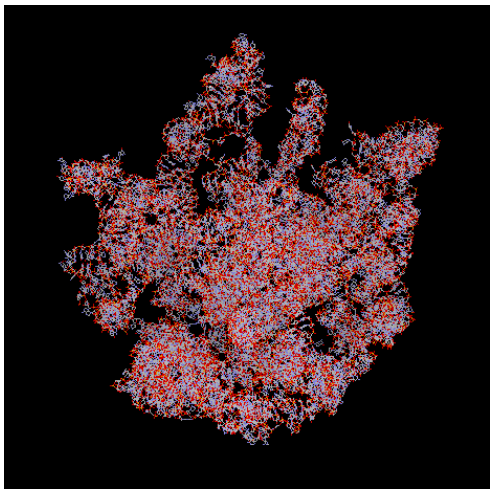

Figure 7: Image of the 23S rRNA

8.7.2. 5S rRNA

The 5S rRNA molecule acts as a seventh domain conferring stability to the large ribosomal subunit. It is vital for the maintenance of the cell health. Particularly it confers stability when it interacts with domain II and V of the 23S molecule which are responsible for translocation and peptide bond formation (Fig. 8). The 5S rRNA is also known to be involved in signal transmission during the translation.

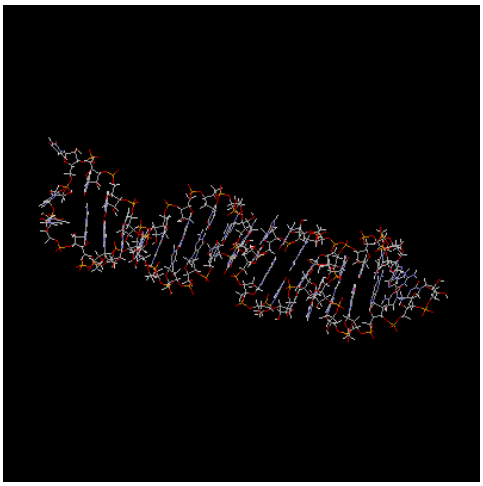

Figure 8: Image of the 5S rRNA

8.8. SUMMARY

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Ribosomal assembly mechanisms: The metabolism and assembly of ribosomes is vital for the synthesis of other cellular components. Ribosomes are large and complex macromolecule and therefore require efficient steps during their assembly. The assembly of bacterial ribosomes proceeds through an alternating series of RNA conformational changes and protein-binding events.

The assembly of these bacterial ribosomes occurs very fast and these steps are simultaneously carried out with the transcription of the ribosomal RNAs. During this binding process the RNA folding process is stabilized by ribosome proteins.

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8.9. CHECK YOUR PROGRESS

1. Explain the general account of the bacterial ribosome structure.
2. What are the steps involved in ribosome genesis?
3. Discuss the ribosomal assembly mechanisms.
4. Explain the structure of the 30S Subunit of bacterial ribosomes.
5. Explain the structure of the 50S Subunit of bacterial ribosomes.
6. Discuss the structure, composition and function of 16S rRNA.
7. Explain the functions of 30S and 50 S ribosomal proteins.

8. Discuss the structure, composition and function of 23S rRNA.
9. Discuss the structure, composition and function of 5S rRNA.

8.10. KEY WORDS

Bacterial ribosomes, mRNA translation and protein synthesis, 30S subunit and 50S subunit, ribosome genesis, ribosomal assembly mechanisms, 16SrRNA, 23S rRNA, 5S rRNA ribosomal proteins.

8.11. FURTHER SUGGESTED READING

1. Alcamo. 2001. Fundamentals of Microbiology Sixth Edition. By, Edward Alcamo. Jones and Bartlett Publishers, London.
2. Aneja K.R., Jain P. and Aneja R. "*A Text Book of Basic and Applied Microbiology*" New Age International Pub. New Delhi (2008).
3. Gerhardt P.R., Murray G.E., Costlow R.N., Nester E.W.,Wood E.A., Kreig N.R., and Phillips G.B.(eds) "*Manual of Methods for General Bacteriology.* American Society for Microbiology, Washington D.C. (1981)
4. James T. Drummond, David White, Clay Fuqua. 2011. The Physiology and Biochemistry of Prokaryotes 0004 Edition. Oxford University Press, USA
5. Pelczar M.J., Chan E.C.S. and Kreig N.R. "*Microbiology* – 5th edn., Tata McGraw-Hill Pub. Co. New Delhi (1986)
6. Purohit, S.S. 2006. Microbiology – Fundamentals and Application. Seventh Edition. Agrobios (India) Publishers, Jodhpur.
7. Ravi Mantha. 2012. All about bacteria. Collins Publications.
8. Stanier, R.Y., Ingraham, J.L., Wheelis, M.L., and Painter, P.R. 2007. General Microbiology Fifth Edition. McMillan Publishers, London.
9. Talaro K.P. and Talaro A. Foundations in Microbiology 6th edn. McGraw Hill (2006).
10. Trivedi, P.C. 2006. Applied Microbiology. Agrobios (India) Publishers, Jodhpur.

8.12. SOURCES

1. Dale, J. W., and Park, S. F. 2004. Molecular Genetics of Bacteria 4th Edition.
2. Kenneth Todar. 2009. Structure and Function of Bacterial Cells. Lectures in Microbiology by Kenneth Todar PhD University of Wisconsin-Madison Department of Bacteriology.
3. Kim, B. H., and Gadd, G. M. 2008. Bacterial Physiology and Metabolism. Cambridge University Press.
4. Loakes, D., Kelley, A.C., and Ramakrishnan, V. 2009. Insights Into Substrate Stabilization from Snapshots of the Peptidyl Transferase Center of the Intact 70S Ribosome. *Nat.Struct.Mol.Biol.* **16**, 528-533
5. Mc Quillen. 1962. Bacterial ribosomes and protein synthesis. General Microbiology. 29: 53-57.
6. Prescott. 2006. Prokaryotic cell structure and function, Chapter 3. pp.39-78.
7. Todar, K. 2003. Structure and function of prokaryotic cells. University of Wisconsin-Madison Department of Bacteriology.

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UNIT 9

STRUCUTRE AND FUNCTION OF BACTERIAL FLAGELLA AND PILI

STRUCTURE

9.1. OBJECTIVES

After reading this section we will be able to learn about

- Different types and arrangement of flagella
- The structure and function of the bacterial flagella and pili
- The bacterial sex pili and their role in conjugation

9.2. INTRODUCTION

Bacterial cell is made up of three essential structures, the cytoplasm, the surface layers and the appendages. The structures found external to the cell wall include capsule, flagella, pili and fimbriae (Fig. 1 and 2). Flagella are small semi-rigid whips that are free at one end and attached to a cell at the other which help in motility of the bacteria. The terms pili and fimbriae are often used interchangeably. Pili are short, hair-like structures on the surfaces of bacterial cells. Pili are found mostly in male cells. Unlike flagella pili grow from the inside of the cell outward, and not from the tip of the fibre. Pili also serve as attachment structures.

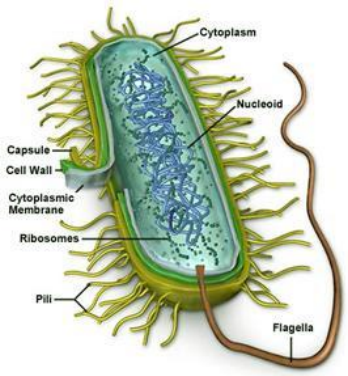

Figure 1: Detailed structure of a bacterial cell with flagella and pili

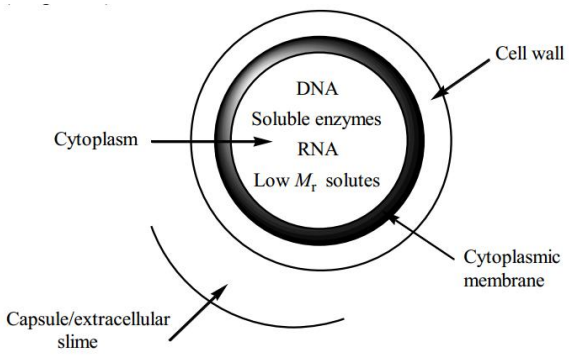

Figure 2: Structure of the bacterial surface layers

9.3. FLAGELLA

Some rod and spiral shaped bacterial possess flagella which serve as locomotory structure. Flagella are special locomotory organelles which are responsible for motility of some bacteria. They are long, rigid proteins that pass through the cell wall. Flagella are composed of several proteins including Flagellin. The size, position and number of flagella vary and are genetically determined. Generally the diameter of a flagellum is thin, 20 nm, and long with some having a length 10 times the diameter of cell. Bacteria can have one or more flagella arranged in clumps or spread all over the cell.

9.3.1. Structure of flagella

Flagella can be thought of as little semi-rigid whips that are free at one end and attached to a cell at the other (Fig. 3). The diameter of a flagellum is thin, 20 nm, and long with some having a length 10 times the diameter of cell. Due to their small diameter, flagella cannot be seen in the light microscope unless a special stain is applied. If a flagellum is cut off it will regenerate until reaches a maximum length. As this occurs the growth is not from base, but from tip. The filament is hollow and subunits travel through the filament and self-assemble at the end.

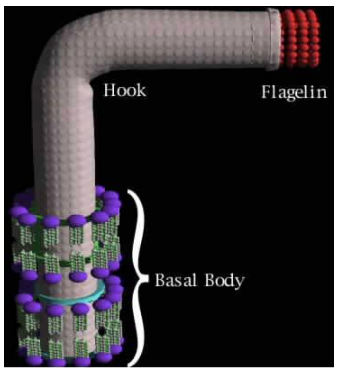

Figure 3: Structure of the bacterial flagella

Flagella are composed of three parts:

1.Filament: Composed of primarily of a single, self-aggregating protein called flagellin. Flagellin proteins twisted together in a helical conformation.

2.Hook: Transition between filament and motor. The hook is used for attachment for a cell

3.Basal body: Anchor in cell wall and motor. The basal body has two sets of rings and rods. In gram negative bacteria, two sets of ring and rods are present, L, P, S, M rings and rods. E.g. *E. coli*. In gram positive bacteria only S and M rings and rods are present. E.g. *Bacillus megaterium*.

In a prokaryotic flagellum, chains of a globular protein are wound in a tight spiral to form a filament (7-15 μm long) and thin (20 nm diam.), which is attached to another protein (the hook), which is inserted into the basal apparatus. Each flagellum rotates 360º around a central axis and affects the surrounding medium much as a ship's propeller would. The motor situated at the base of the flagellum can speed up, slow down, stop and go into reverse. The driving force comes from streams of protons- naked hydrogens stored near the motor and released in volleys by the chemical action of the sensory processing system (e.g. chemotaxis).

The flagellum's motor is much like that of, say, an electric mixer, but the mixer's is driven by electrons instead of protons.

9.3.2. Types of flagella

Based on the number and positioning of the flagella there are several types of flagella (Fig. 4):

Atrich/atrichous= No flagella. E.g., *Streptomyces, Clavibacter*

Monotrichous/Monotrich= single flagellum. E.g., *Vibrio cholerae*

Peritrichous/Peritrich= flagella all around. E.g., *Erwinia, Bacillus cereus*

Amphitrichous/Amphitrich= flagella at both ends. E.g., *Rhodospirillum*
Rhodospirillum rubrum

Lophotrichous/Lophotrich= tuft of many flagella at one end or both ends. E.g., *Pseudomonas savastanoi* pv. *fraxini*

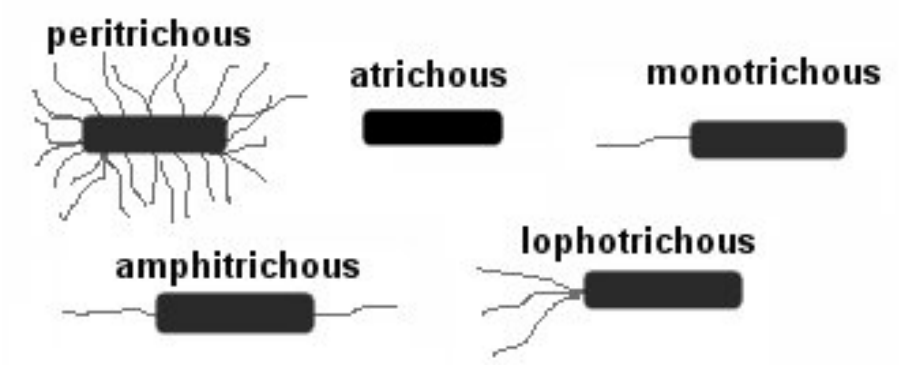

Figure 4: Different types of flagella based on the positioning and arrangement of flagella

9.3.3. Functions of the flagella

The flagella allow the bacteria to spin and propel the bacterial cell allowing both clockwise and anticlockwise movement. The flagella cause chemotaxis, i.e., they are responsible for the swimming mechanisms of the bacteria either towards or away from any chemical stimuli. The chemosensors located in the cell envelope detect these chemicals and signal the flagella to react to the stimuli accordingly.

The flagellum is a rigid structure and rotates like a propeller.

Rings in the basal body rotate relative to each other causing the flagella to turn.

Flagella can rotate:

1. Clockwise (CW): In peritrichous cells, flagella then become limp, cell tumbles or twiddle. **2. Counterclockwise (CCW):** Flagellar bundle then becomes rigid, cell runs. Rotor is always spinning one direction or other Some bacteria are motile w/o flagella.

3. Gliding motility: Depends upon contact with a solid surface, it moves slowly across surfaces, involves sulfur-containing lipids.

9.4. PILI

The terms pili and fimbriae are often used interchangeably. These are short, hair-like structures on the surfaces of bacterial cells. These are found mostly in male

cells. Unlike flagella they grow from the inside of the cell outward, and not from the tip of the fiber.

The site of origin of pili is the cell membranne. Pili are numerous in number and are found all over teh cell surface. Pili are conposed of proteins which have subunits. Pilin is a class of Lectin proteins which bond to teh cell surface polysaccharide. Pili are of two types 1) the common pili or fimbriae which are very fine, rigid, many and help in bacterial attachment. 2) The Sex pili which are much longer and rough, range from 1-4 in number and have a role in bacterial conjugation.

9.4.1. Structure of pili

Pili are short and hollow proteins which bind the bacterial cell to solid surfaces (Fig. 5). These characteristics are important for pathogenic bacteria. Their number varies and usually there will be hundreds of them per cell. Pili are long hollow tubules composed of proteins pilin. A pilus is typically 6 to 7 nm in diameter. Pili can be encoded by chromosomal genes or plasmid genes.

9.4.2. Fimbriae

Fimbriae are extensions made up of a protein called fimbrians. Some fimbriae can contain lectin proteins generally at their tips. Fimbriae originate in the plasma membrane and protrude through the cell wall. Fimbriae are shorter than pili and serve to allow bacteria to attach to various surfaces (Table 1). These structures are sticky, proteinaceous, bristle like projections. These are used by bacteria to adhere to one another, to hosts, and to substances in environment. Their number may be hundreds per cell and are shorter than flagella. Serve an important function in biofilms.

Table 1: Differences between Fimbriae and Pili are given below:

Pili	Fimbriae
Made up of proteins called Pilins	Made up of proteins called Fimbrians
They are longer and thicker	These are shorter
They are present in less numbers	They are present in large numbers
Common in gram negative rods	Found in both gram positive and negative bacteria
Function as receptor sites for bacteriophages	Function in cell adhesion

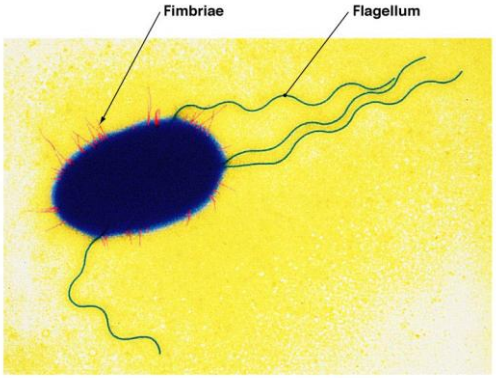

Figure 5: Strucutre of pili and fimbriae in bacteria

The pili Found mostly in Gram negative bacteria Made up of a protein called pilins Longer than fimbriae but shorter than flagella. Bacteria typically only have one or two per cell. There are a variety of different types of pili that differ in structure and function. Special pili (sex pili) involved in transfer of DNA from one cell to another (conjugation). The fertility factor (F+) is required to produce sex pili.

9.4.3. Functions of pili

Numerous different types of pili have been characterized, and various forms of these appendages are involved in diverse activities of bacteria. These are involved in bacterial cell aggregation, adhesion to surfaces of host cells, adhesion to other microbial cells in biofilm, gene and protein injection into other cells, DNA uptake by naturally transformable bacteria, and virulence attributes of pathogenic bacteria.

Pili act as receptors of some bacterial viruses.

Pili produce biofilms (slimy layer covering teeth, tongue) also found in the bottom of ships, trickling filter sewage treatments plants, rocks submerged in water etc.,

These pili help in attachment or adhsision helping the invasion of the host.
These pili prevent phagocytosis.

9.4.4. Sex pili

Sex pili are sex factors; if pili are present they are donors of F factor. Pili or sex factor is major determinant for the bacterial conjugation. Bacterial conjugation is the transfer of DNA from one cell to another. Pili are responsible for the recognition of specific receptor sites on the host cell membrane. In addition, several viruses which infect bacteria specifically infect those Bactria that have F pilus.

The sex pili are usually 1-4 in number. These are relatively longer and are found in G-cells. These are involved in the bacterial conjugation, i.e., transfer of genetic material from one cell to another. The transferred DNA may be plasmid or chromosomal DNA. The bacterial cells that have genes that code for sex pili are said to be male or F+ cells. The genes for sex pili formation are carried by sex plasmids.

9.5. SUMMARY

Bacterial cell is made up of three essential structures, the cytoplasm, the surface layers and the appendages. The structures found external to the cell wall include capsule, flagella, pili and fimbriae.

Flagella: Flagella are special locomotory organelles which are responsible for motility of some bacteria. They are long, rigid proteins that pass through the cell wall. Flagella are composed of several proteins including Flagellin. The size, position and number of flagella vary and are genetically determined. Generally the diameter of a flagellum is thin, 20 nm, and long with some having a length 10 times the diameter of cell. Bacteria can have one or more flagella arranged in clumps or spread all over the cell.

The flagella allow the bacteria to spin and propel the bacterial cell allowing both clockwise and anticlockwise movement. The flagella cause chemotaxis, i.e., they are responsible for the swimming mechanisms of the bacteria either towards or away from any chemical stimuli. The chemosensors located in the cell envelope detect these chemicals and signal the flagella to react to the stimuli accordingly.

Pili: Pili are short, hair-like structures on the surfaces of bacterial cells. These are found mostly in male cells. Unlike flagella they grow from the inside of the cell outward, and not from the tip of the fibre. Pili are of two types 1) the common pili or fimbriae which are very fine, rigid, many and help in bacterial attachment. 2) The Sex pili which are much longer and rough, range from 1-4 in number and have a role in bacterial conjugation.

Numerous different types of pili have been characterized, and various forms of these appendages are involved in diverse activities of bacteria. These are involved in bacterial cell aggregation, adhesion to surfaces of host cells, adhesion to other microbial cells in biofilm, gene and protein injection into other cells, DNA uptake by naturally transformable bacteria, and virulence attributes of pathogenic bacteria.

9.6. CHECK YOUR PROGRESS

5. What are flagella? Discuss their function
6. Explain different types and arrangement of flagella
7. What are fimbriae?
8. What are pili? What is their function?
9. Write a note on the Sex pili
10. What are wall less bacteria?

9.7. KEY WORDS

Flagella, pili, fimbriae, wall-less bacteria, flagella, chemotaxis, types of flagella, gliding motility, pili, sex pili, conjugation, fimbriae, biofilms.

