SRINIVASAN COLLEGE OF ARTS & SCIENCE

(Affiliated to Bharathidasan University, Trichy)

PERAMBALUR – 621 212.

DEPARTMENT OF MICROBIOLOGY

Course : M.Sc Year: **I** Semester: **II**

Course Material on:

MICROBIAL PHYSIOLOGY

Sub. Code : P16MB21

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MICROBIAL PHYSIOLOGY

Unit I Cell structure and function

Bacterial cell wall - Biosynthesis of peptidoglycan - outer membrane, teichoic acid – Exopolysaccharides; cytoplasmic membrane, pili, fimbriae, S-layer. Transport mechanisms – active, passive, facilitated diffusions – uni, sym, antiports. Electron carriers – artificial electron donors – inhibitors – uncouplers – energy bond – phosphorylation.

Unit II Microbial growth

Bacterial growth - Phases of growth curve – measurement of growth – calculations of growth rate – generation time – synchronous growth – induction of synchronous growth, synchrony index – factors affecting growth – pH, temperature, substrate and osmotic condition. Survival at extreme environments – starvation – adaptative mechanisms in thermophilic, alkalophilic, osmophilic and psychrophilic.

Unit III Microbial pigments and photosynthesis

Autotrophs - cyanobacteria - photosynthetic bacteria and green algae – heterotrophs – bacteria, fungi, myxotrophs. Brief account of photosynthetic and accessory pigments – chlorophyll – fluorescence, phosphorescence - bacteriochlorophyll – rhodopsin – carotenoids – phycobiliproteins.

Unit IV Carbon assimilation

Carbohydrates – anabolism – autotrophy – oxygenic – anoxygenic photosynthesis – autotrophic generation of ATP; fixation of CO_2 – Calvin cycle (C3) – C4 pathways. Respiratory metabolism – Embden Mayer Hoff pathway – Entner Doudroff pathway – glyoxalate pathway – Krebs cycle – oxidative and substrate level phosphorylation – reverse TCA cycle – gluconeogenesis – Fermentation of carbohydrates – homo and heterolactic fermentations.

Unit V Spore structure and function

Cell division – endospore – structure – properties – germination. Microbial sporulation and morphogenesis – Bacteria including cyanobacteria and actinobacteria, fungi and algae.

Unit 1

bacterial ultra structure

1. Capsule:

- Capsule is 0.2µm thick viscus layer outer layer to the cell wall.
- Capsule is 98% water and 2% polysaccharide or glycoprotein/ polypeptide or both.
- There are two types of capsule.

i. **Macro-capsule:** thickness of 0.2µm or more, visible under light microscope

ii. **Microcapsule:** thickness less than 0.2µm, visible under Electron microscope

- Capsule is very delicate structure. It can be removed by vigorous washing.Capsule is most important virulence factor of bacteria.

Function:

- It helps in attachments as well as it prevent the cell from desiccation and drying.
- Capsule resist phagocytosis by WBCs

2. Flagella:

- It is 15-20 nm hair like helical structure emerges from cell wall.
- Flagella is not straight but is helical. It is composed of flagellin protein (globular protein) and known as H antigen.
- Flagella has three parts. Basal body, Hook and filament

Function:

- It helps motility of the bacteria

3. Pili or fimbriae:

- Pili are hollow filamentous and non-helical structure.
- They are numerous and shorter than flagella

- Pili is the characteristic feature of gram –ve bacteria.
- Pili is composed of pilin protein.
- Bacteria containing pili: *Shigella, Proteus, Neisseria gonorrhoae, E. coli*

Function:

- Attachment: pili helps the bacteria to attach the host cell surface. Most of the human pathogens of respiratory tract, urinary tract are attached with the help of pili.
- Pili (fimbrae) possess antigenic property
- Specialized function: some pili are modified for specialized function. Eg. Sex pilus (F-pili) help in transfer of DNA from donor to recipient cell during conjugation.
- F-pili also act as receptor for bacteriophage.

4. Sheath:

- Some bacteria forming chain or trichome are enclosed by a hollow tube like structure known as Sheath.
- Aquatic bacteria mostly form sheath
- Some sheathed bacteria are; Sphaerotilus, leptothrix, clonothrix etc

Function:

- Mechanical support
- Sometime sheath is impregnated with ferric or manganese hydroxide which provide strength to sheath.

5. Prosthecae:

- Prosthecae are semi-rigid extension of cell wall and cell membrane
- One bacteria may contains one or many prosthecae.
- Some prosthecae develop bud at the tip and hence helps in reproduction.
- Some prosthecate bacteria are: *Caulibacter, Stella, Prosthecobacter, Hyphomicrobium*

Function:

- Prosthecae increase surface area for nutrition absorption. It is usually formed in bacteria living in very dilute environment where concentration of nutrition is low.
- Helps in adhesion
- Asexual reproduction by budding

6. Stalk:

- It is non-living ribbon like tubular structure.
- It is formed by excretory product of bacteria.
- Some stalked bacteria are: Gallionella, Planctomyces

Function:

- Helps in attachment to solid surface.

7. Cell wall:

- It is an important structure of a bacteria.
- It give shape to the organism.
- On the basis of cell wall composition, bacteria are classified into two major group ie. Gram Positive and gram negative.

Gram positive cell wall

Cell wall composition of gram positive bacteria.

1. Peptidoglycan
2. Lipid
- Teichoic acid

Gram negative cell wall

Cell wall composition of gram negative bacteria

1. Peptidoglycan
2. Outermembrane:
    - Lipid

- Protein
- Lipopopysaccharide (LPS)

Peptidoglycan:

- It consists of glycan backbone formed by repeated unit of NAG (n-acetyl Glucosamine) and NAM (N-acetyl muramic acid) and the glycan backbone is cross linked by peptide bond.
- Peptidoglycan layer is present in cell wall of both gram positive as well as gram negative bacteria. However, gram positive have thick layer of peptidoglycan.

Teichoic acid:

- Teichoic acid is water soluble polymer of glycerol or ribitol phosphate present in gram positive bacteria.
- It constitutes about 50% of dry weight of cell wall.
- It is the major surface antigen of gram positive bacteria

Outer membrane:

- It is an additional layer present in gram negative bacteria.
- It is composed of lipid bilayer, protein and lipo-polysaccharide(LPS)

LPS:

- LPS is composed of lipid-A and polysaccharide.
- Lipid-A: it is phosphorylated glucosamine disaccharide. It is antigenic
- Polysaccharide: it consists of core-polysaccharide and O-polysaccharide.

8. Cell membrane:

- Cell membrane is the inner layer that lies inside the cell wall and encloses the cytoplasm.
- It is also known as cytoplasmic membrane or plasma membrane.
- It is about 80nm thick.
- Cell membrane of bacteria is composed of phospholipid and proteins.

Function:

- It is selectively permeable as it allows to pass selective substances such as sugar, aminoacids across it.

9. Nucleus:

- Nucleus is the most important part of the cell.
- It controls and directs all the cellular activities and stores hereditary information of cell
- Bacterial nucleus is known as nucleoid; it lacks nuclear membrane, nuceloplasm and nucleolus.
- Bacterial DNA is naked (lacked histone protein)

Function:

- It contains and stores hereditary information of the cell.
- It controls all cell activities.

10. Ribosome:

- Bacterial ribosome is of 70s type.
- Ribosomes are rounded granules found freely floating in the cytoplasm
- Ribosomes are known as universal cell organelle because it is found in both bacterial cell and eukaryotic cell.
- Chemically the ribosomes are made up of nucleic acids (particularly RNA and proteins).

Function:

- It helps in protein synthesis

11. Mesosome:

- Mesosome is a spherical or round sac like structure found commonly in gram positive bacteria.
- Function: It is the site for respiration in bacterial cell

12. Cytoplasm:

- It is colorless, viscus fluid present inside cell membrane.
- All the cell organelles and inclusions are found floating in cytoplasmic fluid.
- It contains proteins, lipid, minerals, nucleic acids, glycogen, water etc.

Function:

- It helps to distribute water, oxygen as other substances throughout the cell.
- Literally, all the cellular content including nucleus, and other cell organelle are floating in cytoplasm.