9.8. FURTHER SUGGESTED READING

1. Alcamo. 2001. Fundamentals of Microbiology Sixth Edition. By, Edward Alcamo. Jones and Bartlett Publishers, London.
2. Aneja K.R., Jain P. and Aneja R. "*A Text Book of Basic and Applied Microbiology*" New Age International Pub. New Delhi (2008).
3. Gerhardt P.R., Murray G.E., Costlow R.N., Nester E.W.,Wood E.A., Kreig N.R., and Phillips G.B.(eds) "*Manual of Methods for General Bacteriology.* American Society for Microbiology, Washington D.C. (1981)
4. James T. Drummond, David White, Clay Fuqua. 2011. The Physiology and Biochemistry of Prokaryotes 0004 Edition. Oxford University Press, USA
5. Pelczar M.J., Chan E.C.S. and Kreig N.R. "*Microbiology* – 5th edn., Tata McGraw-Hill Pub. Co. New Delhi (1986)
6. Purohit, S.S. 2006. Microbiology – Fundamentals and Application. Seventh Edition. Agrobios (India) Publishers, Jodhpur.
7. Ravi Mantha. 2012. All about bacteria. Collins Publications.
8. Stanier, R.Y., Ingraham, J.L., Wheelis, M.L., and Painter, P.R. 2007. General Microbiology Fifth Edition. McMillan Publishers, London.
9. Talaro K.P. and Talaro A. Foundations in Microbiology 6th edn. McGraw Hill (2006).
10. Trivedi, P.C. 2006. Applied Microbiology. Agrobios (India) Publishers, Jodhpur.

9.9. SOURCES

1. Ammar et al .,2004. An attachment tip and pili-like structures in insect- and plantpathogenic spiroplasmas of the class Mollicutes. Arch Microbiol 181: 97-105.
2. B. H. Kim and G. M. Gadd. 2008. Bacterial Physiology and Metabolism. Cambridge University Press.
3. Bacteriology. BI 3206 Lecture notes.
4. Cabeen, M. T. and Jacobs-Wagner. C. 2005. Bacterial cell shape. Nature Reviews,Microbiolgy, pp.601-610.

5. J. W. Dale and S. F. Park. 2004. Molecular Genetics of Bacteria 4th Edition.

6. Ken Jarrell. Editor. 2009. Pili and flagella. Caister Academic Press

7. Prescott,2006. Procaryotic cell structure and function, Chapter 3. pp.39-78.

8. Seelke, R.W. 2010. Microbiology Lecture Notes.

Todar, K. 2003. Structure and function of prokaryotic cells. University of

UNIT 10

VACUOLES IN BACTERIA

STRUCTURE

10.1. OBJECTIVES

After reading this unit we will be able to know about:

- Vacuoles and their function in cells
- Occurrence of vacuoles in bacterial cells
- Structure and function of bacterial vacuoles
- Structure and function of gas vacuoles

10.2. INTRODUCTION

Vacuole is a type of vesicle, which is a small bubble enclosed by a lipid bilayer. Vacuoles do not have particular shape of size and usually vary according to the needs of the cell. Vacuoles are usually made up of waste products like carbon dioxide, acids, small molecules and water. In addition in some cases they also help in storage of nutrients and energy products. Some vacuoles also store enzymes in solution form.

Vacuoles plays role in intracellular digestion and release of cellular waste products. Vacuoles also help in restricting materials that might be harmful or a threat to the cell. One of vital cellular function of vacuole is to maintain internal turgor pressure within the cell which helps to retain the shape and buoyancy, mmaintaining an acidic internal pH, exporting unwanted substances from the cell. Vacuoles maintain a balance between production and degradation of various substances within the cell through a process called autophagy. Vacuoles are also known to lyse misfolded proteins.

Certain species of bacteria are also known to possess vacuoles which take part in anaerobic sulfide oxidation with nitrate and store nitrate. *Beggiatoa* spp. and some larger filamentous species and other representatives of big colorless sulfur bacteria, such as *Thioploca* spp. and *Thiomargarita namibiensis*, possess internal vacuoles, which are presumably the main location of nitrate storage. These organisms can use internally stored nitrate as an electron acceptor for anaerobic sulfide oxidation in deep suboxic sediment layers

10.3. BACTERIAL VACUOLES

Prokaryotic cells generally do not have vacuoles except in some sulfurous bacteria. Large vacuoles are found in three genera of sulfur bacteria, the *Thioploca, Beggiatoa* and *Thiomargarita*. These bacterial vacuoles occupy between 40–98% of the cell. In these bacteria vacuoles act as storage organelles and contain high concentrations of nitrate ions. In certain groups of filamentous sulfur bacteria there are giant forms with cell diameters of up to several hundred microns, and members of such group possess a central vacuole. These vacuoles function in restricting the active cytoplasm to a thin outer layer. Most of these sulfur bacteria seem to store nitrate in the vacuole, which they use as an electron acceptor for the oxidation of sulfide. Within these three closely related generat the vacuolated specis form one monophyletic cluster among the smaller not vacuolated speceis of all three general. Aditionally, there are reports of attached vacuolated filamentous sulfur bacteria, which apart from the vacuole resemble morphologically Thiothrix. According to their 16S ribosomal RNA (rRNA) sequence these bactria are the closest relatives toteh other vacuolated sulfur bacteria and are only remotely related to the smaller Thiothrix species. This indicates that the morphological feature of vacuoles in sulfur bacteria has only evolved once.

The vacuolated sulfur bacteria can reach enormous cell diameteres of several hundred microns. In these bacteria, the actual cytoplasm is typically restricted to a thin outer layer of about 0.5 – 2 μm. Thus, apart from their large size, the cells are not diffusion-limited as only a thin layer of cytoplasm is actually metabolically active. Also smaller filaments of Beggiatoa or THioploca with diameters more than 3 μm may contain less conspicuous vacuoles. The vacuole is separted from the cytoplsam by a bilayered membrane, which might originate from an intrusion of the cytoplasmic membrane. In large sulfur bacteria the cytoplasm tends to lsoe continuity and appears as a spongy net surroundign the empty void of the vacuole.

10.3.1. Vacuoles of *Thioploca*

Thioploca spp. is multicellular, filamentous, colorless sulfur bacteria inhabiting freshwater and marine sediments they live in bundles surrounded by a common sheath. Vast communities of large *Thioploca* species live along the Pacific coast of South America and in other upwelling areas of high organic matter sedimentation with bottom waters poor in oxygen and rich in nitrate.

Each cell of these *Thioplocas* harbours a large liquid vacuole which is used as storage for nitrate with a concentration of up to 500 mM (Fig. 1). The vacuole occupies more than 80% of the cell volume. The nitrate is used as an electron acceptor for sulfide oxidation and the bacteria may grow autotrophically or mixotrophically using acetate or other organic molecules as carbon source. The filaments stretch up into the overlying seawater, from which they take up nitrate, and then glide down 5–15 cm deep into the sediment through their sheaths to oxidize sulfide formed by intensive sulfate reduction

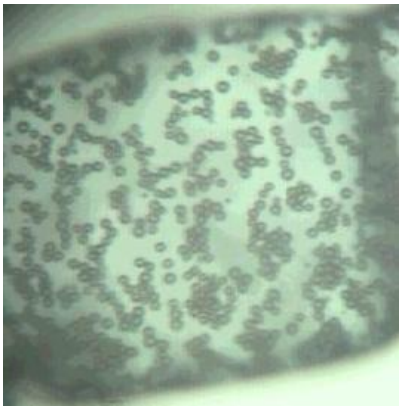

Figure 1: *Thioploca* cells with vacuoles.

10.3.2. Vacuoles of *Beggiatoa*

The genus *Beggiatoa* belongs to teh order Thiotrichales. *Beggiatoa* spp. is large, filamentous, gliding, colorless sulfur bacteria usually inhabiting sulfur-rich marine and fresh-water environments. These bacteria are usually found in cold seeps, suflur springs, sewage water, mud layers of lakes, hydrothermal vents. They

form visible white mats on the surfaces of organic-rich sediments. These bacteria oxidize hydrogen sulfide as an energy source and form sulfur droplets, and in the rhizosphere of swamp plants. The cells are colorless, spherical and arranged in long filaments. They have diameters ranging from 12 – 160 μm. The cells contain a giant centrally located vacuole which is used for accumulation of nitrate, for use as an electron acceptor in anaerobic sulfide oxidation (Fig. 2).

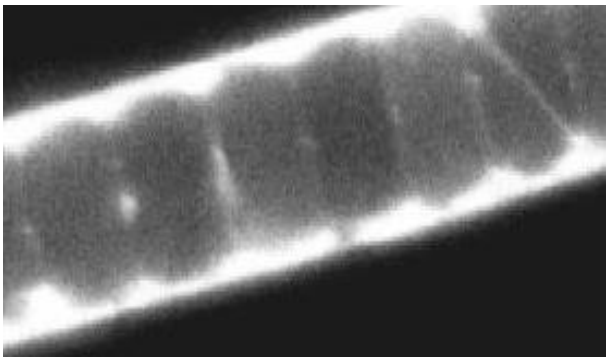

Figure 2: Beggiatoa cells with vacuoles

10.3.3. Vacuoles of *Thiomargarita namibiensis*

One of the largest bacteria is Thiomargarita namibiensis which can be seen with naked eyes. These bacteria are usually found buried in smelly, sulfur-rich, sedimentary seafloors. They grow in long lines of single cells. These cells are filled with reflective white globules of sulfur. These bacteria have evolved very large nitrate-storing vacuoles that allow them to survive long periods of nitrate starvation.

Since the bacterium is sessile, and the concentration of available nitrate fluctuates considerably over time, it stores nitrate at high concentration (up to 800 millimolar) in a large vacuole, which is responsible for some 80% of its size (Fig. 3). When nitrate concentrations in the environment are low, the bacteria use the contents of the vacuole for respiration.

Whenever nitrate is plentiful in surrounding sea water, these bacteria accumulate nitrate in their vacuoles which they use when nitrate supply diminishes. These giant vacuoles give Thiomargarita the ability to survive in nitrate deficient conditions until ocean currents sweep nitrate-containing waters past them again.

The large size of Thiomargarita is also because of these vacuoles. Their cytoplasm forms a thin layer along the peripheral cell membrane, while the nitrate-storing vacuoles occupy almost the complete interior of the cell.

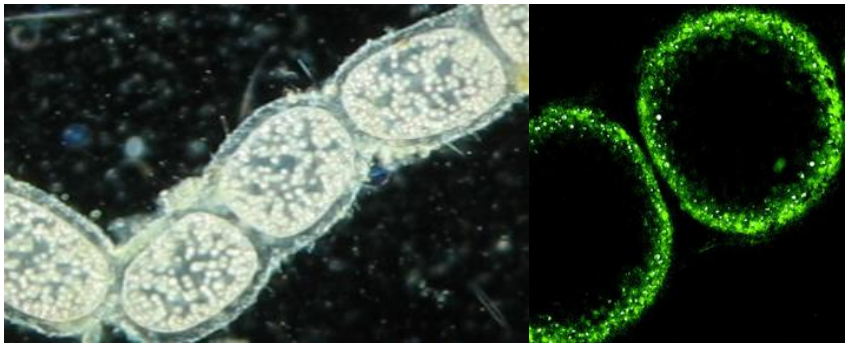

Figure 3: *Thiomargarita namibiensis* cells with vacuoles.

10.4. GAS VACUOLES

Aquatic bacteria like cyanobacteria possess gas vacuoles. They are presnt in the cytoplsam and hence considered as cytoplsamic inclusions. By light microscopy, they appear as brigh refractile bodies and by electron microscopy as hollow cylindrical shapes with conical ends and striated protein boundary (Fig. 4). Protein boundary is impermeable to water but allows exchange of various gases dissovled in water or at the air-water interface. Vacuoles may get collapsed under gas pressure or can be refilled by gases. Their refractility depends on pressure of internal gas. The main function of gas vacuole is to provide buoyancy to organism in aquatic habitat.

Gas vacuoles occur in many aquatic prokaryotic organisms including representatives of the blue-green algae, the photosynthetic green and purple sulfur bacteria, and a few other bacteria. The gas vacuoles are complex organelles consisting of an array of substructures referred to as gas vesicles. The gas vesicles are hollow cylinders with conical ends. The diameter and length vary from 65-115 nm and 0.2-1.2 nm, respectively.

All the vesicles thus far isolated have predominance (30-33%) of amino acids with hydrophobic side chains. It has been hypothesized that the inner surface

of the vesicle membrane is hydrophobic; the outer surface hydrophilic. The membrane is rigid, i.e. it is not inflated with gas, impermeable to water, and freely permeable to all gasses. Its impermeability to water is attributed to the hydrophobic inner membrane surface. The vesicle appears to be a self-assembling structure; the body elongates after formation of the conical ends. The shape is determined by the subunit proteins. The gas vacuoles may function in buoyancy provision, buoyancy regulation, light shielding, surface to volume regulation, or a combination of these functions.

Gas vesicles are found in *Cyanobacteria*, which are photosynthetic and live in aquatic systems. In these lakes and oceans, the *Cyanobacteria* want to control their position in the water column to obtain the optimum amount of light and nutrients.

Gas vesicles are aggregates of hollow cylindrical structures composed of rigid proteins. They are impermeable to water, but permeable to gas. The amount of gas in the vacuole is under the control of the microorganism.

Gas vesicles regulate the buoyancy of the microbes by changing the amount of gas contained within them. Release of gas from the vesicle causes the bacteria to fall in the water column, while filling the vesicle with gas increases their height in the water

Figure 4: Electron micrograph of a bacterial cell showing gas vesicles.

10.5. SUMMARY

Vacuole is a type of vesicle, which is a small bubble enclosed by a lipid bilayer. Vacuoles plays role in intracellular digestion, release of cellular waste products, maintain internal turgor pressure, retain the shape and buoyancy, maintain acidic internal pH.

Prokaryotic cells generally do not have vacuoles except in some sulfurous bacteria. Large vacuoles are found in three genera of sulfur bacteria, the *Thioploca*, *Beggiatoa* and *Thiomargarita*. These bacterial vacuoles occupy between 40–98% of the cell. In these bacteria vacuoles act as storage organelles and contain high concentrations of nitrate ions. Most of these sulfur bacteria seem to store nitrate in the vacuole, which they use as an electron acceptor for the oxidation of sulfide.

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10.6. KEY WORDS

Bacterial vacuoles, sulfur bacteria, gas vacuoles, *Thioploca, Beggiatoa* and *Thiomargarita.*

10.7. CHECK YOUR PROGRESS

1. Give a brief account of bacterial vacuoles
2. What are the main functions of bacterial vacuoles
3. What are gas vacuoles and list their important functions
4. Write short notes on the following:
   a) *Thioploca*
   b) *Beggiatoa*
   c) Thiomargarita namibiensis
   d) Gas Vacuoles

10.8. FURTHER SUGGESTED READING

1. Aneja K.R., Jain P. and Aneja R. "*A Text Book of Basic and Applied Microbiology*" New Age International Pub. New Delhi (2008).
2. Pelczar M.J., Chan E.C.S. and Kreig N.R. "*Microbiology* – 5th edn., Tata McGraw-Hill Pub. Co. New Delhi (1986)
3. Purohit, S.S. 2006. Microbiology – Fundamentals and Application. Seventh Edition. Agrobios (India) Publishers, Jodhpur.
4. Ravi Mantha. 2012. All about bacteria. Collins Publications.
5. Stanier, R.Y., Ingraham, J.L., Wheelis, M.L., and Painter, P.R. 2007. General Microbiology Fifth Edition. McMillan Publishers, London.
6. Trivedi, P.C. 2006. Applied Microbiology. Agrobios (India) Publishers, Jodhpur.

10.9. SOURCES

1. Cohen-Bazire, G., Kunisawa, R., and Pfennig, N. 1969. Comparative Study of the Structure of Gas Vacuoles. Journal of Bacteriology 100: 1049-1061.
2. Heide N. Schulz-Vogt. 2006. Vacuoles. Microbiology Monographs 1: 295-298.
3. Kenneth Todar. 2003. Structure and function of prokaryotic cells. University of Wisconsin-Madison Department of Bacteriology.
4. Kenneth Todar. 2009. Structure and Function of Bacterial Cells. Lectures in Microbiology by Kenneth Todar University of Wisconsin-Madison Department of Bacteriology.
5. Kim, B. H., and Gadd, G. M. 2008. Bacterial Physiology and Metabolism. Cambridge University Press.
6. Prescott. 2006. Prokaryotic cell structure and function, Chapter 3. pp.39-78.
7. Hively, J. M. 1974. Inclusion bodies of prokaryotes. Annu. Rev. Microbiol. 1974.28:167-188.
8. www.iscid.org/encyclopedia/Vacuole

UNIT 11

INCLUSION BODIES IN BACTERIA – METACHROMATIC GRANULES

STRUCTURE

11.1. OBJECTIVES

After reading this unit we will be able to learn in detail about:

- Inclusion bodies found in bacterial cells.
- The different types of inclusion bodies found in bacterial cells.
- Formation and structure of different inclusion bodies.
- Metachromatic granules, Poly-β-hydroxybutyrate (PHB) granules, Polyglucan granules, Sulfur globules, Gas vacuoles.
- Includiosn bodies and thier function, uses of inclusion bodies.
- Staining characteristics of different inclusion granules.

11.2. INTRODUCTION

The cytoplasm of bacteria contain a varieyt of small bodies which are together known as inclusion bodies. When bacteria grow under different environmental conditions they synthesize and accumulate variety of chemical substances as insoluble deposits in the form of inclusion bodies which are food reserves. This food reserve is in the form of concentated organic deposits and are called inclusion bodies. The reserved food is polymeric, high molecular weight and osmotically inert materials. Being osmotically inert do not decrease ore increase osmolarity of cytoplasm and prevent the loss of cytoplasmic contents by cytolysis. Inclusion bodies vary in size, number, and content.

Some of these inclusion bodies are called granules and some are called vesicles. Granules are densely compacted structures without a membrane. These granules may contain glycogen specific substances such as glycogen and polyphosphate or sulfur. Polyphosphate granules are also called volutin granules or metachromatic granules since they exhibit different colors. Polyphosphate or metachromatic granules store phosphate, and sulphur may serve as energy reserve for the bacteria. Many bacteria also accumulate either polyhydroxybutyrate granules (*Ralstonia eutropha*) or glycogen granules as carbon and energy reserve. Some motile aquatic bacteria (e.g. *Aquaspirillum magnetotacticum*) have been found to possess magnetosomes. Magnetosomes are membrane-bound iron-containing

substances that function as tiny magnets. Autotrophic bacteria (*Thiobacillus, Nitrosomonas* etc.) that reduce CO_2 in order to produce carbohydrates, possess carboxysomes containing ribulose bis llipsoid carboxylase, an enzyme used for CO fixation. The green bacteria (e.g. *Chlorobium*) carry out photosynthetic system is located in llipsoidal vesicles called chlorosomes that are independent of the cytoplasmic membrane. The purple bacteria (e.g. *Rhodopseudomonas, Rhodospirillum)* also carry out anoxygenic photosynthesis but their photosynthetic system is located in spherical or lamellar membrane system.

Some bacteria have special inclusion strucurtes called vesicles which have membrane called gas vacuoles. These vaculoes help in bouyancy of aquatic bacteria.

11.3. TYPES OF INCLUSION BODIES

There are five types of inclusion bodies are known to be present in bacteria. They are metachromatic granules, poly-β-hydroxybutyrate and polyglucan granules, sulfur globules and gas vacuoles. The first 3 types are refered to as granules as they have granule like appearance. Inclusison bodies are found dispersed in the cytoplsam or sometimes enclosed by membrane. These inclusion bodies are energy reserves and readily available substrates for metabolic reactions to carry out during unfavaourable conditons.

11.4. METACHROMATIC GRANULES

They are also called as Volutin or Polymetaphosphate granules. known as Volutin granules due to its presene in *Spirillum volutans*. They are composed of polyphosphate, RNA and proteins (Fig. 1). Their main function is to supply phsophate for nucleic acid syntehsis, cell division, energy metabolisms and as a soruce of phosphorous for nutrition. Phosphate is stored in these granules in teh form of linear chains of inorganic pyrophsophate. By staining with methylene blue, they represent different colors like reddish purple, purple, bluish red or maroon under light microscope and hence the name ‘metachromatic’. Under electron

microscope they appear as dark spheres. They are usually detectd in old laboratory cultures stored at room temperature or refrigerator. they occur in species of *Corynebacterium, Spirillum, Rhizobium* and *Bacillus.*

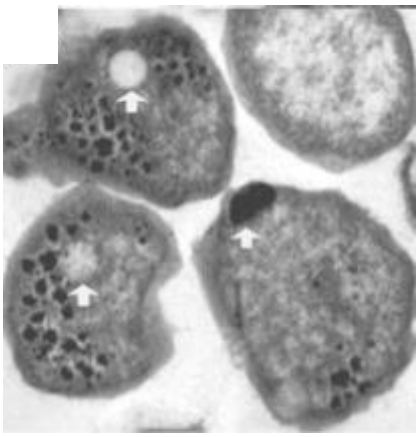

Figure 1: Electron micrographs of metachromatic granules of *Methanosarcina acetivorans*

11.5. POLY-β-HYDROXYBUTYRATE (PHB) GRANULES

They are also known as sudanophilic (stained by lipid stain Sudan) or lipid granules. Chemically, they are polyacetides or polyesters. In some bacterial species, E.g., *Bacillus megatherium*, these granules may make up as much as 60% of the dry weight, especially after growth on acetate or butyrate. For light microscopic obesrvation, they are stained by Sudan Black B or Nile blue to appear as dark blue dots in cell cytoplsam against reddish blue background (Fig. 2). Under elelctron microscope they appear as light round spots. PHB granules are formed during lipid synthesis, acetate or butyrate metabolism, nitrogen deficiency conditiosn or denitrification. In lipid syntehsis, acetyl CoA is condensed to aceto-acetyl CoA and it is further reduced to β-hydroxybutyryl CoA. Polymerization of this compound results in the formation of PHB. Poly-β-hydroxybutyrate granules are importnat source of food during starvation conditons, particularly in soil and rhizosphere envrionment where nutrient stress is always prevalent. PHB granules are found in almost all species of *Rhizobiumm, Bacillus, Alcaligenes* and other soil bacteria.

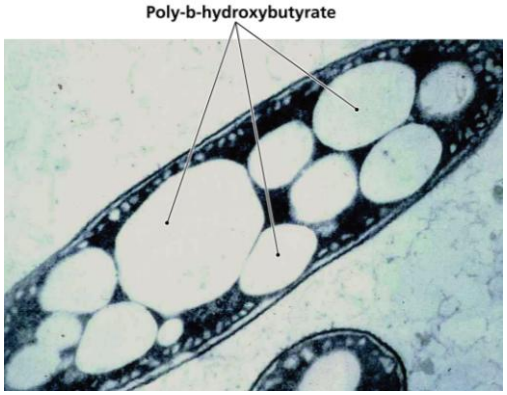

Figure 2: Electron micrographs of Poly-β-hydroxybutyrate (PHB) granules in Bacillus spp.

11.6. POLYGLUCAN GRANULES

They are also known as iodophilic or polysaccharide granules. They are stained by iodine solutio and appear brown or bluish under light microscipe (Fig. 3). They can be seen as dark round spots by electron microscopy. Polyglucan consists of repeated glucan units with α, 1-4 linkage and α, 1-6 branch points. They are deposited by bacteria themseleves inside their cells when simple sugars like glucose, fructose or sucrose are present for polysacchardie (glucan) synthesis. They have been found in clostridia and coliform group of bacteria; they are very important souces of substrate in carbohydrate metabolism during starvation conditons in these bacteria.

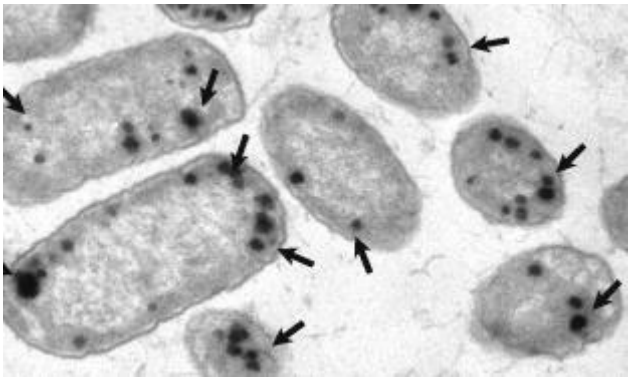

Figure 3: Electron micrographs of polyglucan granules in *Eschrechia coli*

11.7. SULFUR GLOBULES

Sulfur globules are cytoplsamic globules of elemental sulfur (Fig. 4). They are usually found in bacteria growing in environments rich in hydrogen sulfide (H_2S) gas such as hydrothermal vents, thermal geysers, boilign water or sulfur springs. These habitats are always dominated by sulfate reducing (photosynthetic purple and green sulfur) bacteria like *Chromatium* and *Chlorobium*. They use H2S as electron donor to reduce carbon dioxide during photosyntheiss process. The sulfur globules are also found in sulfur oxidising bacteria like extremophile Thiobacillus thioxidans which inhabit sulfur rich environments. They principally oxidize elemental sulfur to sulfates which is then assimilated by plants for syntheiss of sulfur containing amino acids. Both sulfur reducing and oxidising bacteria are integral part of natural elemental sulfur cylce on the Earth.

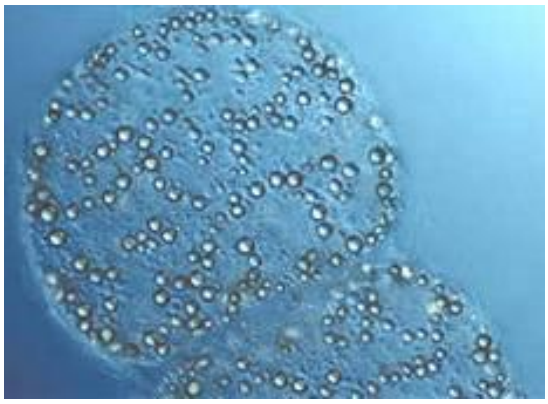

Figure 4: Optical micrograph of the bacterium *Thiomargarita*clearly showing sulfur globules

11.8. GAS VACUOLES

Aquatic bacteria like cyanobacteria possess gas vacuoles. They are presnt in the cytoplsam and hence considered as cytoplsamic inclusions. By light microscopy, they appear as brigh refractile bodies and by electron microscopy as hollow cylindrical shapes with conical ends and striated protein boundary (Fig. 5). Protein boundary is impermeable to water but allows exchange of various gases dissovled in water or at the air-water interface. Vacuoles may get collapsed under gas pressure or

can be refilled by gases. Their refractility depends on pressure of internal gas. The main function of gas vacuole is to provide buoyancy to organism in aquatic habitat.

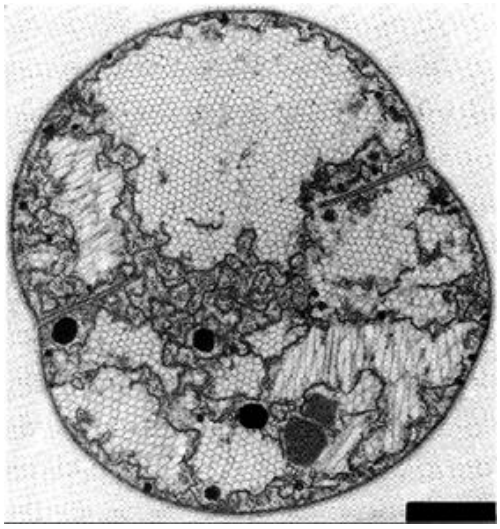

Figure 5: Electron micrographs of a *Cyanobacterium* cell showing gas vacuoles

Comparative analysis of different types of bacterial inclusions bodies, their location, composition and function are presented in Table 1. Staining properties of different bacterial inclusion bodies are presented in Table 2.

Table 1: Different types of bacterial inclusions bodies, their location, composition and function.

Cytoplasmic inclusions	Where found	Composition	Function
Glycogen	many bacteria e.g. *E. coli*	polyglucose	reserve carbon and energy source
Polybetahydroxyutyric acid (PHB)	many bacteria e.g. *Pseudomonas*	polymerized hydroxy butyrate	reserve carbon and energy source

Polyphosphate (volutin granules	many bacteria e.g. *Corynebacterium*	linear or cyclical polymers of PO_4	reserve phosphate; possibly a reserve of high energy phosphate
Sulfur globules	phototrophic purple and green sulfur bacteria and lithotrophic colorless sulfur bacteria	elemental sulfur	reserve of electrons (reducing source) in phototrophs; reserve energy source in lithotrophs
Gas vesicles	aquatic bacteria especially cyanobacteria	protein hulls or shells inflated with gases	buoyancy (floatation) in the vertical water column
Parasporal crystals	endospore-forming bacilli (genus *Bacillus*)	protein	unknown but toxic to certain insects
Magnetosomes	certain aquatic bacteria	magnetite (iron oxide) Fe_3O_4	orienting and migrating along geo- magnetic field lines
Chlorosomes	Green bacteria	lipid and protein and bacteriochlorophyll	light-harvesting pigments and antennae
Carboxysomes	many autotrophic bacteria	enzymes for autotrophic CO_2 fixation	site of CO_2 fixation
Phycobilisomes	cyanobacteria	phycobiliproteins	light-harvesting pigments

Table 2: Staining properties of different bacterial inclusion bodies

Inclusion body	Staining agent	Colour developed
Glycogen	Iodine	Brown
polybetahydroxyutyric acid (PHB)	Lipid soluble dye (nile blue)	Blue

Polyphosphate (volutin granules	Methylene blue	Purple color
Sulfur globules	Phototrophic purple and green sulfur bacteria and lithotrophic colorless sulfur bacteria	Elemental sulfur
Gas vesicles	Light microscope	Bright

11.9. SUMMARY

The cytoplasm of bacteria contain a varieyt of small bodies which are together known as inclusion bodies. When bacteria grow under different environmental conditions they synthesize and accumulate variety of chemical substances as insoluble deposits in the form of inclusion bodies which are food reserves.

There are five types of inclusion bodies are known to be present in bacteria. They are metachromatic granules, poly-β-hydroxybutyrate and polyglucan granules, sulfur globules and gas vacuoles. The first 3 types are refered to as granules as they have granule like appearance.

Metachromatic granules: they are also called as Volutin or Polymetaphosphate granules. They are composed of polyphosphate, RNA and proteins. Their main function is to supply phsophate for nucleic acid syntehsis, cell division, energy metabolisms and as a soruce of phosphorous for nutrition. By staining with methylene blue, they represent different colors like reddish purple, purple, bluish red or maroon under light microscope and hence the name 'metachromatic'. Under electron microscope they appear as dark spheres. they occur in species of *Corynebacterium, Spirillum, Rhizobium* and *Bacillus*.

Poly-β-hydroxybutyrate (PHB) granules: they are also known as sudanophilic (stained by lipid stain Sudan) or lipid granules. Chemically, they are polyacetides or polyesters. In some bacterial species, E.g., *Bacillus megatherium*, these granules may make up as much as 60% of the dry weight, especially after

growth on acetate or butyrate. For light microscopic obesrvation, they are stained by Sudan Black B or Nile blue to appear as dark blue dots in cell cytoplsam against reddish blue background. Under elelctron microscope they appear as light round spots. PHB granules are formed during lipid synthesis, acetate or butyrate metabolism, nitrogen deficiency conditiosn or denitrification. PHB granules are found in almost all species of Rhizobiumm Bacillus, Alcaligenes and other soil bacteria.

Polyglucan granules: they are also known as iodophilic or polysaccharide granules. They are stained by iodine solutio and appear brown or bluish under light microscipe. They can be seen as dark round spots by electron microscopy. Polyglucan consists of repeated glucan units with α, 1-4 linkage and α, 1-6 branch points. They are deposited by bacteria themseleves inside their cells when simple sugars like glucose, fructose or sucrose are present for polysacchardie (glucan) synthesis. They have been found in clostridia and coliform group of bacteria; they are very important souces of substrate in carbohydrate metabolism during starvation conditons in these bacteria.

Sulfur globules: Sulfur globules are cytoplsamic globules of elemental sulfur. They are usually found in bacteria growing in environments rich in hydrogen sulfide (H_2S) gas such as hydrothermal vents, thermal geysers, boilign water or sulfur springs. These habitats are always dominated by sulfate reducing (photosynthetic purple and green sulfur) bacteria like *Chromatium* and *Chlorobium*. They principally oxidize elemental sulfur to sulfates which is then assimilated by plants for syntheiss of sulfur containing amino acids. Both sulfur reducing and oxidising bacteria are integral part of natural elemental sulfur cylce on the Earth.

Gas vacuoles: Aquatic bacteria like cyanobacteria possess gas vacuoles. By light microscopy, they appear as brigh refractile bodies and by electron microscopy as hollow cylindrical shapes with conical ends and striated protein boundary. Protein boundary is impermeable to water but allows exchange of various gases dissovled in water or at the air-water interface. The main function of gas vacuole is to provide buoyancy to organism in aquatic habitat.

11.10. CHECK YOUR PROGRESS

1. What are bactrial inclusion bodies?
2. What are teh different types of inclusion bodies found in bacterila cell?
3. Under what conditons inclusion bodies are formed and what is their main function?
4. What is the difference between granules and vesicles?
5. What are metachromatic granules and what is thier main function?
6. What are Poly-β-hydroxybutyrate (PHB) granules and what is their main function?
7. What are polyglucan granules and what is their main functin?
8. What are sulfur globules and what is their main fucntion?
9. What are gas vacuoles and what is their main funciton?
10. Explain in detail the functions of different inclusion bodies.
11. Add a note of bacterial inclusion bodies with one example each.
12. What are the staining properties of different inclusion granules in bacteria?

11.11. KEY WORDS

Inclusion bodies, granules, vesicles, metachromatic granules, poly-β-hydroxybutyrate and polyglucan granules, sulfur globules and gas vacuoles.

11.12. FURTHER SUGGESTED READING

1. Aneja K.R., Jain P. and Aneja R. "*A Text Book of Basic and Applied Microbiology*" New Age International Pub. New Delhi (2008).
2. Pelczar M.J., Chan E.C.S. and Kreig N.R. "*Microbiology* – 5th edn., Tata McGraw-Hill Pub. Co. New Delhi (1986)
3. Purohit, S.S. 2006. Microbiology – Fundamentals and Application. Seventh Edition. Agrobios (India) Publishers, Jodhpur.
4. Ravi Mantha. 2012. All about bacteria. Collins Publications.

5. Stanier, R.Y., Ingraham, J.L., Wheelis, M.L., and Painter, P.R. 2007. General Microbiology Fifth Edition. McMillan Publishers, London.
6. Trivedi, P.C. 2006. Applied Microbiology. Agrobios (India) Publishers, Jodhpur.

11.13. SOURCES

1. Kenneth Todar. 2003. Structure and function of prokaryotic cells. University of Wisconsin-Madison Department of Bacteriology.
2. Kenneth Todar. Structure and Function of Bacterial Cells. Todar's online textbook of bacteriology. www.textbookofbacteriology.net
3. Kenneth Todar. 2009. Structure and Function of Bacterial Cells. Lectures in Microbiology by Kenneth Todar PhD University of Wisconsin-Madison Department of Bacteriology.
4. Kim, B. H., and Gadd, G. M. 2008. Bacterial Physiology and Metabolism. Cambridge University Press.
5. Prescott. 2006. Prokaryotic cell structure and function, Chapter 3. pp.39-78.
6. Shively, J. M. 1974. Inclusion bodies of prokaryotes. Annu. Rev. Microbiol. 1974.28:167-188.
7. Talaro. 2009. An Introduction to the Prokaryotic Cell, Its Organization, and Members. Foundations of Microbiology, seventh edition. The McGraw-Hill Companies, Inc.
8. The clinical significance of bacterial anatomy. Microbiology: A Clinical Approach. Garland Science

UNIT 12

INCLUSION BODIES IN BACTERIA – POLY BETA-HYDROXY BUTYRATE, POLY BETA-HYDROXY ACETATE

STRUCTURE

12.1. OBJECTIVES

After reading this chapter we will be able to understand:

- Different kinds of inclusion bodies in bacteria
- Poly-β-hydroxybutyrate granules and thier funciton
- Distribution, strucutre and fucntion of Polyphosphate (Volutin, Metachromatic) Granules and Poly-β-hydroxybutyrate Granules

12.2. INTRODUCTION

There are two major types of inclusion bodies namely granules and vesicles. These inclusion bodies may be membrane bound or without membranes. Usually granules are without membrane and vesicles are bound by a membrane. The important five types of inclusion bodies are discussed in detail in this section.

Poly-β-hydroxybutyrate is often found in aerobic bacteria especially under high-carbon, low nitrogen culture condition, They are also known as sudanophilic (stained by lipid stain Sudan) or lipid granules. Chemically, they are polyacetides or polyesters. The ability to accumulate PHB is common among the bacteria and has been reported in the blue-green algae.

Polyglucan granules are of two types, ones with membrane and ones without membrane. they are also known as iodophilic or polysaccharide granules. Polyglucan consists of repeated glucan units with α, 1-4 linkage and α, 1-6 branch points. They are deposited by bacteria themseleves inside their cells when simple sugars like glucose, fructose or sucrose are present for polysacchardie (glucan) synthesis.

Sulfur globules are cytoplsamic globules of elemental sulfur. They are usually found in bacteria growing in environments rich in hydrogen sulfide (H_2S) gas such as hydrothermal vents, thermal geysers, boilign water or sulfur springs. They principally oxidize elemental sulfur to sulfates which is then assimilated by plants for syntheiss of sulfur containing amino acids.

Aquatic bacteria like cyanobacteria possess gas vacuoles. They are presnt in the cytoplsam and hence considered as cytoplsamic inclusions. Protein boundary is impermeable to water but allows exchange of various gases dissovled in water or at the air-water interface.

12.3. POLYPHOSPHATE (VOLUTIN, METACHROMATIC) GRANULES

Polyphosphates are widely distributed in bacteria. They are commonly known as Volutin granules due to its presence in *Spirillum volutans*. They are composed of polyphosphate, RNA and proteins. Their main function is to supply phsophate for nucleic acid syntehsis, cell division, energy metabolisms and as a soruce of phosphorous for nutrition. Phosphate is stored in these granules in teh form of linear chains of inorganic pyrophsophate. By staining with methylene blue, they represent different colors like reddish purple, purple, bluish red or maroon under light microscope and hence the name 'metachromatic'. Under electron microscope they appear as dark spheres (Fig. 1). They are usually detectd in old laboratory cultures stored at room temperature or refrigerator. they occur in species of *Corynebacterium, Spirillum, Rhizobium* and *Bacillus*.