13. Spores (endospore):

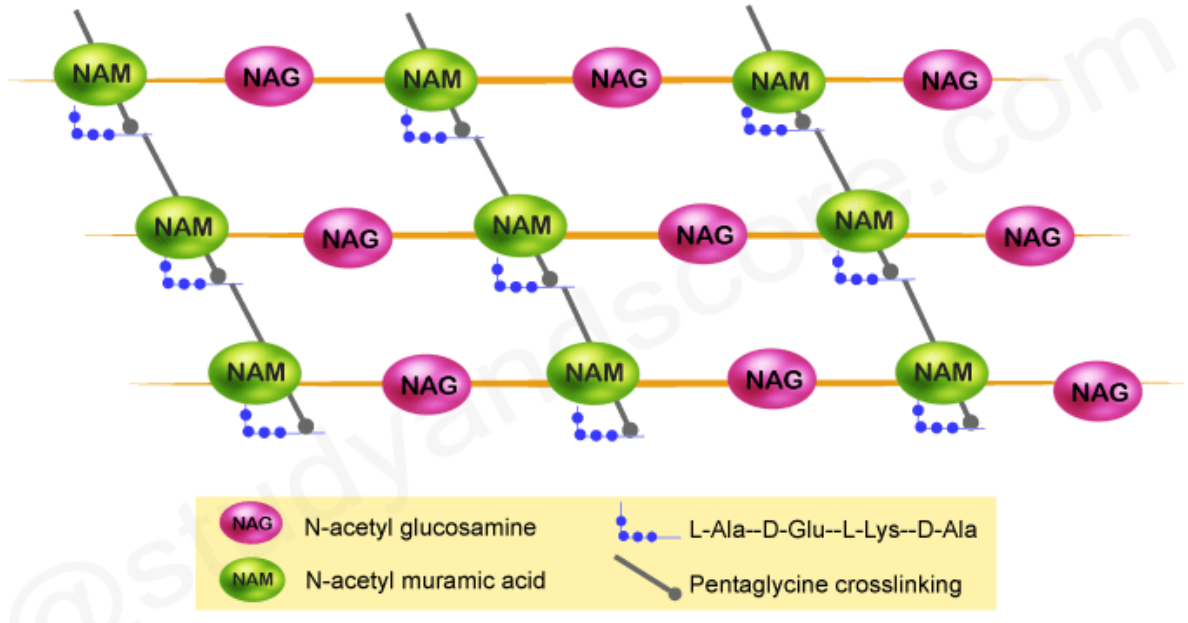
- Spore is metabolically dormant structure produced during unfavourable condition by the process called sporulation
- Sporulation occur during late log phase or early stationary phase
- Under favourable condition spores germinate to give vegetative cell.

STRUCTURE OF BACTERIAL CELL WALL

A Bacterial cell under an electron microscope reveals many components, some of which are external to the cell wall and some are internal to the cell wall. Some of the structures are confined only to certain species and some other structures act as the characteristic features of certain species. Structures external to the cell wall include flagella, capsules and sheaths. Structures internal to the cell include cytoplasmic membrane and all other cytoplasmic inclusions.
Cell wall is a very rigid structure which provides support and gives shape to the cell. It is about 100-140 nm in thickness. Most of the bacteria live in hypotonic environments and tend to take up water due to the variation in the osmotic pressures between the bacterial cells and the existing environment. Hence, the main function of the cell wall is to prevent the cell from expanding and eventually bursting in the hypotonic environments. Bacterial cell has the ability to retain their original shape when subjected to very high pressures, due to the rigidity of the cell wall.

Chemical composition of the bacterial cell wall

Cell wall is composed of two polymers, one consisting of saccharide subunits and the other consisting of amino acid subunits. Thus a bacterial cell wall is glycopeptide which is also known as peptidoglycan. The saccharide component of the cell wall has alternating repeating units of two amino sugars related to glucose. One of these two is NAM (N-Acetyl Muramic acid) and the other is NAG (N-Acetyl glucosamine). Both NAG and NAM form the back bone of the cell wall structure.



CHEMICAL COMPOSITION OF BACTERIAL CELL WALL

The alternating units of NAG and NAM are linked together by a glycosidic bond (β-1, 4 linkages). This linkage gives stability and strength to the cell wall. The NAG and NAM chains are cross-linked to one another by tetra peptides that extend off the NAM unit forming a lattice-like structure.

The four amino acids that compose the tetra peptide are: L-alanine, D-glutamine, L-lysine and D-alanine in Gram positive bacteria and actinomycetes. Whereas in Gram negative bacteria and myxobacteria, L-lysine is replaced by diaminopimelic acid (DAP). The inclusion of both L and D amino acids in the structure provides protection from digesting effect of proteases.

FUNCTIONS OF BACTERIAL CELL WALL

1. The main function of the bacterial cell wall is to provide overall strength to the cell
2. It helps maintain the cell shape, thereby helping the cell to grow, reproduce, obtain nutrients and also move about.
3. Cell wall protects the cell from the osmotic lysis. The cell keeps moving from one environment to other and moreover as water can freely move from both the cell membrane and the cell wall, the cell is at risk of osmotic imbalance thereby causing osmotic lysis of the cell.
4. The cell wall helps in keeping out certain molecules which may be toxic.
5. Bacterial cell wall contributes to the pathogenicity in other words, disease causing ability of bacterial cells.

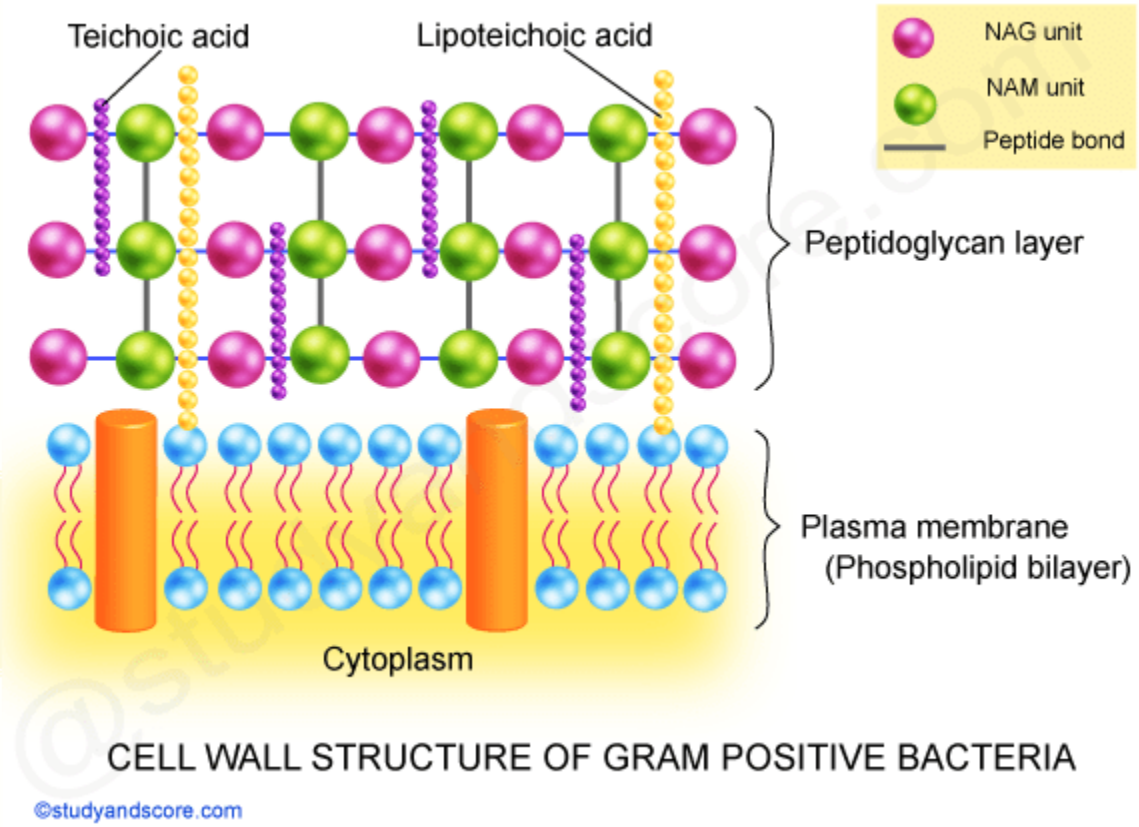
GRAM POSITIVE AND NEGATIVE CELL WALL

In 1884, a scientist named Gram tried to stain the bacterial cells with crystal violet and iodine solution. On washing them with acetone, few bacteria became purple where as others remained colorless. When these bacteria were counter stained with safranin it was observed that some of the bacteria retained purple color and others take up pink color. The bacteria which take purple color are called gram positive bacteria and those which take up pink color are gram negative bacteria.