The bulk of the polyphosphate is present as linear, high molecular weight molecules, e.g. greater than 500 residues per molecule have been reported. Small linear molecules, as well as cyclic tri-, tetra-, penta-, and hexameta-phosphates have been found in some cases. The polyphosphate may constitute 40-50% of the total cell phosphorous. The polymer is commonly deposited as spherical, electron-opaque granules which range in diameter from 48 nm to greater than 1 μm; size depends on the organism examined and on the state of granule development. The granules are usually located in the nucleoplasmic region of the cell but have been seen in association with other cell components. A surrounding membrane it is generally considered to be absent. The granules occur at a fairly constant number during the exponential phase of growth when phosphate is in excess and decrease under limiting phosphate conditions or during periods of cell inactivation. The polymer is synthesized by the enzyme, polyphosphate kinase,

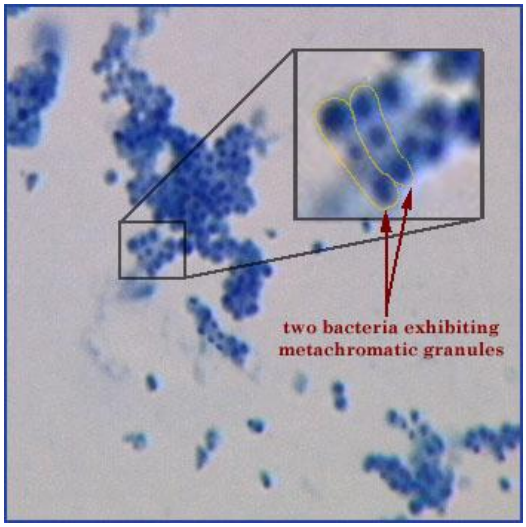

Figure 1: *Corynebacterium xerosis* showing polyphosphate (metachromatic) granules

12.4. POLY -β-HYDROXYBUTYRATE GRANULES

One of the more common storage inclusions is PHA. It is a long polymer of repeating hydrophobic units that can have various carbon chains attached to them. The most common form of this class of polymers is poly-beta-hydroxybutyrate that has a methyl group as the side chain to the molecule. Some PHA polymers have plastic like qualities and there is some interest in exploiting them as a form of biodegradable plastic. The function of PHA in bacteria is as a carbon and energy storage product.

PHB is often found in aerobic bacteria especially under high-carbon, low nitrogen culture condition, E.g., *Ralstonia eutropha*. PHB is a chloroform-soluble, lipid-like material. PHB can be stain with lipid-soluble dyes such as Nile blue.

They are also known as sudanophilic (stained by lipid stain Sudan) or lipid granules. Chemically, they are polyacetides or polyesters. In some bacterial species, E.g., *Bacillus megatherium*, these granules may make up as much as 60% of the dry weight, especially after growth on acetate or butyrate. For light microscopic obesrvation, they are stained by Sudan Black B or Nile blue to appear as dark blue dots in cell cytoplsam against reddish blue background. Under elelctron microscope

they appear as light round spots (Fig. 2). PHB granules are formed during lipid synthesis, acetate or butyrate metabolism, nitrogen deficiency conditiosn or denitrification. In lipid syntehsis, acetyl CoA is condensed to aceto-acetyl CoA and it is further reduced to β-hydroxybutyryl CoA. Polymerization of this compound results in the formation of PHB. Poly-β-hydroxybutyrate granules are importnat source of food during starvation conditons, particularly in soil and rhizosphere envrionment where nutrient stress is always prevalent. PHB granules are found in almost all species of Rhizobiumm Bacillus, Alcaligenes and other soil bacteria.

The ability to accumulate PHB is common among the bacteria and has been reported in the blue-green algae. PHB has recently been found to accumulate in sporulating cells *Clostridium botulinum* type E . The polymer accumulates, sometimes to greater than 50% of the cell dry weight , when carbon and energy sources are in excess. PHB is considered to be cellular reserve of energy, or of carbon and energy. The PHB is deposited in nonunit membrane-enclosed granules which appear electron transparent in thin section. Granule diameters of 100-800 nm have been reported and each granule may contain several thousand PHB molecules. The granules consist of 98% PHB, 2% protein, and trace amounts of lipid and phosphorous. The surrounding membrane 2.0-4.0 nm thick and may arise from one layer of the cytoplasmic membrane.

The granules (240-720 nm in diameter) of *Bacillus cereus* consist of a central core (140-370 nm in diameter) which occupies less than 50% of the granule volume, an outer coat, and a surrounding membrane. This may indicate that the polymer is in different physical states within the granule. The molecular weights of PHB vary to a great degree; values from 1,000 to 256,000 daltons have been reported.

Research indicates that the PHB in the granule is in a crystalline form. The crystalline conformation of the polymer is a right-hand helix stabilized by carbonyl-methyl interaction.

The enzyme for polymerization of o-(-)-fl-hydroxybutyryl CoA, PHB synthetase, and all or part of the depolymerizing complex are associated with the granules, presumably as part of the surrounding membrane. It is postulated that the PHB synthetase aggregates into a micellar form and the PHB is deposited within. The

reutilization of the PHB, under conditions of energy and/or carbon starvation, involves a labile, protein, inhibitor factor (commonly associated with the granules); and activator, which counteracts the effect of the inhibitor; and the depolymerase. The activator and depolymerise may or may not be associated with the granules.

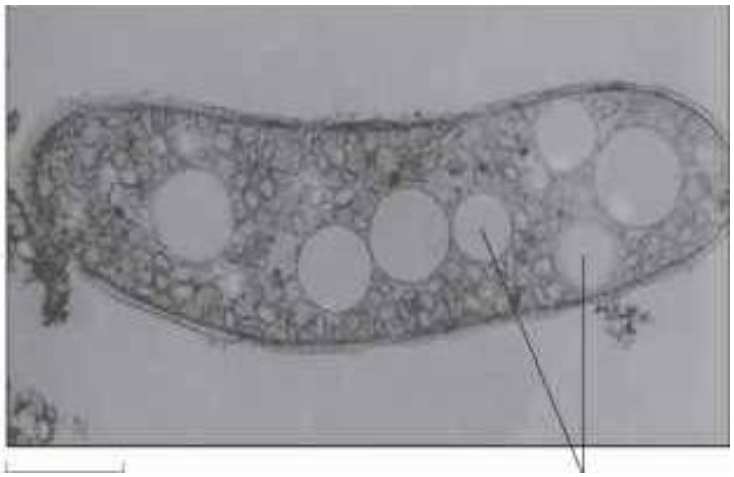

Figure 2: Storage Granules The large unstained areas in the photosynthetic bacterium *Rhodospirillum rubrum* are granules of poly-beta-hydroxybutyrate.

12.5. POLYGLUCAN GRANULES

These granules are of two types, ones with membrane and ones without membrane. they are also known as iodophilic or polysaccharide granules. They are stained by iodine solutio and appear brown or bluish under light microscipe. They can be seen as dark round spots by electron microscopy. Polyglucan consists of repeated glucan units with α, 1-4 linkage and α, 1-6 branch points. They are deposited by bacteria themseleves inside their cells when simple sugars like glucose, fructose or sucrose are present for polysacchardie (glucan) synthesis. They have been found in clostridia and coliform group of bacteria; they are very important souces of substrate in carbohydrate metabolism during starvation conditons in these bacteria.

12.5.1. Polyglucoside (a, Glycogen) Granules (non membrane bound)

Many prokaryotic organisms have been shown to store polymers of glucose. The polyglucoside may be dispersed throughout the cytoplasm or deposited as membrane-enclosed or nonmembrane-enclosed granules. The granules consist of highly branched, high molecular weight polymers which resemble either glycogen or amylopectin. In the bacteria

The granules are 20-100 nm in diameter and commonly have an uneven appearance. The granules of the blue-green algae may be crystals, spheres or rods and are generally observed between the thylakoid membranes

12.5.2. Polyglucoside (Glycogen) Granules (membrane bound)

Many bacteria have been shown to accumulate polyglucose; however, in most instances the polymer accumulates as a nonmembrane bound granule. In several species of *Clostridium* the polyglucose granules appear to be surrounded by a single-layered membrane. The granules first appear in the cells of early log phase cultures and become more numerous as the culture ages, reaching a maximum (c a 15% of cell dry weight) the outset of sporulation. Their initial appearance correlates well with the derepression of ADP-glucose pyrophosphorylase. The polymer appears to be of the amylopectin type and molecular weights of 180,000 daltons have been recorded. Evidence indicates that the enzyme, granulose synthetase, is intimately associated with the granules.

12.6. SULFUR GLOBULES

Sulfur globules are cytoplsamic globules of elemental sulfur. They are usually found in bacteria growing in environments rich in hydrogen sulfide (H_2S) gas such as hydrothermal vents, thermal geysers, boilign water or sulfur springs. These habitats are always dominated by sulfate reducing (photosynthetic purple and green sulfur) bacteria like Chromatium and Chlorobium. They use H2S as electron donor to reduce carbon dioxide during photosyntheiss process. The sulfur globules are also found in sulfur oxidising bacteria like extremophile Thiobacillus thioxidans which inhabit sulfur rich environments. They principally oxidize elemental sulfur to

sulfates which is then assimilated by plants for syntheiss of sulfur containing amino acids. Both sulfur reducing and oxidising bacteria are integral part of natural elemental sulfur cylce on the Earth.

Sulfur globules are a distinctive feature of the *Thiorhodaceae* and of certain other Apochlorotic sulfur bacteria. The sulfur is deposited as the cells oxidize and grow in the presence of hydrogen sulfide. The sulfur globules disappear, i .e. the sulfur is oxidized, when hydrogen sulfide becomes limiting. The globules are generally observed in thin sections as nonunit membrane enclosed holes (Fig. 3); the sulfur is removed during dehydration. They vary from 100 nm to over 1.0 pm in diameter and are most commonly deposited in invaginated pockets of the cytoplasmic membrane i.e., they are inside the cell wall, but outside the cytoplasmic membrane.

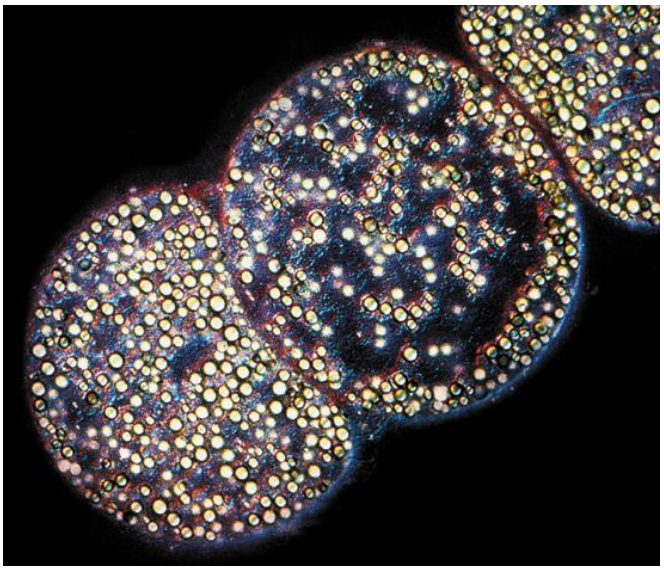

Figure 3: *Thiomargarita namibiensis* showing reflective globules of sulfur

12.7. GAS VACUOLES

Aquatic bacteria like cyanobacteria possess gas vacuoles. They are presnt in the cytoplsam and hence considered as cytoplsamic inclusions. By light microscopy, they appear as brigh refractile bodies and by electron microscopy as hollow cylindrical shapes with conical ends and striated protein boundary (Fig. 4). Protein

boundary is impermeable to water but allows exchange of various gases dissovled in water or at the air-water interface. Vacuoles may get collapsed under gas pressure or can be refilled by gases. Their refractility depends on pressure of internal gas. The main function of gas vacuole is to provide buoyancy to organism in aquatic habitat.

Gas vacuoles occur in many aquatic prokaryotic organisms including representatives of the blue-green algae, the photosynthetic green and purple sulfur bacteria, and a few other bacteria. The gas vacuoles are complex organelles consisting of an array of substructures referred to as gas vesicles. The gas vesicles are hollow cylinders with conical ends. The diameter and length vary from 65-115 nm and 0.2-1.2 nm, respectively.

All the vesicles thus far isolated have predominance (30-33%) of amino acids with hydrophobic side chains. It has been hypothesized that the inner surface of the vesicle membrane is hydrophobic; the outer surface hydrophilic. The membrane is rigid, i.e. it is not inflated with gas, impermeable to water, and freely permeable to all gasses. Its impermeability to water is attributed to the hydrophobic inner membrane surface. The vesicle appears to be a self-assembling structure; the body elongates after formation of the conical ends. The shape is determined by the subunit proteins. The gas vacuoles may function in buoyancy provision, buoyancy regulation, light shielding, surface to volume regulation, or a combination of these functions.

Gas vesicles are found in *Cyanobacteria*, which are photosynthetic and live in aquatic systems. In these lakes and oceans, the *Cyanobacteria* want to control their position in the water column to obtain the optimum amount of light and nutrients.

Gas vesicles are aggregates of hollow cylindrical structures composed of rigid proteins. They are impermeable to water, but permeable to gas. The amount of gas in the vacuole is under the control of the microorganism.

Gas vesicles regulate the buoyancy of the microbes by changing the amount of gas contained within them. Release of gas from the vesicle causes the bacteria to

fall in the water column, while filling the vesicle with gas increases their height in the water

Figure 4: Electron micrograph of a bacterial cell showing gas vesicles

12.8. SUMMARY

Polyphosphate (Volutin, Metachromatic) Granules are common in bacteria. They are also called Volutin granules or Metachromatic granules. They are composed of polyphosphate, RNA and proteins. Their main function is to supply phsophate for nucleic acid syntehsis, cell division, energy metabolisms and as a soruce of phosphorous for nutrition. Phosphate is stored in these granules in teh form of linear chains of inorganic pyrophsophate. By staining with methylene blue, they represent different colors like reddish purple, purple, bluish red or maroon under light microscope and hence the name 'metachromatic'. Under electron microscope they appear as dark spheres. they occur in species of Corynebacterium, Spirillum, Rhizobium and Bacillus.

Poly-β-hydroxybutyrate Granules: Poly-β-hydroxybutyrate is often found in aerobic bacteria especially under high-carbon, low nitrogen culture condition, E.g., *Ralstonia eutropha*. PHB is a chloroform-soluble, lipid-like material. They are also known as sudanophilic (stained by lipid stain Sudan) or lipid granules. Chemically, they are polyacetides or polyesters. For light microscopic obesrvation, they are

stained by Sudan Black B or Nile blue to appear as dark blue dots in cell cytoplsam against reddish blue background. Under elelctron microscope they appear as light round spots. PHB granules are formed during lipid synthesis, acetate or butyrate metabolism, nitrogen deficiency conditiosn or denitrification. Granule diameters of 100-800 nm have been reported and each granule may contain several thousand PHB molecules. The granules consist of 98% PHB, 2% protein, and trace amounts of lipid and phosphorous. PHB granules are found in almost all species of Rhizobiumm Bacillus, Alcaligenes and other soil bacteria.

Polyglucan granules : These granules are of two types, ones with membrane and ones without membrane. they are also known as iodophilic or polysaccharide granules. They are stained by iodine solutio and appear brown or bluish under light microscipe. They can be seen as dark round spots by electron microscopy. Polyglucan consists of repeated glucan units with α, 1-4 linkage and α, 1-6 branch points. They are deposited by bacteria themseleves inside their cells when simple sugars like glucose, fructose or sucrose are present for polysacchardie (glucan) synthesis. They have been found in clostridia and coliform group of bacteria; they are very important souces of substrate in carbohydrate metabolism during starvation conditons in these bacteria.

Sulfur globules: Sulfur globules are cytoplsamic globules of elemental sulfur. They are usually found in bacteria growing in environments rich in hydrogen sulfide (H_2S) gas such as hydrothermal vents, thermal geysers, boilign water or sulfur springs. These habitats are always dominated by sulfate reducing (photosynthetic purple and green sulfur) bacteria like *Chromatium* and *Chlorobium*. They use H_2S as electron donor to reduce carbon dioxide during photosyntheiss process. The sulfur globules are also found in sulfur oxidising bacteria like extremophile Thiobacillus thioxidans which inhabit sulfur rich environments. They principally oxidize elemental sulfur to sulfates which is then assimilated by plants for syntheiss of sulfur containing amino acids. They vary from 100 nm to over 1.0 pm in diameter and are most commonly deposited in invaginated pockets of the cytoplasmic membrane i.e., they are inside the cell wall, but outside the cytoplasmic membrane.

Gas vacuoles: Aquatic bacteria like cyanobacteria possess gas vacuoles. They are presnt in the cytoplsam and hence considered as cytoplsamic inclusions. By light microscopy, they appear as brigh refractile bodies and by electron microscopy as hollow cylindrical shapes with conical ends and striated protein boundary. Protein boundary is impermeable to water but allows exchange of various gases dissovled in water or at the air-water interface. Vacuoles may get collapsed under gas pressure or can be refilled by gases. Their refractility depends on pressure of internal gas. The main function of gas vacuole is to provide buoyancy to organism in aquatic habitat. The gas vesicles are hollow cylinders with conical ends. The diameter and length vary from 65-115 nm and 0.2-1.2 nm, respectively. The gas vacuoles may function in buoyancy provision, buoyancy regulation, light shielding, surface to volume regulation, or a combination of these functions.

12.9. CHECK YOUR PROGRESS

1. What are the different kinds of inclusion bodies in bacteria?
2. What are Poly-β-hydroxybutyrate granules and thier funciton?
3. Write an essay on distribution, strucutre and fucntion of Polyphosphate and Poly-β-hydroxybutyrate granules.
4. Explain the composition of polyphosphate granules and poly beta hydroxybutyrate granules.
5. What are polyglucan granules and give examples of their distribution?
6. What are sulfur globules and give examples of their distribution?
7. What are gas vacuoles and give examples of their distribution?

12.10. KEY WORDS

Inclusion bodies in bacteria, Polyphosphate granules, Poly beta-hydroxybutyrate granules, Sulfur globules, Gas vacuoles.

12.11. FURTHER SUGGESTED READING

1. Aneja K.R., Jain P. and Aneja R. "*A Text Book of Basic and Applied Microbiology*" New Age International Pub. New Delhi (2008).
2. Pelczar M.J., Chan E.C.S. and Kreig N.R. "*Microbiology* – 5th edn., Tata McGraw-Hill Pub. Co. New Delhi (1986)
3. Purohit, S.S. 2006. Microbiology – Fundamentals and Application. Seventh Edition. Agrobios (India) Publishers, Jodhpur.
4. Ravi Mantha. 2012. All about bacteria. Collins Publications.
5. Stanier, R.Y., Ingraham, J.L., Wheelis, M.L., and Painter, P.R. 2007. General Microbiology Fifth Edition. McMillan Publishers, London.
6. Trivedi, P.C. 2006. Applied Microbiology. Agrobios (India) Publishers, Jodhpur.

12.12. SOURCES

1. Kenneth Todar. 2003. Structure and function of prokaryotic cells. University of Wisconsin-Madison Department of Bacteriology.
2. Kenneth Todar. Structure and Function of Bacterial Cells. Todar's online textbook of bacteriology. www.textbookofbacteriology.net
3. Kenneth Todar. 2009. Structure and Function of Bacterial Cells. Lectures in Microbiology by Kenneth Todar PhD University of Wisconsin-Madison Department of Bacteriology.
4. Kim, B. H., and Gadd, G. M. 2008. Bacterial Physiology and Metabolism. Cambridge University Press.
5. Prescott. 2006. Prokaryotic cell structure and function, Chapter 3. pp.39-78.
6. Shively, J. M. 1974. Inclusion bodies of prokaryotes. Annu. Rev. Microbiol. 1974.28:167-188.
7. Talaro. 2009. An Introduction to the Prokaryotic Cell, Its Organization, and Members. Foundations of Microbiology, seventh edition. The McGraw-Hill Companies, Inc.
8. The clinical significance of bacterial anatomy. Microbiology: A Clinical Approach. Garland Science

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UNIT 13

INCLUSION IN BACTERIA – VOLUTIN AND OTHER BODIES

STRUCTURE

13.1. OBJECTIVES

After reading this unit we will be able to learn about:

- The other miscellaneous types of incusion bodies found in bactrial cells.
- The specific functions of different inclusion bodies
- The details of different inclusion bodies like cyanophycin granules, carboxysomes, magnetosomes, phycobilisomes, crystals and paracrystalline arrays, chlorosomes and other inclusion bodies
- The functions and applications of different types of inclusion bodies

13.2. INTRODUCITON

Apart from the commonly found inclusion bodies which are meant for storage, some bacteria also possess other types of includison bodies which perform specific functions. These include cyanophycin granules, carboxysomes, magnetosomes, phycobilisomes Etc., Cyanobacteria contain large inclusion bodies called cyanophycin granules that store nitrogen for the bacteria. Cyanobacteria, thiobacilli, and nitrifying bacteria, organisms that reduce CO_2 in order to produce carbohydrates, possess carboxysomes containing an enzyme used for CO_2 fixation. Some motile aquatic bacteria are able to orient themselves by responding to the magnetic fields of the earth because they possess magnetosomes, membrane-bound crystals of magnetite or other iron-containing substances that function as tiny magnets. Several members of the genus *Bacillus* form parasporal inclusions. Inclusions with polygonal profiles have been observed in the blue-green Algae, and in many, but not all, of the nitrifying bacteria and thiobacilli. Lipid deposits have been repeatedly reported in a number of bacteria and blue-green algae.

13.3. CYANOPHYCIN GRANULES

Cyanobacteria contain large inclusion bodies called cyanophycin granules that store nitrogen for the bacteria. Cyanophycin granules are observed in most, if

not all, of the blue-green algae. When well preserved by proper fixation, they appear in thin sections "tightly packed, undulating, flattened sacs". These inclusions are without limiting membrane and are variable in size and shape. The granules *Anabaena cylindrica* consist of 25,000-100,000 dalton molecular weight polypeptides containing arganine and aspartic acid in a 1:1 ratio. The number of granules is lowest in cells of exponentially growing cultures and highest in cells of stationary phase cultures and akinetes. The number decreases upon germination of the akinete or upon transfer of the maximum stationary phase culture to conditions suitable for growth. These granules are cellular nitrogen reserves. During normal growth, amino acids are incorporated into protein, but upon cessation of growth, nitrogen fixed from the atmosphere is stored in the polypeptide

13.4. PHYCOBILISOMES

The cyanobacteria carry out oxygenic photosynthesis, that is, they use water as an electron donor and generate oxygen during photosynthesis. The photosynthetic system is located in an extensive thylakoid membrane system that is lined with particles called phycobilisomes. Three biliproteins have been reported in the blue-green algae: C-phycocyanin (C-PC), allophycocyanin (allo-PC), and C-phycoerythrin (C-PE). C-PC and PC appear to be universal constituents; C-PE may or may not be present. The chromophores, the tetrapyrrole pigments phycocyanobilin and phycoerythrobilin, are readily separated from their apoproteins. Phycobilisomes, high molecular weight aggregates of C-PC, allo-PC, and frequently C-PE, were originally demonstrated in the Rhodophyta, but have subsequently been observed in many blue-green algae. These inclusions are 35-50 nm in diameter and are attached to the photosynthetic lamellae. It is still to be resolved whether all of the biliproteins are attached, or if a certain quantity is free in the cytoplasm. The C-PC containing phycobilisomes of *Synechococcus lividus* are described as rods, 35 nm in diameter, consisting of heptamers of smaller rods each of which is composed of a stack of dimeric discs. Each dimeric disc is 3.0-3.5 nm thick and 12.0-12.5 nm in diameter. It is proposed that these discs are hexamers of phycocyanin. The phycobilisomes of *Anacystis nidulans* also lack C-PE and are rod shaped, while those of *Nostoc* species, which possess all three biliproteins, are compact structures

with a rounded surface and a flattened base (40 nm in diameter) which attaches the complex to the photosynthetic lamellae. Isolated phycobilisomes are variable in size and shape. This is the result of natural heterogeneity or damage during isolation.

13.5. CRYSTALS AND PARACRYSTALLINE ARRAYS

Several members of the genus *Bacillus* form parasporal inclusions. The best characterized, because of its toxicity to *Lepidoptera* larvae, is the parasporal crystal of *Bacillus thuringiensis*. The crystal, a bipyrimidal octahedral with a square base plane, is composed of rod-shaped subunits 4.7 nm by 11.8 nm. With a molecular weight of 230,000 daltons and break down to smaller subunits upon treatment with alkali. The formation of the crystal is definitely linked to sporulation and evidence indicates that the crystal protein may result from the over production of spore coat proteins. The crystal forms in 1-2% of the cells that do not form spores (85% of calls sporulate normally). Parasporal crystals have also been reported in clostridia. *Clostridium cochlearium* This crystal was formed only in the sporangium. A second crystal was formed (6.5 nm periodicity) in both vegetative cells and sporangia.

13.6. CARBOXYSOMES

Cyanobacteria, thiobacilli, and nitrifying bacteria, organisms that reduce CO2 in order to produce carbohydrates, possess carboxysomes containing an enzyme used for CO2 fixation. Inclusions with polygonal profiles have been observed in the blue-green Algae, and in many, but not all, of the nitrifying bacteria and thiobacilli. They have also been observed *Beggiatoa*. There are commonly several bodies per cell; t-15 (4-6 most common) in *Thiobacillus neapolitanus*, 60-70 in older cells of *Thiobacillus thioparus*, and 200 in *Nitrococcus mobilis*. As seen in thin section, the bodies are 90-500 nm in diameter, have a granular substructure of medium electron density, are commonly located in the nucleoplasmic region of the cell and appear to be bounded by a nonunit membrane 3.0-4.0 nm thick.

13.7. CHLOROSOMES

The green bacteria carry out anoxygenic photosynthesis. They use reduced molecules such as H2, H2S, S, and organic molecules as an electron source and generate NADH and NADPH. The photosynthetic system is located in ellipoidal vesicles called chlorosomes that are independent of the cytoplasmic membrane. The chlorophyll, principally chlorobium chlorophyll, but also bacteriochlorophyl *a,* of the green photosynthetic bacteria is contained within vesicles 30-40 nm wide by 100-150 nm long, which are bounded by a nonunit membrane 2-3 nm thick. The vesicles immediately underly the cytoplasmic membrane and more or less completely line the internal periphery of the cell. The vesicles may appear either transparent or opaque in thin section depending on the embedding medium utilized. The vesicles themselves may be interconnected, but they have little, if any, association with the cytoplasmic membrane of the cell. Under normal growth conditions the vesicles constitute about 12% of the total cell dry weight.

13.8. MAGNETOSOMES

Some motile aquatic bacteria are able to orient themselves by responding to the magnetic fields of the earth because they possess magnetosomes, membrane-bound crystals of magnetite or other iron-containing substances that function as tiny magnets. These inclusion bodies are used for purpose other than storage. Some magnetotactic bactrium like *Aquaspirillium magnetotacticum* stores Magnetitite (Ferric oxide). The presence of such magntic inclusiosn enables these bacteria to respond to magnetic fields. Bacteria use magnetosomes to orient in the earth's magnetic field

13.9. OTHER INCLUSIONS

Lipid deposits have been repeatedly reported in a number of bacteria and blue-green algae. Many of these will probably be identified as poly-fl-hydroxybutyrate, but the occurrence of other lipid deposits is entirely possible. Other inclusions in prokaryotes include the cylindrical bodies of *Trichodesmiuemr ythraeum* and *Symplocam uscorum*; the membrane-associated protein inclusions of

Bacillus subtilis; the striated organelles of *Halobacteriuhma lobium*; the fibrillar organelle and greenish bodies *Thiovulum muasj,"* the filamentous inclusions, spheroids, rosette-like inclusions ,and intrathylakoidal granules of representatives of the blue-green algae; the large granulated body of *Corynebacterium;* the hydrocarbon inclusion of *dcinetobacter*; the calcium carbonate inclusions of *Achromatiu*m; and the ribosome studded inclusions of the myxospores of *Stigmatella arthantiaca*.

13.10. SUMMARY

Apart from the commonly found inclusion bodies which are meant for storage, some bacteria also possess other types of includison bodies which perform specific functions. These include cyanophycin granules, carboxysomes, magnetosomes, chlorosomes, phycobilisomes Etc.,

Cyanophycin Granules: Cyanobacteria contain large inclusion bodies called **cyanophycin granules** that store nitrogen for the bacteria. Cyanophycin granules are observed in most, if not all, of the blue-green algae. These inclusions are without limiting membrane and are variable in size and shape. The granules. E.g., *Anabaena cylindrical.* These granules are cellular nitrogen reserves. During normal growth, amino acids are incorporated into protein, but upon cessation of growth, nitrogen fixed from the atmosphere is stored in the polypeptide

Phycobilisomes: The photosynthetic system of cyanobacteria is located in an extensive thylakoid membrane system that is lined with particles called phycobilisomes. Three biliproteins have been reported in the blue-green algae: C-phycocyanin (C-PC), allophycocyanin (allo-PC), and C-phycoerythrin (C-PE). C-PC and PC appear to be universal constituents; C-PE may or may not be present. These inclusions are 35-50 nm in diameter and are attached to the photosynthetic lamellae. E.g., *Anacystis nidulans* Isolated phycobilisomes are variable in size and shape.

Crystals and Paracrystalline Arrays: Several members of the genus *Bacillus* form parasporal inclusions. E.g., *Bacillus thuringiensis*. The formation of the crystal is

linked to sporulation and evidence indicates that the crystal protein may result from the over production of spore coat proteins. The crystal forms in 1-2% of the cells that do not form spores (85% of calls sporulate normally). Parasporal crystals have also been reported in clostridia. *Clostridium cochlearium.*

Carboxysomes: Cyanobacteria, thiobacilli, and nitrifying bacteria, organisms that reduce CO_2 in order to produce carbohydrates, possess carboxysomes containing an enzyme used for CO_2 fixation. Inclusions with polygonal profiles have been observed in the blue-green Algae, and in many, but not all, of the nitrifying bacteria and thiobacilli . They have also been observed *Beggiatoa*. There are commonly several bodies per cell.

Chlorosomes: The green bacteria carry out anoxygenic photosynthesis. They use reduced molecules such as H_2, H_2S, S, and organic molecules as an electron source and generate NADH and NADPH. The photosynthetic system is located in ellipoidal vesicles called chlorosomes that are independent of the cytoplasmic membrane. The chlorophyll, principally chlorobium chlorophyll, but also bacteriochlorophyl *a,* of the green photosynthetic bacteria is contained within vesicles. Under normal growth conditions the vesicles constitute about 12%o f the total cell dry weight.

Magnetosomes: Some motile aquatic bacteria are able to orient themselves by responding to the magnetic fields of the earth because they possess magnetosomes, membrane-bound crystals of magnetite or other iron-containing substances that function as tiny magnets. Some magnetotactic bactrium like Aquaspirillium magnetotacticum stores Magnetitite (Ferric oxide). The presence of such magntic inclusiosn enables these bacteria to respond to magnetic fields.

Other inclusions: Other inclusions in prokaryotes include the cylindrical bodies of *Trichodesmiuemr ythraeum* and *Symplocam uscorum*; the membrane-associated protein inclusions of *Bacillus subtilis*; the striated organelles of *Halobacteriuhma lobium*; the fibrillar organelle and greenish bodies *Thiovulum muasj,"* the filamentous inclusions, spheroids, rosette-like inclusions ,and intrathylakoidal granules of representatives of the blue-green algae; the large granulated body of *Corynebacterium;* the hydrocarbon inclusion of *dcinetobacter*; the calcium carbonate inclusions of *Achromatiu*m; and the ribosome studded inclusions of the myxospores of *Stigmatella arthantiaca*.

13.11. CHECK YOUR PROGRESS

1. What are the different kinds of miscellaneous inclusion bodies found in bacterial cells?
2. Explain briefly about cyanophycin granules, carboxysomes, magnetosomes, phycobilisomes, crystals and paracrystalline arrays, chlorosomes and other inclusion bodies.
3. What are the functions and applications of different types of inclusion bodies?

13.12. KEY WORDS

Cyanophycin granules, carboxysomes, magnetosomes, phycobilisomes, crystals and paracrystalline arrays, chlorosomes and other inclusion bodies

13.13. Further suggested reading

1. Aneja K.R., Jain P. and Aneja R. "*A Text Book of Basic and Applied Microbiology*" New Age International Pub. New Delhi (2008).
2. Pelczar M.J., Chan E.C.S. and Kreig N.R. "*Microbiology* – 5th edn., Tata McGraw-Hill Pub. Co. New Delhi (1986)
3. Purohit, S.S. 2006. Microbiology – Fundamentals and Application. Seventh Edition. Agrobios (India) Publishers, Jodhpur.
4. Ravi Mantha. 2012. All about bacteria. Collins Publications.
5. Stanier, R.Y., Ingraham, J.L., Wheelis, M.L., and Painter, P.R. 2007. General Microbiology Fifth Edition. McMillan Publishers, London.
6. Trivedi, P.C. 2006. Applied Microbiology. Agrobios (India) Publishers, Jodhpur.

13.14. SOURCES

1. Kenneth Todar. 2003. Structure and function of prokaryotic cells. University of Wisconsin-Madison Department of Bacteriology.
2. Kenneth Todar. Structure and Function of Bacterial Cells. Todar's online textbook of bacteriology. www.textbookofbacteriology.net
3. Kenneth Todar. 2009. Structure and Function of Bacterial Cells. Lectures in Microbiology by Kenneth Todar PhD University of Wisconsin-Madison Department of Bacteriology.
4. Kim, B. H., and Gadd, G. M. 2008. Bacterial Physiology and Metabolism. Cambridge University Press.
5. Prescott. 2006. Prokaryotic cell structure and function, Chapter 3. pp.39-78.
6. Shively, J. M. 1974. Inclusion bodies of prokaryotes. Annu. Rev. Microbiol. 1974.28:167-188.
7. Talaro. 2009. An Introduction to the Prokaryotic Cell, Its Organization, and Members. Foundations of Microbiology, seventh edition. The McGraw-Hill Companies, Inc.
8. The clinical significance of bacterial anatomy. Microbiology: A Clinical Approach. Garland Science

UNIT 14

BACTERIAL PIGMENTS

STRUCTURE

14.1. OBJECTIVES

After reading this chapter we will be able to understand:

- The diversity of pigments produced by bacterial colonies
- The array of pigmentation and their representative colours in bacteria
- The factors that influence pigmentation in bacteria
- Details of some of the important pigments in bacteria and their functions
- Applications of bacterial pigments in various fields

14.2. INTRODUCTION

Pigments are molecules that have color. Some bacteria produce pigments as part of their normal metabolism. Pigment producing bacteria are called Chromobacteria. The specific color of the pigment is characteristic for each bacterium. Chromogenic bacteria may be the ones which produce pigmnets intracellular or those which produce extracellular soluble pogments. Intracellular pigments which are produced which can be seen after they grow into colonies. Extracellular pigments are secreted outside into the surrounding medium. Most pigments have a function in bacterial metabolic or physiological acts. Pigments can help identify bacteria (Fig. 1)

Figure 1: Range of pigmentation produced by bacterial strains

14.3. FACTORS AFFECTING PIGMENT PRODUCTION

Bacteria produce pigments for various reasons and it plays an important role. Some bacteria such as cyanobacteria have phycobilin pigments to carry out photosynthesis. Other example for pigment-producing bacterial strains includes *Serratia marcescens* that produces prodigiosin, *Streptomyces coelicolor* (prodigiosin and actinorhodin), *Chromobacterium violaceum* (violacein) and *Thialkalivibrio versutus* (natronochrome and chloronatronochrome).

Production of pigments is influenced by various factors like light, temperature, pH, and constitution of media. Bacterial pigments may be water soluble or water insoluble. Bacterial pigments are derivatives of xanthophylls, caretenoids, pyrrole, phenazine, or quinone. Pigments are synthesised in the periplasmic space or in cell walls of bacteria. Anaerobic bacteria do not produce pigments.

For example, some bacteria produce water soluble pigments which spread through the medium in which they grow. Others produce pigments that are soluble in fat. To determine this one can remove some of a pigmented colony and shake it in oil. If the oil becomes pigmented the pigment is fat soluble. Sometimes a species of bacteria will only produce their pigments under certain environmental circumstances. For example, *Serrattia marsescens* produces a brick red pigment when grown at room temperature, but no pigment when grown at body temperature (37^0 C). Other species produce pigment as the colonies age or when a particular nutrient is present in the media.

14.4. RANGE OF BACTERIAL PIGMENT AND COLONY COLORS

Bacterial pigments produce a wide range of colors as follows (Fig. 2):

Since, pigmentation requires oxygen, usually, pigments are produced by aerobic or facultative aerobic bacteria.

Table 1: Range of pigments and their color produced by differnet bacteria.

Bacteria	Pigment	Colony color
Staphylococcus epidermidis	Astaxanthin	White
Prevotela melaninogenica	Melanin	Black
Proteus vulgaris	Astaxanthin	Cream
Serratia marcescens	Prodigiosin	Red
Rugamonas rubra	prodigiosin	Maroon
Micrococcus roseus	Astaxanthin	Pink
Streptomyces coelicolor	Actinorhodin	Blue
Chlorobium tepidum	Bacteriochlorophyll(BChl)	Green
Sarcina aurentiaca	Carotene	Orange
Xanthomonas campestris	Xanthomonadin	Yellow
Spirillum rubrum	violacein	Purple
Chromobacterium violacein	violacein	Violet
Janthinobacterium lividum	violacein	Indigo
Rhizobium elti	Melanin	Brown
Staphylococcus aureus	Staphyloxanthin Zeaxanthin	Golden
Actinomyces sp.	Melanoid	Silver
Pseudomonas aeruginosa	Pyocyanin	Fluorescent blue/green
Pseudomonas fluorescens	Pyoverdin/Fluorescin	Fluorescent yellow

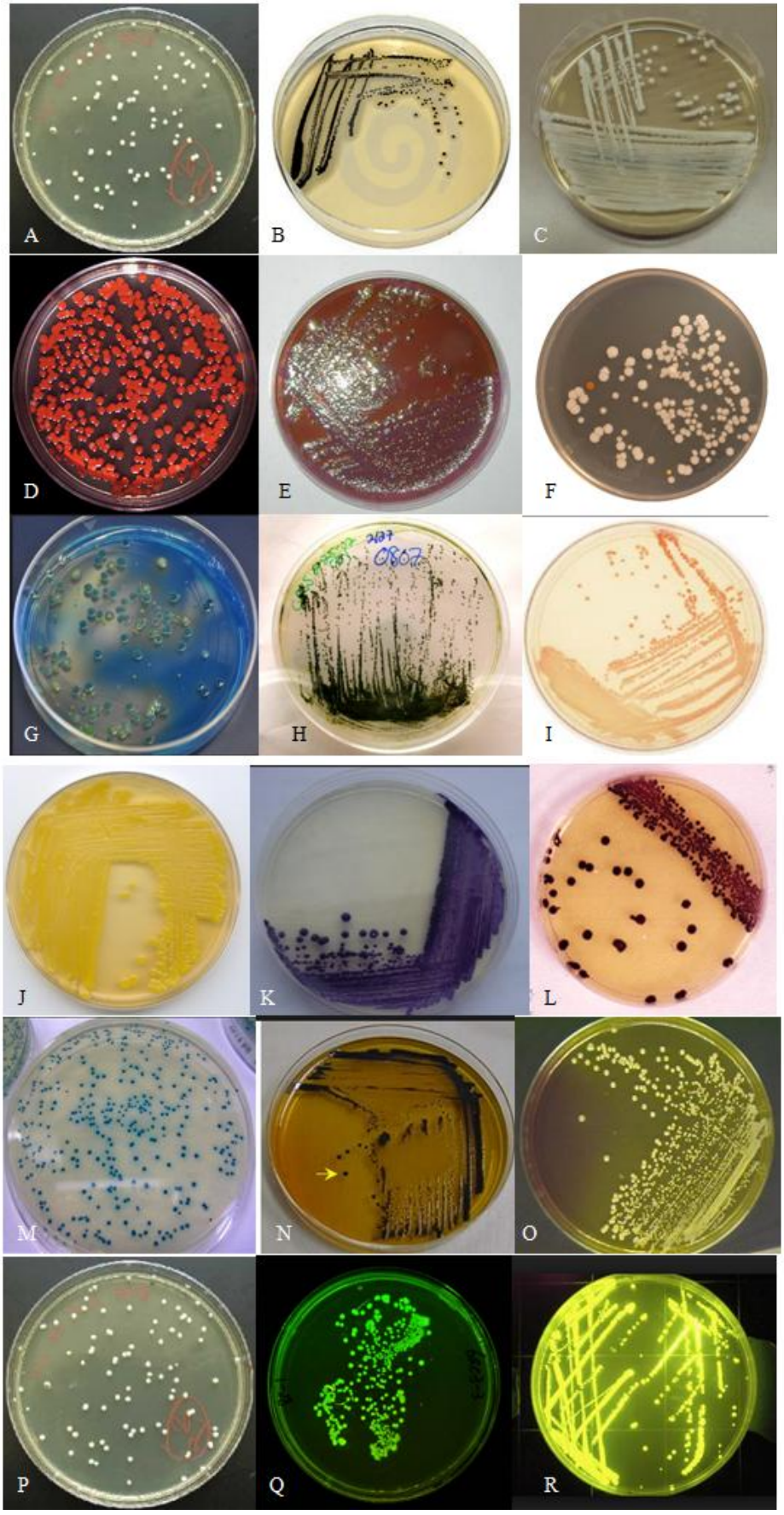
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Figure 2: Differnet kinds of pigments produced by bacteria

A. White colored colonies of *Staphylococcus epidermidis*
B. Black colored colonies of *Prevotela melaninogenica*
C. Cream colored colonies *Proteus vulgaris*
D. Red colored colonies of *Serratia marcescens*
E. Maroon colored colonies of *Rugamonas rubra*
F. Pink colored colonies of *Micrococcus roseus*
G. Blue colored colonies of *Streptomyces coelicolor*
H. Green colored colonies of *Chlorobium tepidum*
I. Orange colored colonies *Sarcina aurentiaca*
J. Yellow colored colonies of *Xanthomonas campestris*
K. Purple colored colonies of *Spirillum rubrum*
L. Violet colored colonies of *Chromobacterium violacein*
M. Indigo colored colonies of *Janthinobacterium lividum*
N. Brown colored colonies of *Rhizobium elti*
O. Golden colored colonies of *Staphylococcus aureus*
P. Silver colored colonies of *Actinomyces sp.*
Q. Fluorescent green colored colonies of *Pseudomonas aeruginosa*
R. Fluorescent yellow colored colonies of *Pseudomonas fluorescens*

14.5. CULTURING PIGMENT PRODUCING BACTERIA

Several kinds of indicator or differential media are used for culturing pigment producing bacteria. Such media will be supplemented with pH indicator dyes like methylene blue, methyl red, eosin, and certain chemiclas like sodium sulphite, potassium tellurite. Colonies of bacteria on such media appear colored. E.g., MacConkey agar, EMB agar. Lactose fermenting bacteira appear pink on MacCnkey agar.