The bacterial cell wall is not only a protective layer but serves an important purpose of distinguishing bacteria into two major groups namely gram-negative and gram-positive. The walls of gram-positive bacteria have simpler chemical structures compared to gram-negative bacteria.

Gram-positive cell wall

Gram-positive cell wall is thick measuring about 15-80 nm and more homogenous compared to gram-negative cell wall. This cell wall consists of large amount of peptidoglycan arranged in several layers. Peptidoglycan in gram-positive cell wall constitutes about 40-80% of the dry weight.



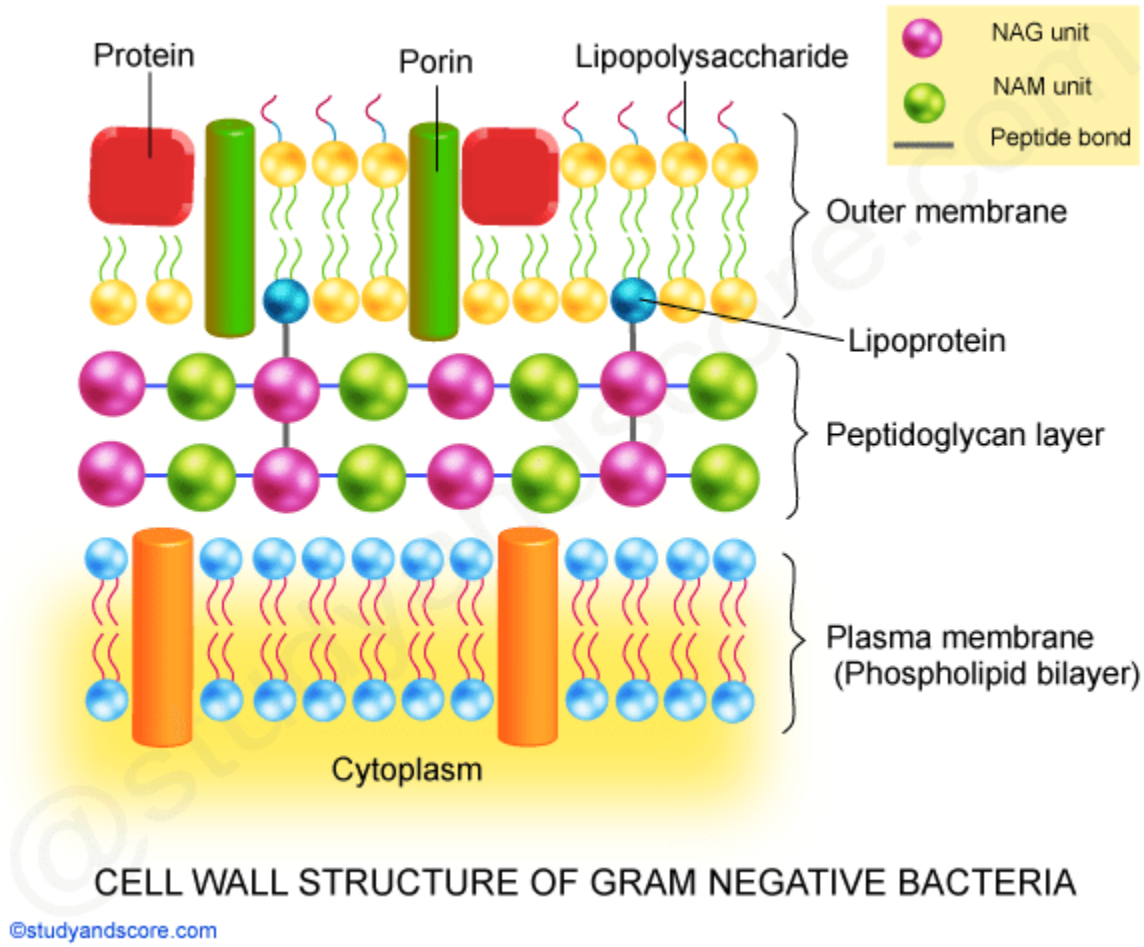
Gram positive cell wall consists of teichoic acid and teichuronic acid. Along with these acids, gram positive cell wall also consists of neutral sugars and acidic sugars which occur as subunits of polysaccharides.

Teichoic acids: Teichoic acids are polymers of polyribitol phosphate and polyglycerol phosphate containing ribitol and glycerol. These polymers may have sugar or amino acid substitutes either as side chains or within the chains. Teichoic acids can be of two types namely, wall teichoic acid and lipoteichoic acid. Teichoic acids are connected to the peptidoglycan chain by covalent bond.

Teichuronic acids: Teichuronic acid comprises of the repeated units of sugar acids. These are synthesized as substitutes of teichoic acids, when the phosphate supply in the cell is not sufficient.

Gram Negative cell wall

Gram negative cell wall more complex compared to gram positive cell wall. The amount of peptidoglycan is also considerable less in gram negative cell wall. Not more than two layers of peptidoglycan are present just outside the gram negative cell wall.



CELL WALL STRUCTURE OF GRAM NEGATIVE BACTERIA

Outside the peptidoglycan layer three main components namely lipoprotein layer, outer membrane and lipopolysaccharides are present.

Lipoprotein layer: this layer is mainly composed of special lipoproteins called as Braun's lipoproteins. These lipoproteins are small and are covalently joined to the underlying peptidoglycan layer. They are embedded in the outer membrane by its hydrophobic end.

Outer membrane: This membrane is a bilayered structure. The inner part resembles the cell membrane in composition while the outer part is distinct. The proteins in the outer membrane include porins and outer membrane proteins.

Lipopolysaccharides: Lipopolysaccharides are complex molecules. They are adhesive in nature and help the gram negative bacteria to adhere. This layer consists of three main components namely, lipid A, Core oligosaccharide and O polysaccharide.

GRAM POSITIVE CELL WALL Vs GRAM NEGATIVE CELL WALL

Character	Gram +ve cell wall	Gram-ve cell wall
Thickness of cell wall	15-80nm	2nm
Lipid content	2-5%	15-20%
Teichoic acid	Present	Absent
Amino acid variety	Few	Several
Aromatic amino acids	Absent	Present
Sulfur containing amino acids	Absent	Present
Treatment with lysozyme	Protoplast	

Function of Peptidoglycan

Peptidoglycan prevents osmotic lysis. As seen earlier under the cytoplasmic membrane, bacteria concentrate dissolved nutrients (solute) through active transport. As a result, the bacterium's cytoplasm is usually hypertonic to its surrounding environment and the net flow of free water is into the bacterium. Without a strong cell wall, the bacterium would burst from the osmotic pressure of the water flowing into the cell.

Structure and Composition of Peptidoglycan

With the exceptions above, members of the domain *Bacteria* have a cell wall containing a semirigid, tight knit molecular complex called peptidoglycan. Peptidoglycan, also called murein, is a vast polymer consisting of interlocking chains of identical peptidoglycan monomers (Figure 1). A peptidoglycan monomer consists of two joined amino sugars, N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), with a pentapeptide coming off of the NAM (Figure 2). The types and the order of amino acids in the pentapeptide, while almost identical in gram-positive and gram-negative bacteria, show some slight variation among the domain *Bacteria*.