14.6. IMPORTNAT BACTERIAL PIGMENTS AND THEIR FUNCTIONS

Bacterial pigments are associated with bacteria in several ways like defining thier morphological characters, cellular metabolism, pathogenesis, protection and survival. Bacterial pigments help in photosynthesis. Cyanobacteria have green pigment Chlorophyll, Phycobilin, Chlorophyll b which are useful in photosynthesis. Other photosynthetic bacteria have bacteriochlorophyll, proteorhodopsin and bacteriorhodopsin useful for photosynthesis.

Carotenoids are the most widespread pigments in bacteria and there are hundreds of types. Carotenoid pigments protect bacteria from light and oxidative damage from activated forms of oxygen. Phycobiliproteins are red or blue Carotenoid-like pigments of photosynthetic bacteria like cyanobacteria help in light harvesting.

Carotenoid or carotenoid-like compounds are produced by halophilic bacteria related to the *Cytophaga-Flavobacterium- Bacteroides* group. Astaxanthin is one of the carotenoids that have commercial value as a food supplement for humans and as food additives for animals and fish. Recently, another astaxanthin-producing marine bacterium was isolated and identified as *Paracoccus haeundaensis*.

Prodiginines – red pigment isolated from Pseudoalteromonas denitrificans produce cycloprodigiosin. This compound has immunosuppressive, antimalarial, and apoptosis-inducing activities Prodiginines share a common pyrrolyldipyrromethene core structure and have a wide variety of biological properties, including antibacterial, antifungal, antimalarial, antibiotic, immunosuppressive, and anticancer activities.

Violacein- The violet pigment violacein is an indole derivative, predominantly isolated from bacteria of the genus *Chromobacterium* that inhabit the soil and water of tropical and subtropical areas. Violacein has a variety of biological activities, including antiviral, antibacterial, antiulcerogenic, antileishmanial, and anticancer properties. Use of violacein as a chemical defense against eukaryotic predators has also been reported.

Phenazine Compounds- Phenazines are redox-active, small nitrogen-containing aromatic compounds produced by a diverse range of bacterial genera, including *Streptomyces* (terrestrial), *Pseudomonas* (ubiquitous), *Actinomycetes* (terrestrial and aquatic), *Pelagibacter* (aquatic), and *Vibrio* (aquatic), under the control of quorum sensing. Due to the abundance

and biotechnological application of *Pseudomonas aeruginosa* phenazines, pyocyanin and pyorubrin have also been suggested as food colorant pigments.

Quinones- Quinones are additional colored compounds with an aromatic ring structure that have been isolated from marine environment. Quinone derivatives range in color from yellow to red, exhibit antiviral, antiinfective, antimicrobial, insecticidal, and anticancer activities,
and have many commercial applications as natural and artificial dyes and pigments.

Tambjamines- It has long been noticed that marine bacteria have the ability to prevent biofouling. The marine *Pseudoalteromonas* species, *P. tunicata* has the widest range of antibiofouling activities against microorganisms, including bacteria, invertebrate larvae, algal spores, protozoan, and fungi, and provides protection for host marine organisms. The tambjamines also exhibit antibiotic activity against *E. coli*, *Staphylococcus*, *Vibrio anguillarum, Bacillus subtilis,* and *Candida albicans,* and displayed cytotoxic activity against several tumor cell lines.

Pigments are light-absorbing compounds that are responsible for the colors that organisms display. For example, the pigment xanthomonadin protects the Bacteria "Xanthomonas oryzae" from damage due to light (photo damage)

Food Colorants: A blue pigment produced by the bacteria "Streptomyces coelicolor" was isolated and named as λ-actinorhodin, related to the bacteria-produced antibiotic actinorhodin. In a toxicity trial, this pigment was shown to have an LD50 (median lethal dosage) greater than 15000 mg/kg, thus making it non-toxic.

Textile and Other Colorants: yellow-orange, red, and purple pigments were extracted from "Chryseobacterium sp.", "Serratia marceseus", and Chromobacterium_violaceum "Chromobacterium violaceum" respectively. The red and purple pigments, known as prodigiosin and violacein respectively, are used as colorants on different fabrics (acrylic fiber, silk, cotton, polyester, and polyester microfiber),

Fluorescence-based Indicators: Bacterial pigments with fluorescence are used in laboratories to label antibodies and also indicate the progress of specific reactions. A key example is phycoerythrin, used to detect the rate of damage caused by free radicals. A specific type of Phycoerthyrin, known as R-Phycoerthyrin, can be used to fluorescently label antibodies.

Human Health: Some bacterial pigments are used to promote human health, providing key nutrients and compounds that are needed by the body. Carotenes, β-carotene, are essential in maintaining the yellow color of the retinal giving it the ability to act as sun block on certain parts of the retina.

Common bacteria pigments such as prodigiosin (red color), carotene, and xanthophylls have carcinogenesis-prevention roles, as these pigments have anti-oxidative, anti-free radical, and apoptosis-inducing activity.

Melanin, a common pigment that creates the black, brown, and grey colors in many bacteria, has also shown significant antioxidant activity. Furthermore, melanin is used in sunblock to protect the skin from harmful UV radiation.

Bacterial pigments help in cell protection by absorbing UV radiation and quenching oxygen free radicals.

Certain bacerial pigments are antibiotic in nature which protect the bacteria against pathogens. E.g., Prodigiosin (Serratia), Erythromycin (Streptomyces), Pyocyanin, Pyoverdin and Pyochelin from Pseudomonas, Spirilloxanthin from Spirillum.

Bacterial pigments help in overcoming stress conditions. E.g., rhizobacteria Pseudomonads produce iron chelating siderophore which draw iron from rhizosphere. This iron chelating process deprives iron for pathogenic bacteria and fungi. E.g., Pyochelin, Pyoveridin.

Extremophilic bacteria are bright colored and their pigments protect them from oxidative stress, maintain membrane integrity and stability, help in respiration and photosynthesis.

Bacterial pigments confer antibacteiral and heavy metal resistance. Staphylococci are multidrug tolerant because of their pigment which protect cell wall and membrance from antibiotics. Heavy metal resistant bacteria have pigments which help in remediation of water and soil from arscenic, mercury, cadmium and nickel.

Pigmented bactria act as biosensors and are useful in detecting environemtnal pollution like oil spills, pesticide and heavy metal recalcitrance.

14.7. SUMMARY

Pigments are molecules that have color. Some bacteria produce pigments as part of their normal metabolism. Pigment producing bacteria are called Chromobacteria. The specific color of the pigment is characteristic for each bacterium.

Chromogenic bacteria may be the ones which produce pigmnets intracellular or those which produce extracellular soluble pogments. Intracellular pigments which are produced which can be seen after they grow into colonies. Extracellular pigments are secreted outside into the surrounding medium.

Production of pigments is influenced by various factors like light, temperature, pH, and constitution of media. Bacterial pigments may be water soluble or water insoluble. Bacterial pigments are derivatives of xanthophylls, caretenoids, pyrrole, phenazine, or quinone. Pigments are synthesised in the periplasmic space or in cell walls of bacteria. Anaerobic bacteria do not produce pigments.

Range of bacterial pigment and colony colours

Bacterial pigments produce a wide range of colors as follows:

Since, pigmentation requires oxygen, usually, pigments are produced by aerobic or facultative aerobic bacteria.

Bacteria	Pigment	Colony color
Staphylococcus epidermidis	Astaxanthin	White
Prevotela melaninogenica	Melanin	Black
Proteus vulgaris	Astaxanthin	Cream
Serratia marcescens	Prodigiosin	Red

Rugamonas rubra	prodigiosin	Maroon
Micrococcus roseus	Astaxanthin	Pink
Streptomyces coelicolor	Actinorhodin	Blue
Chlorobium tepidum	Bacteriochlorophyll(BChl)	Green
Sarcina aurentiaca	Carotene	Orange
Xanthomonas campestris	Xanthomonadin	Yellow
Spirillum rubrum	violacein	Purple
Chromobacterium violacein	violacein	Violet
Janthinobacterium lividum	violacein	Indigo
Rhizobium elti	Melanin	Brown
Staphylococcus aureus	Staphyloxanthin Zeaxanthin	Golden
Actinomyces sp.	Melanoid	Silver
Pseudomonas aeruginosa	Pyocyanin	Fluorescent blue/green
Pseudomonas fluorescens	Pyoverdin/Fluorescin	Fluorescent yellow

Several kinds of indicator or differential media are used for culturing pigment producing bacteria. Such media will be supplemented with pH indicator dyes like methylene blue, methyl red, eosin, and certain chemiclas like sodium sulphite, potassium tellurite. Colonies of bacteria on such media appear colored. E.g., Lactose fermenting bacteira appear pink on MacCnkey agar.

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Applications of bacterial pigments

Bacterial pigments have several applications.

1. Bacterial pigments offer resistance to phagocytosis. Bacterial pigments make the bacteria unpalatable tobacteriophagous species of protozoa and nematodes.

1. Bacterial pigments offer heat resistance and acid stability
2. Bacterial pigments enhance in vitro antibody formation.
3. Bacterial pigments have anticancer properties.
4. Bacterial pigments are used in paint manufacturing
5. Bacterial pigments are used in food industries as additives and colorants
6. Bacterial pigments are used in textile industries for coloring/dyeing
7. Bacterial pigments are a rich source of Vitamin A.
8. Bacterial pigments are used in various therapeutics
9. Bacterial pigments are used as indicators of oil spills
10. Bacterial pigments are used as biosensors for detecting air, water and soil pollution
11. Bacterial pigments are used in bacterial identification

14.8. CHECK YOUR PROGRESS

1. What are bacterial pigments? Explain the different types of pigments.
2. What are the importnat factors that affect bacterial pigment production?
3. Give an account of differnet types of pigments and their colors in bacteria.
4. Name some of the importnat bacterial pigments and their functions
5. Write a note on the photosynthetic pigments of bacteria.
6. Write a note on carotenoids pigments in bacteria and their applications.
7. What are Prodiginines? Mention their functions.
8. What are Violaceins? Name their uses.
9. What are Quinones? Name their applications
10. What are Tambjamines and what are their uses?

11. Add a note on use of pigments as food and textile colorants.
12. Discuss in detail the applications of bacterial pigments in various fields.

14.9. KEY WORDS

Bacterial pigments, Chromobacteria, Chlorophyll, Phycobilin, Carotenoids, Prodiginines, Violacein, Phenazine Compounds, Quinones, Tambjamines, applications of bacterial pigments

14.10. FURTHER SUGGESTED READING

1. Ball, P. 2002. Bright earth: art and the invention of color; 1st American ed. New York: Farrar, Straus and Giroux.
2. Dapson, R.W. 2007. The history, chemistry and modes of action of carmine and related dyes. Biotech Histochem 82: 173–187.
3. Joshi, V.K., Attri, D., Bala, A. and Bhushan, S. 2003. Microbial Pigments. Indian J. Biotech., 2: 362-369.
4. Madigan, M T; Martinko J M and Parker J (2000). Brock: Biology of Microorganisms. 9th Ed. Prentice-Hall International. London. Pp 573 -589.
5. Nagpal, N., Munjal, N. and Chatterjee, S. 2011. Microbial Pigments with Health Benefits - A Mini Review. Trends Biosci, 4: 157-160.
6. Pfeifer, B.A., and Khosla, C. 2001. Biosynthesis of polyketides in heterologous hosts. Microbiol Mol Biol Rev 65: 106–118.

14.11. SOURCES

1. Ahmad, W.A., et al., 2012. Chapter 2-Isolation of Pigment-Producing Bacteria and Characterization of the Extracted Pigments. In: Application of Bacterial Pigments as Colorant, Springer Briefs in Molecular Science.
2. Aberoumand, A. 2011. A Review Article on Edible Pigments Properties and Sources as Natural Biocolorants in Foodstuff and Food Industry. World J. Dairy Food Sci., 6 (1): 71-78.

3. Ancient Rock Art's Colours Come from Microbes." http://www.bbc.co.uk/news
4. Charkoudian LK, Fitzgerald JT, Khosla C, Champlin A (2010) In Living Color: Bacterial Pigments as an Untapped Resource in the Classroom and Beyond. PLoS Biol 8(10): e1000510. doi:10.1371/journal.pbio.1000510
5. Kamla Malik, Jayanti Tokkas and Sneh Goyal. 2012. Microbial Pigments: A review. International Journal of Microbial Resource Technology 1: 361-365.
6. Maurice Moss. 2002. Bacterial pigments. Microbiologist December 2002, 10-12 www.sfam.org.uk

UNIT 15

FINE STRUCTURE AND HYDRO-DYNAMICS OF BACTERIAL FLAGELLA

STRUCTURE

15.1. OBJECTIVES

After reading this section we will be able to understand:

- The structural features of bacterial flagella
- Types and arrangements of bacterial flagella
- Functioning of the bacterial flagella during bacterial motility
- Flagellar assembly
- The hydrodynamics of bacterial flagellar movement
- The fine structure of different parts of bacterial flagella
- Functions of the bacterial flagella

15.2. INTRODUCTION

The flagellum consists of three parts: the filament (helical propeller), the hook (universal joint), and the basal structure (rotary motor).

Filament: In a prokaryotic flagellum, chains of a globular protein are wound in a tight spiral to form a filament (7-15 μm long) and thin (20 nm diam.), which is attached to another protein (the hook), which is inserted into the basal apparatus.

Hook: It comprises of the region between filament and basal structure. It is short, slightly curved, and has a diameter somewhat greater than the filament. The hook ranges from 70-90 nm in length and consists principally of single polypeptide.

Basal Body: Gram negative bacteria possess MS-ring system in cell membrane. 1. P-ring system seen in peptidoglycan layer. 2. L-ring system that is present in lipopolysaccharide layer is also seen. 3. Gram positive bacteria possess MS ring and P-ring. The C ring (switch) bound to MS ring. 4. Motor itself consist of stator and rotor known as Mot complex. Most complex (a pair of proteins) is involved in driving rotatory motion of the basal body. A set of proteins present between MS rings known as Fli proteins those act as molecular switches and are responsible for changing direction of rotation.

Each flagellum rotates 360º around a central axis and affects the surrounding medium much as a ship's propeller would. The motor situated at the base of the flagellum can speed up, slow down, stop and go into reverse. The driving force comes from streams of protons- naked hydrogens stored near the motor and released in volleys by the chemical action of the sensory processing system (e.g. chemotaxis). The flagellum's motor is much like that of, say, an electric mixer, but the mixer's is driven by electrons instead of protons.

Flagellum is propeller in action. The energy used to drive the flagellar rotation comes from the proton motive force. As a proton enters the cell through the mot complex, its energy is coupled to movement. In order to achieve a single rotation, 1000 protons must be translocated. The speed of rotation is directly proportional to the proton motive force. With flagellar activity a bacterium can attain a speed of 100 μ/second. Basal body rotates typically at 20,000 RPM, but when detatched from the filament can go 100K RPM.

Flagella and proton motive force: Rotation of the filament is driven by the diffusion of protons into the cell through the basal apparatus after the protons have been actively transported by proton pumps in the plasma membrane. The electron transport system is shown oxidizing NAD by removal of a pair of electrons, passing them through its sequence of carriers eventually to O2. ATPase is the transmembranous protein enzyme which is utilizing protons from the outside to synthesize ATP on the inside of the membrane.

15.3. FINE STRUCTURE OF THE FLAGELLUM

The flagellum has three morphologically and chemically distinguishable parts namely 10 The filament 2) hook and 3) the basal body (Fig. 1).

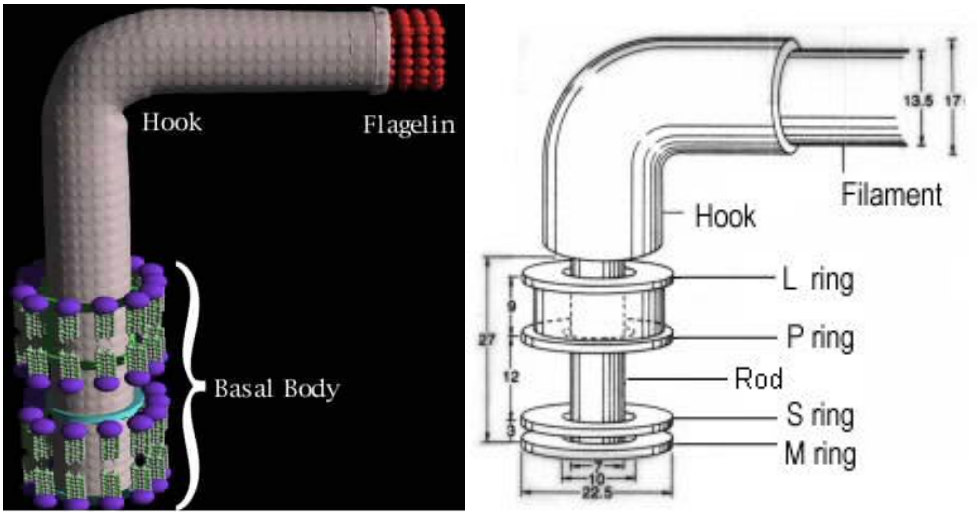

Figure 1: The detailed fine structure of the bacterial flagella

15.3.1. Filament

Filament is the largest part of the flagellum. Generally the filament is helical in structure. The filament is located outside the cell surface and lies between the hook and the distal end of the flagellum. The filament is around 20 nm in diameter, 10-20 μm in length and wavy in nature. Composed of primarily of a single, self-aggregating protein called flagellin. Flagellins are low molecular weight proteins twisted together in a helical conformation or around an axial cylinder depending on the bacterial species. Flagellin molecules are synthesised in the cytoplasm. The flagellum grows in length due to the addition of flagellin molecules towards the growing end. In Salmonella the filament grows to a length of around 15 mm and is composed of as many as 30,000 copies of a single protein named flagellin. Some bacteria, for example Vibrio, have several closely related flagellins that form the filament. The flagellin subunits are self-assembled to form a hollow concentric double-tubular structure (inner and outer tubes) consisting of 11 protofilaments, which are arranged approximately parallel to the filament axis Formation of a helical structure is achieved by a mixture of the protofilaments of two distinct conformations, the R- and L-type, distinguished by their helical handedness right or left. Each protofilament switches between these two conformations by responding to a variety of factors including pH, ionic strength, mechanical stress, and mutations. Intersubunit hydrophobic interactions in the inner tube make the filament structure mechanically stable, and the diameter of central channel is only 2 nm. This central

channel serves as a transport pathway of flagellins that will polymerize at the tip of the growing filament. In some bacteria like *Bdellovibrio* and *Vibrio cholerae*, a sheath surrounds the flagellar filament.