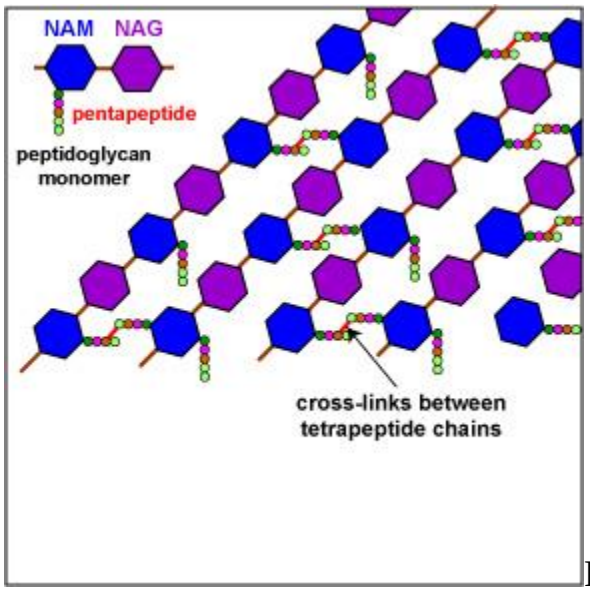


Figure 1: Peptidoglycan is composed of cross-linked chains of peptidoglycan monomers (NAG-NAM-pentapeptide). Transglycosylase enzymes join these monomers join together to form chains. Transpeptidase enzymes then cross-link the chains to provide strength to the cell wall and enable the bacterium to resist osmotic lysis. (left) In a peptidoglycan monomer of S. aureus, the pentapeptide coming off the NAM is composed of the amino acids L-alanine, D-glutamine, L-lysine, and two D-alanines. The peptide cross-link forms by formation of a short peptide interbridge consisting of 5 glycines. In the process the terminal D-alanine is cleaved from the pentapeptide to form a tetrapeptide in the peptidogycan. (right) In a peptidoglycan monomer of E. coli, the pentapeptide coming off the NAM is composed of the amino acids L-alanine, D-glutamic acid, meso-diaminopimelic acid, and two D-alanines. The peptide cross-link forms between the diaminopimelic acid of one peptide chain with the D-alanine of another and in the process the terminal D-alanine is cleaved from the pentapeptide to form a tetrapeptide in the peptidogycan.

The peptidoglycan monomers are synthesized in the cytosol of the bacterium where they attach to a membrane carrier molecule called bactoprenol. As discussed below, The bactoprenols transport the peptidoglycan monomers across the cytoplasmic membrane and work with the enzymes discussed below to insert the monomers into existing peptidoglycan enabling bacterial growth following binary fission.

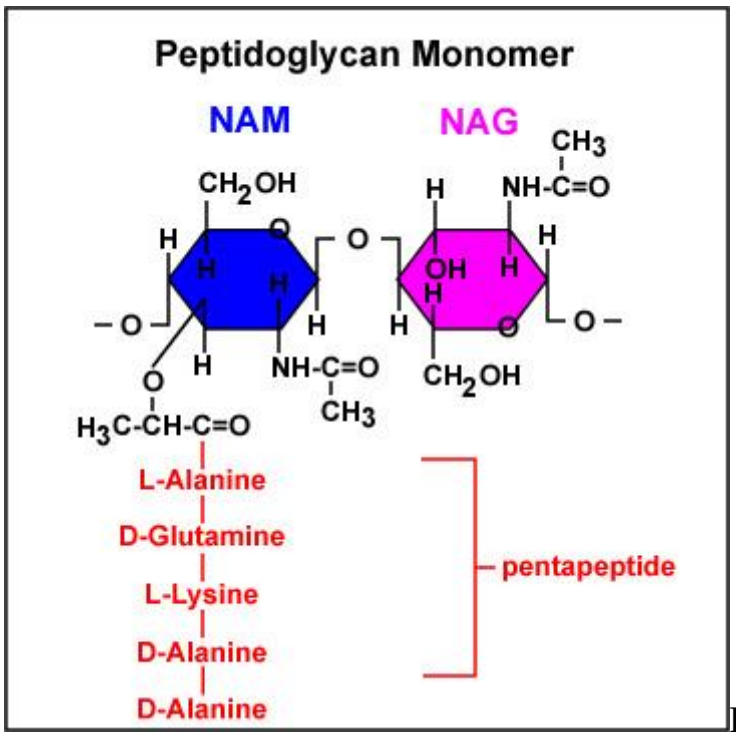


Figure 2: (left) A peptidoglycan monomer consists of two joined amino sugars, N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), with a pentapeptide coming off of the NAM. In E. coli, the pentapeptide consists of the amino acids L-alanine, D-glutamic acid, meso diaminopimelic acid, and two D-alanines. (right) A peptidoglycan monomer consists of two joined amino sugars, N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), with a pentapeptide coming off of the NAM. In S. aureus, the pentapeptide consists of the amino acids L-alanine, D-glutamine, L-lysine, and two D-alanines.

Once the new peptidoglycan monomers are inserted, glycosidic bonds then link these monomers into the growing chains of peptidoglycan. These long sugar chains are then joined to one another by means of peptide cross-links between the peptides coming off of the NAMs. By linking the rows and layers of sugars together in this manner, the peptide cross-links provide tremendous strength to the cell wall, enabling it to function similar to a molecular chain link fence around the bacterium (see Figure 1).

Synthesis of Peptidoglycan

In order for bacteria to increase their size following binary fission, links in the peptidoglycan must be broken, new peptidoglycan monomers must be inserted, and the peptide cross links must be resealed. The following sequence of events occur:

Step 1. Bacterial enzymes called autolysins:

a) Break the glycosidic bonds between the peptidoglycan monomers at the point of growth along the existing peptidoglycan .

b) Break the peptide cross-bridges that link the rows of sugars together .

Step 2. The peptidoglycan monomers are synthesized in the cytosol (and bind to bactoprenol. The bactoprenols transport the peptidoglycan monomers across the cytoplasmic membrane and interacts with transglycosidases to insert the monomers into existing peptidoglycan.

Step 3. Transglycosylase (transglycosidase) enzymes insert and link new peptidoglycan monomers into the breaks in the peptidoglycan .

Step 4. Finally, transpeptidase enzymes reform the peptide cross-links between the rows and layers of peptidoglycan to make the wall strong .

In *Escherichia coli*, the terminal D-alanine is cleaved from the pentapeptides to form a tetrapeptides. This provides the energy to bond the D-alanine of one tetrapeptide to the diaminopimelic acid of another tetrapeptide. In the case of *Staphylococcus aureus*, the terminal D-alanine is cleaved from the pentapeptides to form a tetrapeptides. This provides the energy to bond a pentaglycine bridge (5 molecules of the amino acid glycine) from the D-alanine of one tetrapeptide to the L-lysine of another .

In the center of the bacterium, a group of proteins called Fts (filamentous temperature sensitive) proteins interact to form a ring at the cell division plane. These proteins form the cell division apparatus known as the divisome and are directly involved in bacterial cell division by binary fission .

The divisome is responsible for directing the synthesis of new cytoplasmic membrane and new peptidoglycan to form the division septum.

Antimicrobial Agents that Inhibit Peptidoglycan Synthesis Causing Bacterial Lysis

Many antibiotics work by inhibiting normal synthesis of peptidoglycan in bacteria causing them to burst as a result of osmotic lysis. As just mentioned, in order for bacteria to increase their size following binary fission, enzymes called

autolysins break the peptide cross links in the peptidoglycan, transglycosylase enzymes then insert and link new peptidoglycan monomers into the breaks in the peptidoglycan, and transpeptidase enzymes reform the peptide cross-links between the rows and layers of peptidoglycan to make the wall strong.

Interference with this process results in a weak cell wall and lysis of the bacterium from osmotic pressure. Examples include the penicillins (penicillin G, methicillin, oxacillin, ampicillin, amoxicillin, ticarcillin, etc.), the cephalosporins (cephalothin, cefazolin, cefoxitin, cefotaxime, cefaclor, cefoperazone, cefixime, ceftriaxone, cefuroxime, etc.), the carbapenems (imipenem, metropenem), the monobactems (aztreonem), the carbacephems (loracarbef), and the glycopeptides (vancomycin, teichoplanin).