15.3.2. Hook

Transition between filament and motor. It comprises of the region between filament and basal structure. It is short, slightly curved, and has a diameter somewhat greater than the filament. The hook ranges from 70-90 nm in length and consists principally of single polypeptide. The hook is thought to function as a universal joint to smoothly transmit the torque produced by the motor to the filament. The hook structure of Salmonella is composed of about 120 copies of a single protein FlgE. The junction between hook and filament consists of the two proteins, FlgK (HAP1) and FlgL (HAP3). About 13 molecules of each protein are present in each flagellum. Mutational studies suggested that the junction acts as a buffering structure connecting two filamentous structures (hook and filament) with distinct mechanical properties.

15.3.3. Basal body

Anchor in cell wall and motor. It is the part that anchors or attaches the flagellum in the cytoplasmic membrane and the cell wall. It is a complex structure consisting of a small central rod and a series of discs or rings. The basal body functions like a motor system which helps the flagellum to rotate and propel the bacterium in the liquid environment. The proximal end of the hook is connected to the basal body structure, consisting of the rod and three coaxially mounted rings, termed as MS, P, and L ring (Fig. 2 and 3). The MS ring is embedded in the cytoplasmic membrane and made of a single protein FliF, the P and L rings are associated with the peptidoglycan layer and the outer membrane, respectively, and are composed of FlgI and FlgH. The rod structure is composed of three proximal rod proteins FlgB, FlgC, FlgF, and a distal rod protein FlgG, and fully traverses the periplasmic space. The L and P rings together form a quite rigid assembly resistant to stringent chemical treatments, and the LP-ring complex is believed to act as a molecular bushing for the flagellar axial structure. The basal body of Gram-positive bacteria is composed of only the MS ring and rod, and the LP ring is not present,

probably because Gram-positive bacteria do not have the outer membrane but have a thick peptidoglycan layer (Fig. 2). When the basal body is isolated with more gentle treatment, a drum-shaped structure, called C ring, was found on the MS ring facing the cytoplasm. It is composed mostly of FliM and FliN proteins. These proteins, together with FliG which is located beneath the MS ring, have been known to form a complex, referred to as the switch complex. They are also important for rotation, and mutational studies revealed that FliG most closely participates in torque generation. A central protrusion within the C ring is probably the export apparatus essential for assembly of flagellum.

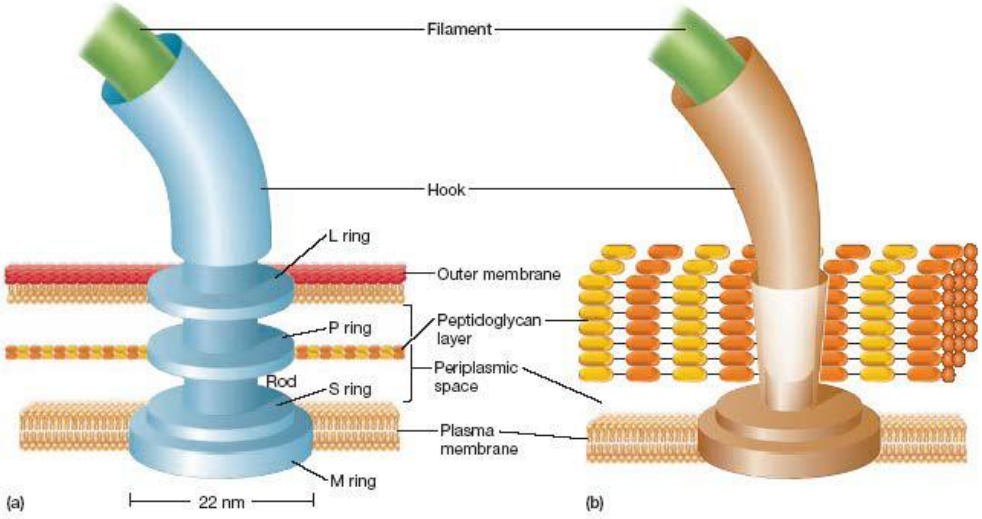

Figure 2: Differences between the flagellar structure of Gram positive and Gram negative bacteria

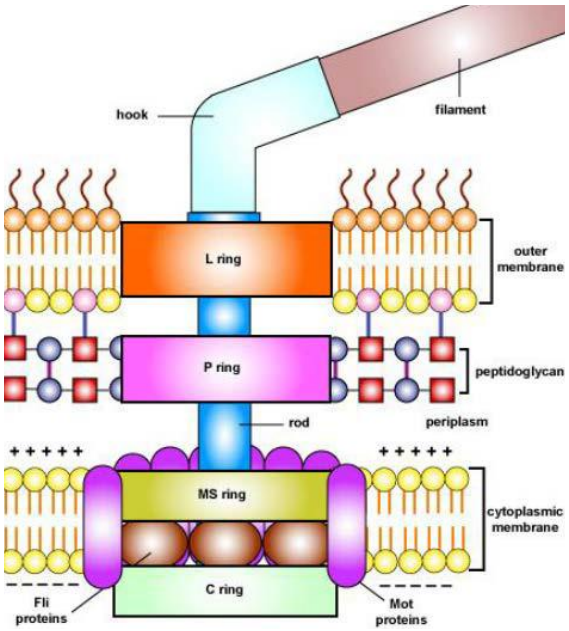

Figure 3: Basal body and ring systems of the bacterial flagella

15.4. FUNCTIONS OF THE FLAGELLUM

Flagella, the organelles of locomotion impart motility by rotating in clockwise and anticlockwise manner controlled by the basal body. The movement of the basal body is driven by a proton motive force rather than by ATP directly. Bacteria swim through liquid by means of the propeller-like action of the flagella in response to environmental stimulus. The stimulus may be due to chemicals (chemotaxis), light (phototaxis), osmotic pressure (osmotaxis), oxygen (aerotaxis), and temperature (thermotaxis). Chemotaxis, referred to as movement in response to attractant and repellent substances in the environment help bacterial pathogens to move through the mucous layer and colonize the mucous membranes and thereby facilitate bacterial pathogenesis.

Bacteria move towards the stimuli in two ways:
Runs – movements of cell in single direction for some time due to counterclockwise flagellar rotation; increase with favorable stimuli (positive chemotaxis, positive phototaxis)
Tumbles – abrupt, random, changes in direction due to clockwise flagellar rotation; increase with unfavorable stimuli (negative chemotaxis, negative phototaxis) (Fig. 4)

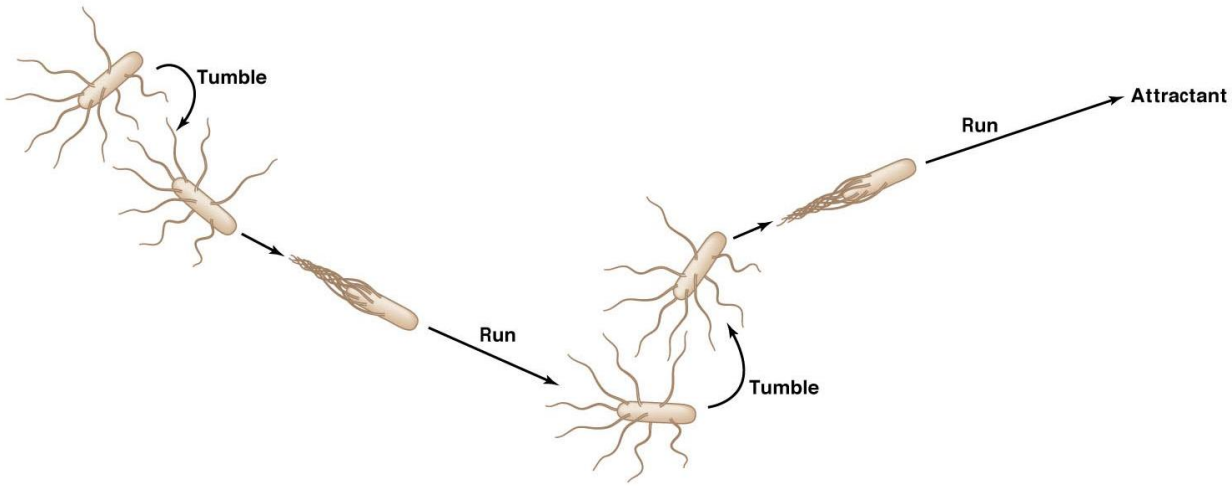

Figure 4: Tumble and Run movement of bacteria with flagella

15.5. FLAGELLAR ASSEMBLY

More than 50 genes are required for flagellar formation and function. Because the flagellum is such a big organelle, a large amount of energy is consumed during the assembly process. Bacteria deal with this problem by developing highly organized and regulated systems for flagellar assembly. Its characteristic feature is that the flagellar gene regulation temporally and tightly couples to the assembly process.

In general, assembly starts at the inner structure of the basal body then proceeds to the outer ones. The first built structure is the MS ring and proximal rod, which is formed by a single protein FliF. The MS ring is the core structure of the rotor and is embedded in the cytoplasmic membrane. The C ring attaches on the cytoplasmic face of MS ring. The C ring contains mostly two switch proteins FliM and FliN, and is associated with MS ring via another switch/motor protein FliG, which probably contributes to a part of the face of MS ring.

Assembly of these three proteins on the basal body requires the MS-ring platform, and mutations give rise to the nonflagellate phenotype. Inside the MSring, there is the flagellum-specific export apparatus which appears as a protrusion inside the C ring. When the export apparatus is established in the flagellar base is still unclear. After the export apparatus is constructed, structural proteins for the basal body, expressed from class 2 operons, are secreted through the export apparatus. First, the proximal rod, composed of FlgB, FlgC, and FlgF, is added on the MS ring. FliE is needed for this assembly, joining FliF, and proximal rod as an adaptor. Then the distal rod, made of FlgG, is assembled on the proximal rod. Formation of the rod requires FlgJ, which is exported to the periplasmic space via export apparatus and acts as a cap on the growing rod to facilitate the polymerization at the tip. FlgJ also has a muramidase activity at its C-terminal half, hydrolyzing the peptidoglycan adjacent to the MS ring to allow the rod to penetrate the peptidoglycan layer. Then the P ring (made of FlgI) is formed around the distal rod, followed by L-ring (FlgH) formation. FlgI and FlgH are not secreted through the export apparatus, but through the Sec pathway using a signal sequence at their N-termini. P-ring formation requires the Dsb system, which is involved in intramolecular disulfide bond

formation in the periplasm. FlgI protein contains two cysteine residues important for protein stability. P-ring formation also requires the FlgA protein that acts as a periplasmic chaperone, assisting a polymerization reaction of FlgI into the P ring through FlgI–FlgI interaction. The hook assembles next, from about 120 copies of FlgE proteins at the distal end of the growing hook, with the aid of hook-capping protein FlgD. Hook elongation proceeds to the well-controlled length of 55 _ 6 nm by a sophisticated export switching mechanism. After hook reached to the defined length, FlgD dissociates from the tip of the hook, then replaced by the three HAPs, FlgK, FlgL, and FliD in this order . Addition of FlgK and FlgL is facilitated by the chaperone FlgN, whereas that of FliD is facilitated by another chaperone FliT . Finally, the FliC filament subunits (flagellin) are inserted at the distal end. FliD acts as a cap to facilitate the filament elongation by inserting each FliC subunit between the FlgL and FliD zones, with rotary cap mechanism. Initial growth rate is about 30 nm/min, which corresponds to one flagellin incorporated per second,
suggesting that to reach the 10 mm long of the filament of wild-type cells, it takes several generations. The mechanism for directing the MS ring (or to initiate MS-ring assembly) at the pole has remained unknown, but recent studies revealed that two proteins, FlhF and FlhG, are somehow involved in the process. FlhF functions in polar location of the flagellum.

15.6. HYDRODYNAMICS OF FLAGELLAR MOVEMENT

Most flagellar motors are reversible rotary machines, able to rotate both clockwise (CW) and anticlockwise (ACW). Rotational switching completes very quickly, within only 1 millisecond. Rotational direction is controlled by environmental stimuli, such as pH, temperature, and chemicals like sugars and amino acids. Methyl-accepting chemotaxis proteins (MCPs) sense these stimuli and transmit signals to the motor through a two-component phosphorelay signaling cascade. When a repellent signal is sensed by the MCP, autophosphorylation activity of the CheA protein, associated with MCP on the cytoplasmic side, is activated and a histidine residue of CheA is phosphorylated. Then this phospho group is transferred to the Asp residue of the response regulator CheY. Phosphorylated CheY

protein (CheY-P) then associated with the motor to trigger CW rotation. On the other hand, when an attractant signal is sensed by the MCP, autophosphorylation activity of CheA is repressed, so that the level of CheY-P decreases and the motor rotates in its default direction, ACW. The target of CheY-P in the motor is the switch complex, composed of FliG, FliM, and FliN. FliG/FliM/FliN complex is also called ‘‘the switch complex’’ because mutations in these proteins cause defects in switching the CCW/CW rotation in response to tactic stimuli.

FliM functions most directly in regulation of the switching frequency by binding to CheY-P . This binding of CheY-P to FliM probably changes the FliG–FliM interaction, and causes movement of the C-terminal domain of FliG (FliGC) that interacts with the stator protein MotA, thereby altering the rotor–stator interface to switch the direction of rotary motion (Fig. 5).

Studies of intracellular level of CheY-P in a single cell that causes switching fromCCWtoCWrevealed that switching is a highly cooperative event, showing a Hill coefficient of about 10, suggesting that chemotactic signal is amplified within the switch. binding of CheY-P to FliM is much less cooperative than motor switching. The chemotactic signal is amplified within the switch, but subsequent to the CheY-P binding to FliM. Some bacteria respond to tactic stimuli using modes, other than directional switching. Rhodobacter sphaeroides has a unidirectional flagellar motor that alternates between CW rotation and brief stops, during which the bacterium is reoriented by Brownian motion and changes in flagellar filament morphology. In the case of Sinorhizobium meliloti, the motor also rotates unidirectionally in theCWdirection and swimming cells respond to tactic stimuli by modulating the flagellar rotary speed. The marine bacterium V. alginolyticus has dual flagellar systems, Naþ-driven polar flagellum (Pof) and Hþ-driven lateral flagella (Laf), and their switching modes are different: the Laf motor rotates unidirectionally inCCWand responds to tactic signals by slowing down, whereas the Pof motor turns in both directions . In each case described, tactic signals are transmitted through the two-component signalling pathway, and CheY-P association to the motor modulates rotation.

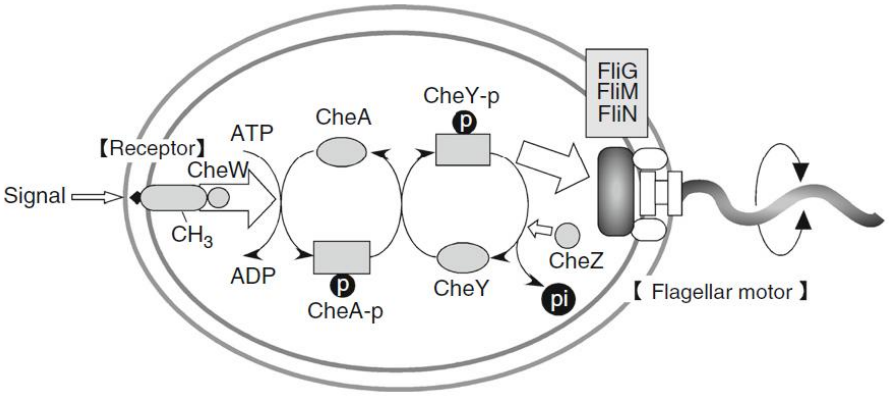

Figure 5: Schematic diagram of signal transduction of *Escherichia coli* chemotaxis.

15.7. SUMMARY

The flagellum has three morphologically and chemically distinguishable parts namely 1) the filament 2) hook and 3) the basal body.

Filament: The filament is located outside the cell surface and lies between the hook and the distal end of the flagellum. The filament is around 20 nm in diameter, 10-20 μm in length and wavy in nature. Composed of primarily of a single, self-aggregating protein called flagellin. Flagellins are low molecular weight proteins twisted together in a helical conformation or around an axial cylinder depending on the bacterial species. Flagellin molecules are synthesised in the cytoplasm. The flagellum grows in length due to the addition of flagellin molecules towards the growing end. Formation of a helical structure is achieved by a mixture of the protofilaments of two distinct conformations, the R- and L-type. Each protofilament switches between these two conformations by responding to a variety of factors including pH, ionic strength, mechanical stress, and mutations. Intersubunit hydrophobic interactions in the inner tube make the filament structure mechanically stable, and the diameter of central channel is only 2 nm. This central channel serves as a transport pathway of flagellins that will polymerize at the tip of the growing filament.

Hook: It comprises of the region between filament and basal structure. It is short, slightly curved, and has a diameter somewhat greater than the filament. The hook ranges from 70-90 nm in length and consists principally of single polypeptide. The hook is thought to function as a universal joint to smoothly transmit the torque produced by the motor to the filament. Studies suggested that the junction acts as a buffering structure connecting two filamentous structures (hook and filament) with distinct mechanical properties.

Basal body: It is the part that anchors or attaches the flagellum in the cytoplasmic membrane and the cell wall. It is a complex structure consisting of a small central rod and a series of discs or rings. The basal body functions like a motor system which helps the flagellum to rotate and propel the bacterium in the liquid environment. The proximal end of the hook is connected to the basal body structure, consisting of the rod and three coaxially mounted rings, termed as MS, P, and L ring. The MS ring is embedded in the cytoplasmic membrane and made of a single protein FliF, the P and L rings are associated with the peptidoglycan layer and the outer membrane, respectively, and are composed of FlgI and FlgH. The rod structure is composed of three proximal rod proteins FlgB, FlgC, FlgF, and a distal rod protein FlgG, and fully traverses the periplasmic space. The basal body of Gram-positive bacteria is composed of only the MS ring and rod, and the LP ring is not present. A drum-shaped structure, called C ring, is found on the MS ring facing the cytoplasm. It is composed mostly of FliM and FliN proteins. These proteins, together with FliG which is located beneath the MS ring, have been known to form a complex, referred to as the switch complex. They are also important for rotation.