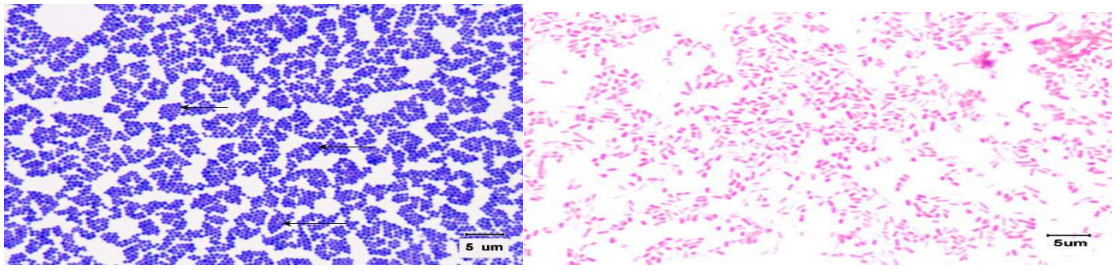
- For example, penicillins and cephalosporins bind to the transpeptidase enzymes (also called penicillin-binding proteins) responsible for resealing the cell wall as new peptidoglycan monomers are added during bacterial cell growth. This blocks the transpeptidase enzymes from cross-linking the sugar chains and results in a weak cell wall and subsequent osmotic lysis of the bacterium.

Antimicrobial chemotherapy will be discussed in greater detail later in Unit 2 under Control of Bacteria by Using Antibiotics and Disinfectants.

Gram-Positive, Gram-Negative, and Acid-Fast Bacteria

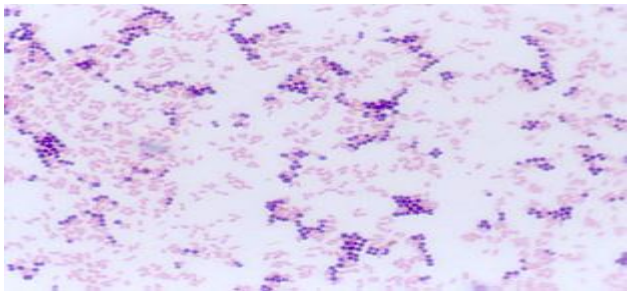
Most bacteria can be placed into one of three groups based on their color after specific staining procedures are performed: Gram-positive, Gram-negative, or acid-fast.

- **Gram-positive Bacteria**: These retain the initial dye crystal violet during the Gram stain procedure and appear purple when observed through the microscope. Common Gram-positive bacteria of medical importance include *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Staphylococcus aureus, Enterococcus faecalis,* and *Clostridium* species.



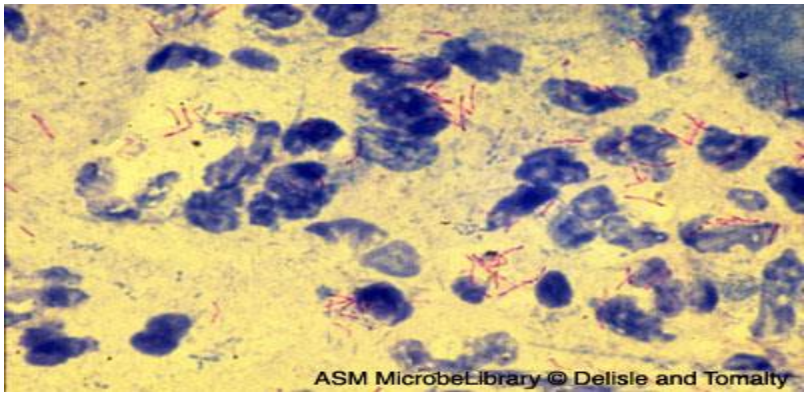
(left) Gram Stain of Staphylococcus aureus which are gram-positive (purple) cocci in clusters. (right) Gram Stain of Escherichia coli which are Gram-negative (pink) bacilli.

- **Gram-negative Bacteria**: These decolorize during the Gram stain procedure, pick up the counterstain safranin, and appear pink when observed through the microscope. Common Gram-negative bacteria of medical importance
  include *Salmonella* species, *Shigella* species, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* species, and *Pseudomonas aeruginosa*. Also see gram stain of a mixture of gram-positive and gram-negative bacteria.



A Gram Stain of a Mixture of Gram-Positive and Gram-Negative Bacteria. Note Gram-negative (pink) bacilli and Gram-positive (purple) cocci.

- **acid-fast Bacteria**: These resist decolorization with an acid-alcohol mixture during the acid-fast stain procedure, retain the initial dye carbolfuchsin and appear red when observed through the microscope. Common acid-fast bacteria of medical importance
  include *Mycobacterium tuberculosis*, *Mycobacterium leprae*, and *Mycobacterium avium-intracellulare* complex.



Acid-Fast Stain of Mycobacterium tuberculosis in Sputum. Note the reddish acid-fast bacilli among the blue normal flora and white blood cells in the sputum that are not acid-fast.

These staining reactions are due to fundamental differences in their cell wall as will be discussed in Lab 6 and Lab 16. We will now look at each of these three bacterial cell wall types.

The S-layer

1. Structure and Composition

The most common cell wall in species of *Archaea* is a paracrystalline surface layer (S-layer). It consists of a regularly structured layer composed of interlocking glycoprotein or protein molecules. In electron micrographs, has a pattern resembling floor tiles. Although they vary with the species, S-layers generally have a thickness between 5 and 25 nm and possess identical pores with 2-8 nm in diameter. Several species of *Bacteria* have also been found to have S-layers.

To view electron micrographs of S-layers see the following:

- S-Layer Proteins, the Structural Biology Homepage at Karl-Franzens University in Austria.
- Characteristic Properties of S-layer Proteins, at Foresight Nanotech Institute in Austria.

2. Functions and Significance to Bacteria Causing Infections

The S-layer has been associated with a number of possible functions. These include the following:

a. The S-layer may protect bacteria from harmful enzymes, from changes in pH, from the predatory bacterium *Bdellovibrio*, a parasitic bacterium that actually uses its motility to penetrate other bacteria and replicate within their cytoplasm, and from bacteriophages.

b. The S-layer can function as an adhesin, enabling the bacterium to adhere to host cells and environmental surfaces, colonize, and resist flushing.

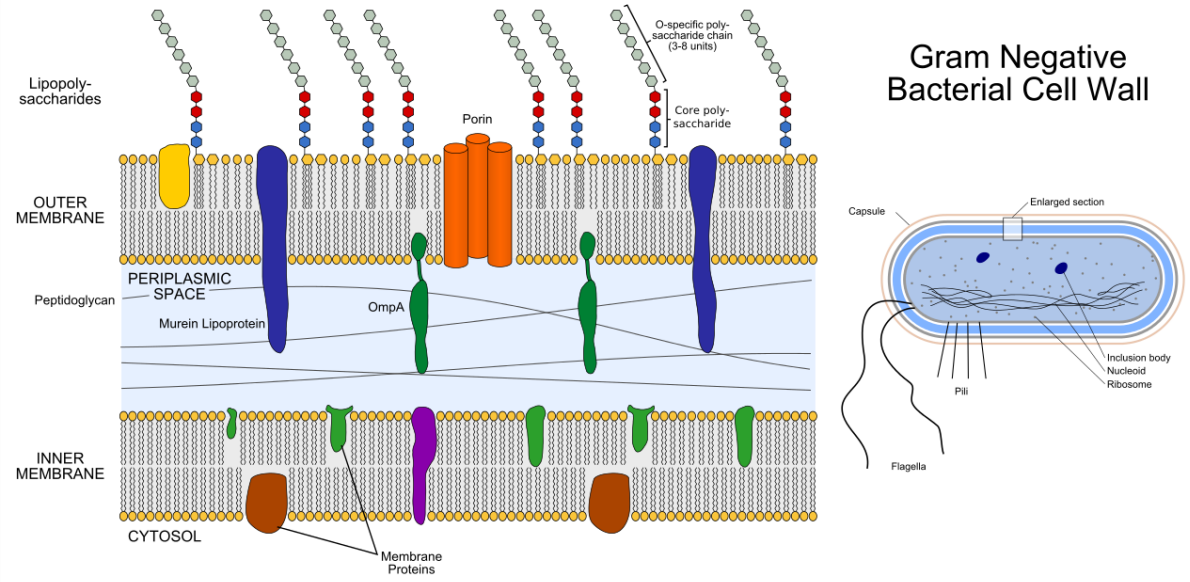
c. The S-layer may contribute to virulence by protecting the bacterium against complement attack and phagocytosis.

d. The S-layer may act as a as a coarse molecular sieve.

Bacterial outer membrane

The **bacterial outer membrane** is found in gram-negative bacteria. Its composition is distinct from that of the inner cytoplasmic cell membrane - among other things, the outer leaflet of the outer membrane of many gram-negative bacteria includes a complex lipopolysaccharide whose lipid portion acts as an endotoxin - and in some bacteria such as *E. coli* it is linked to the cell's peptidoglycan by Braun's lipoprotein.

Porins can be found in this layer.



Clinical significance

If lipid A, part of the lipopolysaccharide, enters the circulatory system it causes a toxic reaction by activating toll like receptor TLR 4. Lipid A is very pathogenic and not immunogenic. However, the polysaccharide component is very immunogenic, but not pathogenic, causing an aggressive response by the immune system. The sufferer will have a high temperature and respiration rate and a low blood pressure. This may lead to endotoxic shock, which may be fatal. The bacterial outer membrane is physiologically shed as the bounding membrane of outer membrane vesicles in cultures, as well as in animal tissues at the host-pathogen interface, implicated in translocation of gram-negative microbial biochemical signals to host or target cells.

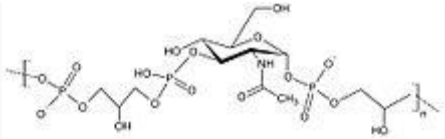
Biogenesis

The biogenesis of the outer membrane requires that the individual components are transported from the site of synthesis to their final destination outside the inner membrane by crossing both hydrophilic and hydrophobic compartments. The machinery and the energy source that drive this process are not yet fully understood. The lipid A-core moiety and the O-antigen repeat units are synthesized at the cytoplasmic face of the inner membrane and are separately exported via two independent transport systems, namely, the O-antigen transporter Wzx (RfbX) and the ATP binding cassette (ABC) transporter MsbA that flips the lipid A-core moiety from the inner leaflet to the outer leaflet of the inner membrane.[2][3][4][5][6] O-antigen repeat units are then polymerised in the periplasm by the Wzy polymerase and ligated to the lipid A-core moiety by the WaaL ligase.[7][8]

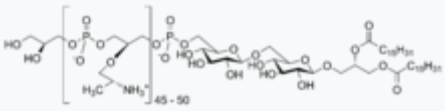
The LPS transport machinery is composed of LptA, LptB, LptC, LptD, LptE. This supported by the fact that depletion of any one of these proteins blocks the LPS assembly pathway and results in very similar outer membrane biogenesis defects. Moreover, the location of at least one of these five proteins in every cellular compartment suggests a model for how the LPS assembly pathway is organised and ordered in space.[8]

LptC is required for the translocation of lipopolysaccharide (LPS) from the inner membrane to the outer membrane.[8] LptE forms a complex with LptD, which is involved in the assembly of LPS in the outer leaflet of the outer membrane and is essential for envelope biogenesis.

Teichoic acid



Structure of a teichoic acid repeat unit from *Micrococcaceae*



Structure of the lipoteichoic acid polymer

Teichoic acids (*cf.* Greek τεῖχος, *teīkhos*, "wall", to be specific a fortification wall, as opposed to τοῖχος, *toīkhos*, a regular wall)[1] are bacterial copolymers [2] of glycerol phosphate or ribitol phosphate and carbohydrates linked via phosphodiester bonds.

Teichoic acids are found within the cell wall of most Gram-positive bacteria such as species in the genera *Staphylococcus*, *Streptococcus*, *Bacillus*, *Clostridium*, *Corynebacterium*, and *Listeria*, and appear to extend to the surface of the peptidoglycan layer. They can be covalently linked to *N*-acetylmuramic acid or a terminal D-alanine in the tetrapeptide crosslinkage between *N*-acetylmuramic acid units of the peptidoglycan layer, or they can be anchored in the cytoplasmic membrane with a lipid anchor.

Teichoic acids that are anchored to the lipid membrane are referred to as **lipoteichoic acids**(LTAs), whereas teichoic acids that are covalently bound to peptidoglycan are referred to as **wall teichoic acids** (WTA).

Structure

The most common structure of Wall teichoic acids are a ManNAc(β1→4)GlcNAc disaccharide with one to three glycerol phosphates attached to the C4 hydroxyl of the ManNAc residue followed by a long chain of glycerol- or ribitol phosphate repeats. Variations come in the long chain tail, which generally include sugar subunits being attached to thee sides or the body of the repeats. Four types of WTA repeats have been named, as of 2013.

Lipoteichoic acids follow a similar pattern of putting most variation in the repeats, although the set of enzymes used are different, at least in the case of Type I LTA. The repeats are anchored onto the membrane via a (di)glucosyl-

diacylglycerol ($Glc_{(2)}$DAG) anchor. Type IV LTA from *Streptococcus pneumoniae* represents a special case where both types intersect: after the tail is synthesized with an undecaprenyl phosphate (C_{55}-P) intermediate "head", different TagU/LCP (LytR-CpsA-Psr) family enzymes either attaches it to the wall to form a WTA or to the GlcDAG anchor.

Function

The main function of teichoic acids is to provide rigidity to the cell-wall by attracting cations such as magnesium and sodium. Teichoic acids can be substituted with D-alanine ester residues, or D-glucosamine, giving the molecule zwitterionic properties. These zwitterionic teichoic acids are suspected ligands for toll-like receptors 2 and 4. 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.

Lipoteichoic acids may also act as receptor molecules for some Gram-positive bacteriophage; however, this has not yet been conclusively supported. It is an acidic polymer and contributes negative charge to the cell wall.

Biosynthesis

WTA and Type IV LTA

Enzymes involved in the biosynthesis of WTAs have been named: TarO, TarA, TarB, TarF, TarK, and TarL. Their roles are:

- TarO (O34753, EC 2.7.8.33) starts off the process by connecting GlcNAc to a biphospho-undecaprenyl (bactoprenyl) in the inner membrane.
- TarA (P27620, EC 2.4.1.187) connects a ManNAc to the UDP-GlcNac formed by TarO via a β-(1,4) linkage.
- TarB (P27621, EC 2.7.8.44) connects a single glycerol-3-phosphate to the C4 hydroxyl of ManNAc.
- TarF (P13485, EC 2.7.8.12) connects more glycerol-3-phosphate units to the glycerol tail. In Tag-producing bacteria, this is the final step (a long glycerol tail). Otherwise it only adds one unit.
- TarK (Q8RKJ1, EC 2.7.8.46) connects the initial ribitol-5-phosphate unit. It is necessary in *Bacillus subtilis* W23 for Tar production, but *S. aureus* has both functions in the same TarL/K enzyme.
- TarL (Q8RKJ2, EC 2.7.8.47) constructs the long ribitol-5-phosphate tail.

Following the synthesis, the ATP-binding cassette transporters (teichoic-acid-transporting ATPase) TarGH (P42953, P42954) flip the cytoplasmic complex to the external surface of the inner membrane. The redundant TagTUV enzymes link this product to the cell wall. The enzymes TarI (Q8RKI9) and TarJ (Q8RKJ0) are responsible for producing the substrates that lead to the polymer tail. Many of these proteins are located in a conserved gene cluster.

Later (2013) studies have identified a few more enzymes that attach unique sugars to the WTA repeat units. A set of enzymes and transporters named DltABCE that adds alanines to both wall and lipo-teichoic acids were found.

Note that the set of genes are named "Tag" (teichoic acid glycerol) instead of "Tar" (teichoic acid ribitol) in *B. subtilis* 168, which lacks the TarK/TarL enzymes. TarB/F/L/K all bear some similarities to each other, and belong to the same family (InterPro: *IPR007554*). Due to the role of *B. subtilis* as the main model strain, some linked UniProt entries are in fact the "Tag" ortholog as they are better annotated. The "similarity search" may be used to access the genes in the Tar-producing *B. substilis* W23 (BACPZ).

As an antibiotic drug target

This was proposed in 2004. A further review in 2013 has given more specific parts of the pathways to inhibit given newer knowledge.

The Plasma Membrane

The **plasma membrane**, also called the **cytoplasmic membrane**, is the most dynamic structure of a procaryotic cell. Its main function is a s a **selective permeability barrier** that regulates the passage of substances into and out of the cell. The plasma membrane is the definitive structure of a cell since it sequesters the molecules of life in a unit, separating it from the environment. The bacterial membrane allows passage of water and uncharged molecules up to mw of about 100 daltons, but does not allow passage of larger molecules or any charged substances except by means special membrane **transport processes** and **transport systems**.