Functions of the flagellum

Flagella, the organelles of locomotion impart motility by rotating in clockwise and anticlockwise manner controlled by the basal body. The movement of the basal body is driven by a proton motive force rather than by ATP directly. Bacteria swim through liquid by means of the propeller-like action of the flagella in response to environmental stimulus. The stimulus may be due to chemicals (chemotaxis), light

(phototaxis), osmotic pressure (osmotaxis), oxygen (aerotaxis), and temperature (thermotaxis). Chemotaxis, referred to as movement in response to attractant and repellent substances in the environment help bacterial pathogens to move through the mucous layer and colonize the mucous membranes and thereby facilitate bacterial pathogenesis.

Hydrodynamics of flagellar movement

Most flagellar motors are reversible rotary machines, able to rotate both clockwise (CW) and anticlockwise (ACW). Rotational switching completes very quickly, within only 1 millisecond. Rotational direction is controlled by environmental stimuli, such as pH, temperature, and chemicals like sugars and amino acids. Methyl-accepting chemotaxis proteins (MCPs) sense these stimuli and transmit signals to the motor through a two-component phosphorelay signaling cascade. When a repellent signal is sensed by the MCP, autophosphorylation activity of the CheA protein, associated with MCP on the cytoplasmic side, is activated and a histidine residue of CheA is phosphorylated. Then this phospho group is transferred to the Asp residue of the response regulator CheY. Phosphorylated CheY protein (CheY-P) then associated with the motor to trigger CW rotation. On the other hand, when an attractant signal is sensed by the MCP, autophosphorylation activity of CheA is repressed, so that the level of CheY-P decreases and the motor rotates in its default direction, ACW.

15.8. CHECK YOUR PROGRESS

1. What are the different parts of the bacterial flagellum?
2. Explain the basic features of the bacterial flagellar filament, hook and the basal body.
3. Discuss the fine structure of the bacterial flagella.
4. Write a note on the flagellar assembly
5. Discuss in detail the functions of the bacterial flagellum
6. Explain the differences in the flagellar structure in Gram positive and Gram negative bacteria.

15.9. KEY WORDS

Ultrastructure of bacterial flagellum, filament, hook, basal body, locomotion, running and tumbling movements, flagellar assembly, hydrodynamics of flagellar movement, functions of the flagellum.

15.10. FURTHER SUGGESTED READING

1. Samatey, F. A., Matsunami, H., Imada, K., Nagashima, S., Shaikh, T. R., Thomas, D. R., Chen, J. Z., Derosier, D. J., Kitao, A., and Namba, K. 2004. Structure of the bacterial flagellar hook and implication for the molecular universal joint mechanism. Nature 431, 1062–1068.
2. Silverman, M., and Simon, M. I. 1974. Flagellar rotation and the mechanism of bacterial motility. Nature 249, 73–74.
3. Sowa, Y., Rowe, A. D., Leake, M. C., Yakushi, T., Homma, M., Ishijima, A., and Berry, R. M. 2005. Direct observation of steps in rotation of the bacterial flagellar motor. Nature 437, 916–919.
4. Xing, J., Bai, F., Berry, R., and Oster, G. 2006. Torque-speed relationship of the bacterial flagellar motor. Proc. Natl. Acad. Sci. USA 103, 1260–1265
5. Yamashita, I., Hasegawa, K., Suzuki, H., Vonderviszt, F., Mimori-Kiyosue, Y., and Namba, K. 1998. Structure and switching of bacterial flagellar filaments studied by X-ray fiber diffraction. Nat. Struct. Biol. 5, 125–132.
6. Yonekura, K., Maki-Yonekura, S., and Namba, K. 2003. Complete atomic model of the bacterial flagellar filament by electron cryomicroscopy. Nature 424, 643–650

15.11. SOURCES

1. Francesco Sala. 1972. Structure and Function of Bacterial Flagella, Bolletino di zoologia, 39: 111-118.
2. Hiroyuki Terashima, Seiji Kojima, and Michio Homma. 2008. Flagellar Motility in Bacteria: Structure and Function of Flagellar Motor. International Review of Cell and Molecular Biology, Volume 270: 39-85.
3. Iino, T. 1969. Genetics and chemistry of bacterial flagella. Bacteriol. Rev. 33, 454–475.
4. James Lighthill. 1976. Flagellar hydrodynamics. SIAM Review 18: 161-230.
5. Kojima, S., and Blair, D. F. 2004. The bacterial flagellar motor: Structure and function of a complex molecular machine. Int. Rev. Cytol. 233, 93–134.
6. Leifson, E. 1960. Atlas of Bacterial Flagellation. Academic Press, New York and London.
7. Saverio E. Spagnolie and Eric Lauga. 2011. Comparative Hydrodynamics of Bacterial Polymorphism. Physical Review Letters 106: 1-4.

UNIT 16

BACTERIAL SPORES AND CYSTS

STRUCTURE

16.1. OBJECTIVES

After reading this unit we will be able to learn about:

- The resting structures of bacteria
- Endospores structure and function
- The process of endospore development
- Specialized kinds of spores and their functions
- Different kinds of bacterial cysts and their functions

16.2. INTRODUCTION

Bacterial endospores and cysts are resting structures. At this state the bacterial cells slow down metabolism and stop its regular activities. Bacteria adapt various means to overcome the unfavourable environmental conditions. Endospore formation is one of the extreme survival strategy employed by bacteria, particularly the low G+C Gram-positive bacteria. Non-availability of essential nutrients is one of the important causes for the formation of endospores. Endospores allow bacteria to produce a dormant and highly resistant cell to preserve the cells genetic material in times of extreme stress. Endospores can withstand environmental changes like high temperature, high UV irradiation, desiccation, chemical damage and enzymatic destruction. Endospores due to their tolerant nature resistant antimicrobial treatments. Some bacteria make less durable resting structures called cysts. These cysts are resistant to desiccation and chemicals but do not resist high temperatures.

16.3. ENDOSPORES

Endospores are found everywhere, are easily dispersed throughout the environment and can be difficult to remove. Endospores typically have a dormant bacterium surrounded by several layers of hard protective coatings. Within these membranes and the hard coating, the dormant bacterium is able to survive for weeks, even years, through drought, heat and even radiation. These spores germinate

when there is availability of water and food. Some bacterial spores have possibly been revived after they lay underground for more than 250 million years. Endospores found in great tombs of the Egyptian Pharaohs were able to germinate and grow when placed in appropriate medium. Viable endospores were recovered from bees trapped in amber that is 25-40 million years old. All bacteria do not form spores. Soil bacteria like *Bacillus subtilis* and *Clostridium clastriduium* can form endospores. Endospores of the gram positive bacteria *Epulopiscium* are the largest endospores containing large amounts of dipicolinic acid. These endospores are 4000 times larger than a *Bacillus subtilis* endospore.

16.4. ENDOSPORE STRUCTURE

Endospore has unique cellular structure. Endospores contain four layers namely core, cortex, coat and exosporium (Fig. 1). There is an outer proteinaceous coat that surrounds the spore. This outer coat provides chemical and enzymatic resistance. Inner to this outer coat is the cortex which is made up of a thick layer of specialized peptidoglycan. During high temperatures the cortex plays an important role in dehydration. Beneath the cortex is the germ cell wall, which is a peptidoglycan layer that forms the bacterial cell wall during endospore germination. Under the germ cell wall, lies the inner membrane which is a major permeability barrier against several potentially damaging chemicals. The central region of the endospore is the core, which is highly dehydrated and contains DNA, ribosomes and dipicolinic acid (Calcium dipicolinate). Dipicolinic acid is an endospore specific chemical comprising 10% of the spore's dry weight and appears to play a role in maintaining spore dormancy. In additions, some small acid-soluble proteins (SASPs) are also exclusively found in endospores. These SASPs tightly bind and condense the DNA, and resist damages due to UV light and DNA-damaging chemicals. Sometimes, there are also other species-specific structures and chemicals associated with endospores like stalks, toxin crystals, or an additional outer glycoprotein layer called the exosporium. The model organism used to study endospore formation is *Bacillus subtilis*.

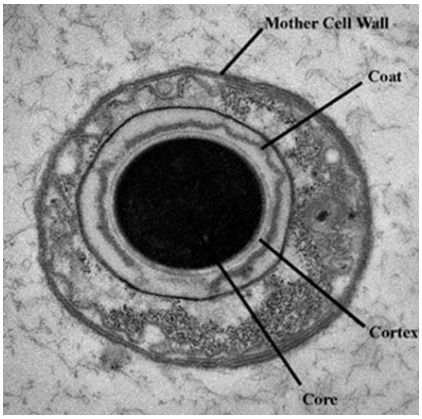

Figure 1: Typical structure of a bacterial endospore

16.5. ENDOSPORE DEVELOPMENT

Endospore formation is a complex process and takes several hours to complete. When a bacterium senses unfavourable conditions like food or water scarcity, rising temperature etc., it soon makes a copy of its chromosome, the string of DNA that carries all its genes. Initially, the cell divides asymmetrically resulting in formation of two compartments namely the larger mother cell and smaller daughter cell/forespore. Next, the septum of peptidoglycan layer between these two cells is degraded and the mother cell engulfs the forespore forming a cell inside a cell. The activities of the mother cell and forespore lead to the synthesis of the endospore-specific compounds, formation of the cortex and deposition of the coat. Later, final dehydration and maturation of the endospore takes place. Finally, the mother cell is destroyed in a programmed cell death, and the endospore is released into the environment. The endospore will remain dormant until favourable conditions are back.
Sigma Factor is a small protein that directs RNA polymerase to specific sites on the DNA to initiate gene expression (Fig. 2).

These endospores are involved in symbiosis between the *Epulopiscium*-like bacteria and their surgeonfish. The host surgeon fish provides food the bacteria with food in the gut at night. The fish also benefits by these bacteria which help in digestion and also gains nutrients from microbial products released during spore germination.

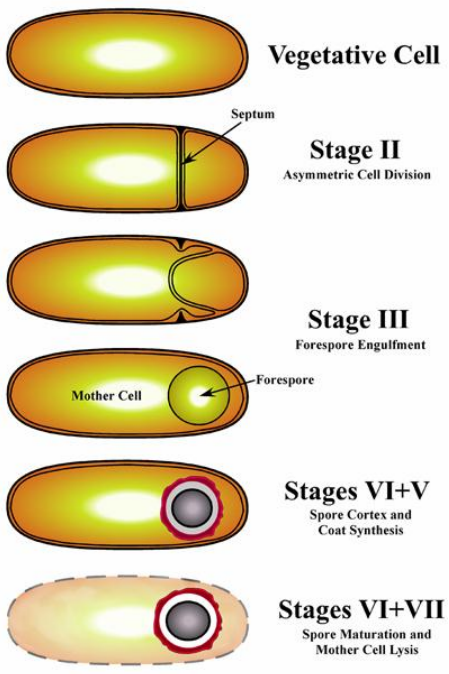

Figure 2: Stages of endospore development

16.6. EXOSPORES

Methylosinus bacteria produces spores called exospores. Unlike endospores which are formed inside the cell, exospores are formed outside by growing or budding out from one end of the cell. Exospores also have different structural composition.

16.7. FILAMENTS

Actinomycetes produce spores which are long tubules called filaments. Under nutrient poor conditions these filaments differentiate into round resting structures termed spores. Such spores are formed by formation of cross walls that divide the filament into sections, each containing a chromosome. These then differentiate into mature spores. Cytoplasm converts to a dormant state becoming resistant to heat and chemicals.

16.8. BACTERIAL CYSTS

Cysts are resting or dormant stage of bacteria which helps to overcome the unfavourable environmental conditions. Cyst formation is usually trigged by unfavourable environmental conditions such as lack of nutrients or oxygen, extreme temperatures, lack of moisture and presence of toxic chemicals. During this period the metabolic processes of the cell are slowed down and the cell stops its activities. When favourable conditions return the cyst wall breaks down by a process known as *excystation***.** Encystment also helps the microbe to disperse easily, from one host to another or to a more favourable environment.

During cyst formation the cytoplasm contracts and the cell wall thickens. The composition of the cyst wall is variable in different organisms. The cyst walls of bacteria are formed by the thickening of the normal cell wall with added peptidoglycan layers.

Thick walled protective structures called cysts are formed by members of the *Azotobacter, Bdellovibrio, Myxococcus* and *Cyanobacteria* (Fig. 3). They are less durable than endospores.

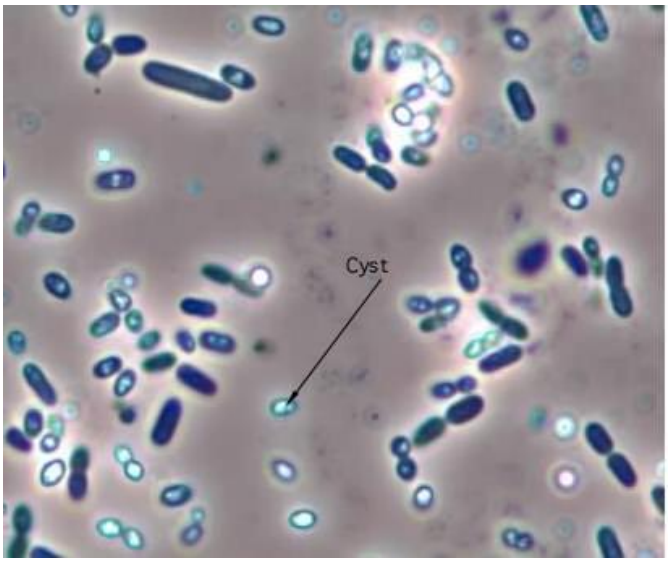

Figure 3: Micrograph of the *Azotobacter vinelandii* cysts

Photosynthetic filamentous cyanobacteria produce specialized structures called heterocysts (Fig. 4). These are rounded structures distributed at regular

intervals along the string of vegetative cells or at one end. Heterocysts fix N_2, while the rest of the cells perform photosynthesis. Heterocysts are common in several different groups of cyanobacteria and are the sites of nitrogen fixation. These heterocysts are slightly enlarged in size and distinctly shaped when compared to the vegetative cells.

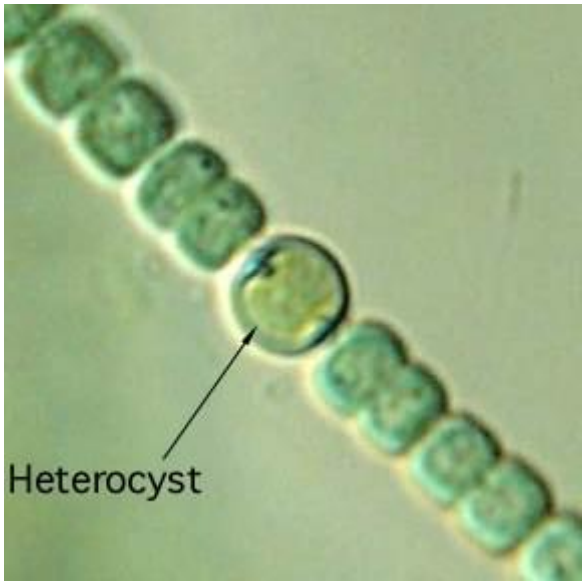

Figure 4: The heterocyst of the filamentous cyanobacteria *Anabaena*

16.9. SUMMARY

Bacterial endospores and cysts are resting structures. At this state the bacterial cells slow down metabolism and stop its regular activities. Endospore formation is one of the extreme survival strategy employed by bacteria.

Non-availability of essential nutrients is one of the important causes for the formation of endospores. Endospores allow bacteria to produce a dormant and highly resistant cell to preserve the cells genetic material in times of extreme stress.

Endospores can withstand environmental changes like high temperature, high UV irradiation, desiccation, chemical damage and enzymatic destruction.

Some bacteria make less durable resting structures called cysts. These cysts are resistant to desiccation and chemicals but do not resist high temperatures.

Endospore has unique cellular structure. Endospores contain four layers namely core, cortex, coat and exosporium. There is an outer proteinaceous coat that surrounds the spore. This outer coat provides chemical and enzymatic resistance. Inner to this outer coat is the cortex which is made up of a thick layer of specialized peptidoglycan. During high temperatures the cortex plays an important role in dehydration. Beneath the cortex is the germ cell wall, which is a peptidoglycan layer that forms the bacterial cell wall during endospore germination. Under the germ cell wall, lies the inner membrane which is a major permeability barrier against several potentially damaging chemicals. The central region of the endospore is the core, which is highly dehydrated and contains DNA, ribosomes and dipicolinic acid (Calcium dipicolinate).

Endospore formation is a complex process and takes several hours to complete. When a bacterium senses unfavourable conditions like food or water scarcity, rising temperature etc., it soon makes a copy of its chromosome, the string of DNA that carries all its genes. Initially, the cell divides asymmetrically resulting in formation of two compartments namely the larger mother cell and smaller daughter cell/forespore. Next, the septum of peptidoglycan layer between these two cells is degraded and the mother cell engulfs the forespore forming a cell inside a cell. The activities of the mother cell and forespore lead to the synthesis of the endospore-specific compounds, formation of the cortex and deposition of the coat. Later, final dehydration and maturation of the endospore takes place. Finally, the mother cell is destroyed in a programmed cell death, and the endospore is released into the environment.

Methylosinus bacteria produces spores called exospores. Unlike endospores which are formed inside the cell, exospores are formed outside by growing or budding out from one end of the cell. Exospores also have different structural composition.

Actinomycetes produce spores which are long tubules called filaments. Under nutrient poor conditions these filaments differentiate into round resting structures termed spores. Such spores are formed by formation of cross walls that divide the filament into sections, each containing a chromosome.

Cysts are resting or dormant stage of bacteria which helps to overcome the unfavourable environmental conditions. Cyst formation is usually trigged by unfavourable environmental conditions such as lack of nutrients or oxygen, extreme temperatures, lack of moisture and presence of toxic chemicals. During this period the metabolic processes of the cell are slowed down and the cell stops its activities. When favourable conditions return the cyst wall breaks down by a process known as *excystation***.** Encystment also helps the microbe to disperse easily, from one host to another or to a more favourable environment.

Photosynthetic filamentous cyanobacteria produce specialized structures called heterocysts. Heterocysts are common in several different groups of cyanobacteria and are the sites of nitrogen fixation. These heterocysts are slightly enlarged in size and distinctly shaped when compared to the vegetative cells.

16.10. CHECK YOUR PROGRESS

1. What are the different types of resting structures in bacteria?
2. Explain the detailed structure of a typical bacterial endospore?
3. What are the functions of a bacterial endospore?
4. Discuss the process of endospore development.
5. What are exospores and filaments?
6. What are bacterial cysts? Explain their structure and function.
7. What are heterocysts? What is their function?

16.11. KEY WORDS

Bacterial endospores and cysts, resting structures, **endospore structure, endospore development,** exospores, filaments, bacterial cysts, heterocysts.

16.12. FURTHER SUGGESTED READING

1. Arthur L. Koch. 2007. The Bacteria: Their Origin, Structure, Function and Antibiosis. Springer.

2. Darralyn McCall, David Stock. 2001. 11th Hour: Introduction to Microbiology 1st Edition. Blackwell Science.

3. Hans G Schiegel. 2008. General Microbiology. 7th Edition. Cambridge University Press.

4. Jennifer M. Warner, I. Edward Alcamo. 2009. Schaum's Outline of Microbiology 0002 Edition. McGraw-Hill Publications.

5. Rickford Grant, Horikoshi. 1998. Extremophiles: Microbial Life in Extreme Environments (Hardcover). John Wiley and Sons.

16.13. SOURCES

1. Alcamo. 2001. Fundamentals of Microbiology Sixth Edition. By, Edward Alcamo. Jones and Bartlett Publishers, London.

2. Betsey Dexter Dyer. 2003. A field guide to bacteria. Comstock Publishing.

3. Purohit, S.S. 2008. Microbiology – Fundamentals and Application. Sixth Edition. Student Edition Publishers, Jodhpur.

4. Ravi Mantha. 2012. All about bacteria. Collins Publications.

5. Stanier, R.Y., Ingraham, J.L., Wheelis, M.L., and Painter, P.R. 2007. General Microbiology Fifth Edition. McMillan Publishers, London.