Bacterial membranes are composed of 40 percent phospholipid and 60 percent protein. The phospholipids are amphiphilic molecules with a polar hydrophilic glycerol "head" attached via an ester bond to two nonpolar hydrophobic fatty acid tails, which naturally form a bilayer in aqueous environments. Dispersed within the bilayer are various structural and enzymatic proteins which carry out

most membrane functions. At one time, it was thought that the proteins were neatly organized along the inner and outer faces of the membrane and that this accounted for the double track appearance of the membrane in electron micrographs. However, it is now known that while some membrane proteins are located and function on one side or another of the membrane, most proteins are partly inserted into the membrane, or possibly even traverse the membrane as channels from the outside to the inside. It is possible that proteins can move laterally along a surface of the membrane, but it is thermodynamically unlikely that proteins can be rotated within a membrane, which discounts early theories of how transport systems might work. The arrangement of proteins and lipids to form a membrane is called the **fluid mosaic model**, and is illustrated in Figure 20.

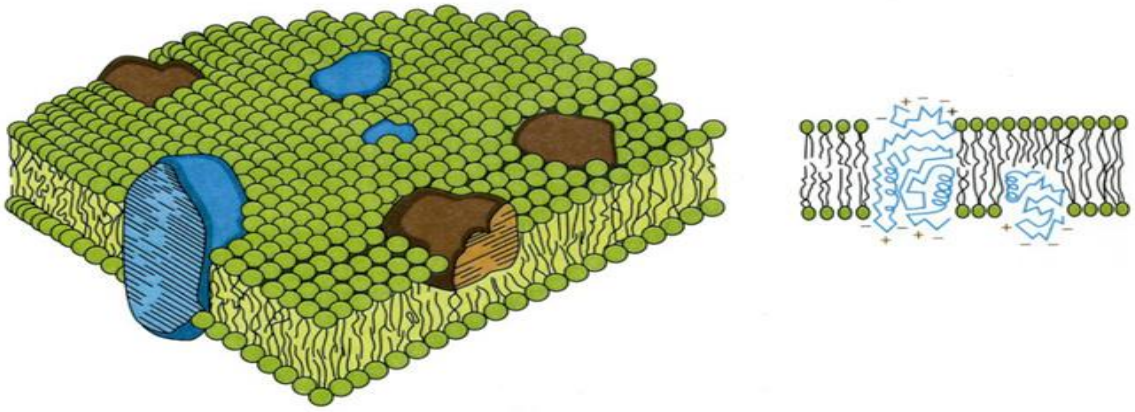


Figure 20. Fluid mosaic model of a biological membrane. In aqueous environments membrane phospholipids arrange themselves in such a way that they spontaneously form a fluid bilayer. Membrane proteins, which may be structural or functional, may be permanently or transiently associated with one side or the other of the membrane, or even be permanently built into the bilayer, while other proteins span the bilayer and may form transport channels through the membrane.

The membranes of **Bacteria** are structurally similar to the cell membranes of eucaryotes, except that bacterial membranes consist of saturated or monounsaturated fatty acids (rarely, polyunsaturated fatty acids) and do not normally contain sterols. The membranes of **Archaea** form bilayers functionally equivalent to bacterial membranes, but archaeal lipids are saturated, branched, repeating isoprenoid subunits that attach to glycerol via an ether linkage as opposed to the ester linkage found in glycerides of eukaryotic and bacterial membrane lipids (Figure 21). The structure of archaeal membranes is thought to be an adaptation to their existence and survival in extreme environments.

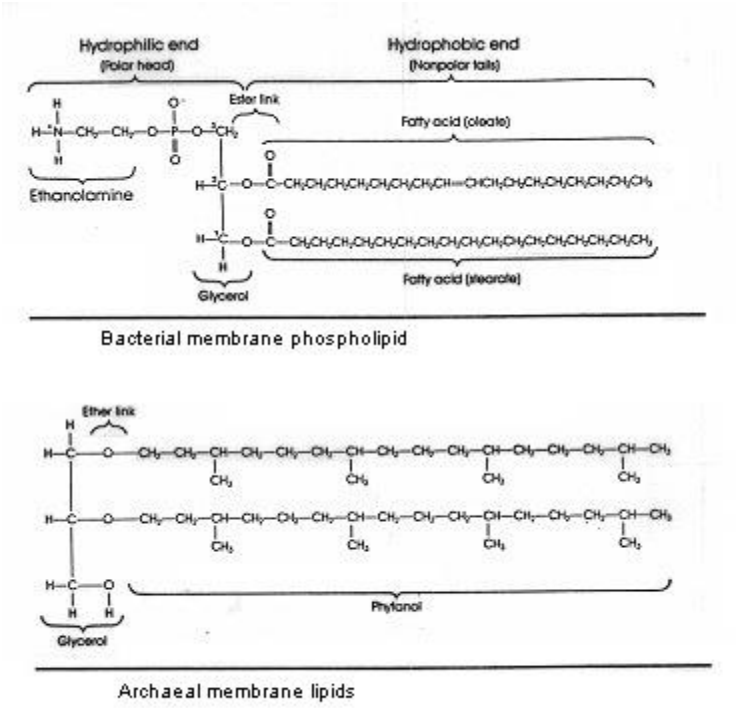


Figure 21. Generalized structure of a membrane lipids. (top). A phospholipid in the membrane of the bacterium *Escherichia coli*. The R1 and R2 positions on glycerol are substituted with saturated or monounsaturated fatty acids, with ester linkages to the glyceride. The R3 position is substituted with phosphatidylethanolamine, the most common substituent in this position in Bacteria. (bottom). An Archaeal membrane lipid. In contrast to bacterial phospholipids, which are glycerol esters of fatty acids, the lipids in membranes of Archaea are diethers of glycerol and long-chain, branched, saturated hydrocarbons called isoprenoids or which are made up of repeating C5 subunits. One of the major isoprenoids is the C20 molecule phytanol. The R3 position of glycerol may or may not be substituted. The structure of archaeal membrane lipids is thought to be an adaptation to extreme environments such as hot and acidic conditions where Archaea prevail in nature.

Functions of the Cytoplasmic Membrane

Since procaryotes lack any intracellular organelles for processes such as respiration or photosynthesis or secretion, the plasma membrane subsumes these processes for the cell and consequently has a variety of functions in **energy generation**, and **biosynthesis**. For example, the **electron transport system** that couples **aerobic respiration** and **ATP synthesis** is found in the procaryotic membrane. The **photosynthetic chromophores** that harvest light energy for conversion into chemical energy are located in the membrane. Hence, the plasma membrane is the site of **oxidative phosphorylation** and **photophosphorylation** in procaryotes, analogous to the functions of mitochondria and chloroplasts in eukaryotic cells.
Besides **transport proteins** that selectively mediate the passage of substances into and out of the cell, procaryotic membranes may contain **sensing**

proteins that measure concentrations of molecules in the environment or **binding proteins** that translocate signals to genetic and metabolic machinery in the cytoplasm. Membranes also contain **enzymes** involved in many metabolic processes such as cell wall synthesis, septum formation, membrane synthesis, DNA replication, CO_2fixation and ammonia oxidation.

Functions of the procaryotic plasma membrane

1. Osmotic or permeability barrier

2. Location of transport systems for specific solutes (nutrients and ions)

3. Energy generating functions, involving respiratory and photosynthetic electron transport systems, establishment of proton motive force, and transmembranous, ATP-synthesizing ATPase

4. Synthesis of membrane lipids (including lipopolysaccharide in Gram-negative cells)

5. Synthesis of murein (cell wall peptidoglycan)

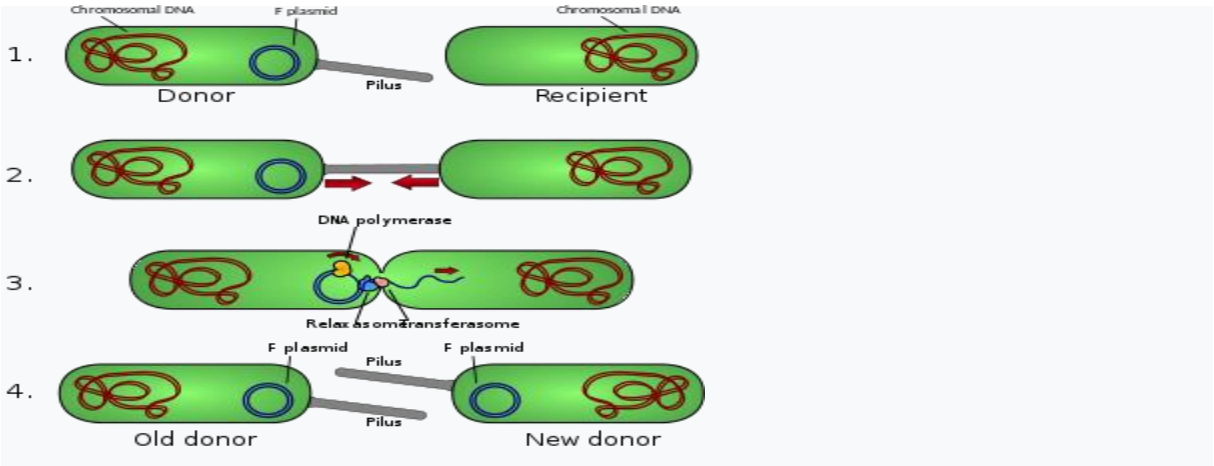
6. Assembly and secretion of extracytoplasmic proteins

7. Coordination of DNA replication and segregation with septum formation and cell division

8. Chemotaxis (both motility per se and sensing functions)

9. Location of specialized enzyme system

Pilus



Schematic drawing of bacterial conjugation. **1-** Donor cell produces pilus. **2-** Pilus attaches to recipient cell, brings the two cells together. **3-** The mobile plasmid is nicked and a single strand of DNA is then transferred to the recipient cell. **4-** Both cells recircularize their plasmids, synthesize second strands, and reproduce pili; both cells are now viable donors.

A **pilus** (Latin for 'hair'; plural: *pili*) is a hair-like appendage found on the surface of many bacteria and archaea. The terms *pilus* and *fimbria* (Latin for 'fringe'; plural: *fimbriae*) can be used interchangeably, although some researchers reserve the term *pilus* for the appendage required for bacterial conjugation. All pili in the latter sense are primarily composed of pilinproteins, which are oligomeric.

Dozens of these structures can exist on the bacterial and archaeal surface. Some bacteria, viruses or bacteriophages attach to receptors on pili at the start of their reproductive cycle.

Pili are antigenic. They are also fragile and constantly replaced, sometimes with pili of different composition, resulting in altered antigenicity. Specific host responses to old pili structure are not effective on the new structure. Recombination genes of pili code for variable (V) and constant (C) regions of the pili (similar to immunoglobulin diversity).

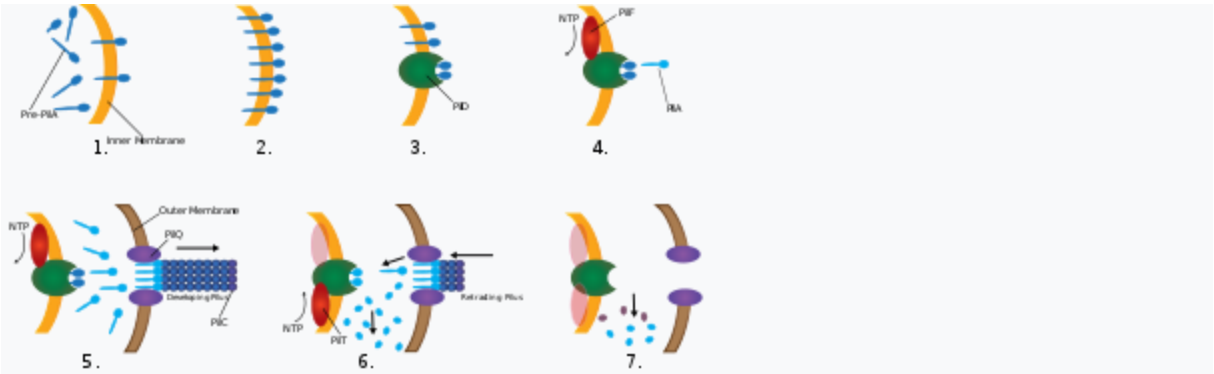
Types

Conjugative pili

Conjugative pili allow for the transfer of DNA between bacteria, in the process of bacterial conjugation. They are sometimes called "sex pili", in analogy to sexual reproduction, because they allow for the exchange of genes via the formation of "mating pairs". Perhaps the most well-studied is the pilus of *Escherichia coli*, encoded by the fertility F sex factor.

A pili is typically 6 to 7 nm in diameter. During conjugation, a pilus emerging from the donor bacterium ensnares the recipient bacterium, draws it in close, and eventually triggers the formation of a mating bridge, which establishes direct contact and the formation of a controlled pore that allows transfer of DNA from the donor to the recipient. Typically, the DNA transferred consists of the genes required to make and transfer pili (often encoded on a plasmid), and so is a kind of selfish DNA; however, other pieces of DNA are often co-transferred and this can result in dissemination of genetic traits throughout a bacterial population, such as antibiotic resistance. Not all bacteria can make conjugative pili, but conjugation can occur between bacteria of different species.

Type IV pili



Type IV Pilus Twitching Motility **1.** Pre-PilA is made in the cytoplasm and moves into the inner membrane. **2.** Pre-PilA is inserted into the inner membrane. **3.** PilD, a peptidase, removes a leader sequence, thus making the Pre-PilA shorter and into PilA, the main building-block protein of Pili. **4.** PilF, a NTP-Binding protein that provides energy for Type IV Pili Assembly. **5.** The secretin protein, PilQ, found on the outer membrane of the cell is necessary for the development/extension of the pilus. PilC is the first proteins to form the pilus and are responsible for overall attachment of the pilus. **6.** Once the Type IV Pilus attaches or interacts with what it needs to, it begins to retract. This occurs with the PilT beginning to degrade the last parts of the PilA in the pilus. The mechanism of PilT is very similar to PilF. **7.** Degradation of the pilus into the components to be utilized and synthesized into PilA again.

Some pili, called type IV pili (T4P), generate motile forces. The external ends of the pili adhere to a solid substrate, either the surface to which the bacterium is attached or to other bacteria. Then, when the pili contract, they pull the bacterium forward like a grappling hook. Movement produced by type IV pili is typically jerky, so it is called twitching motility, as opposed to other forms of bacterial motility such as that produced by flagella. However, some bacteria, for example *Myxococcus xanthus*, exhibit gliding motility. Bacterial type IV pili are similar in structure to the component flagellins of archaella (archaeal flagella). Besides archaella, many archaea produce adhesive type 4 pili, which enable archaeal cells to adhere to different substrates. The N-terminal alpha-helical portions of the archaeal type 4 pilins and archaellins are homologous to the corresponding regions of bacterial T4P; however, the C-terminal beta-strand-rich domains appear to be unrelated in bacterial and archaeal pilins.

Genetic transformation is the process by which a recipient bacterial cell takes up DNA from a neighboring cell and integrates this DNA into its genome

by homologous recombination. In *Neisseria meningitidis* (also called meningococcus), DNA transformation requires the presence of short DNA uptake sequences (DUSs) which are 9-10 monomers residing in coding regions of the donor DNA. Specific recognition of DUSs is mediated by a type IV pilin. Menningococcal type IV pili bind DNA through the minor pilin ComP via an electropositive stripe that is predicted to be exposed on the filament's surface. ComP displays an exquisite binding preference for selective DUSs. The distribution of DUSs within the *N. meningitides* genome favors certain genes, suggesting that there is a bias for genes involved in genomic maintenance and repair.

Fimbriae

To initiate formation of a biofilm, fimbriae must attach bacteria to host surfaces for colonization during infection. A fimbria is a short pilus that is used to attach the bacterium to a surface. They are sometimes called "attachment pili". Fimbriae are either located at the poles of a cell or are evenly spread over its entire surface. Mutant bacteria that lack fimbriae cannot adhere to their usual target surfaces, and thus cannot cause diseases.

Some fimbriae can contain lectins. The lectins are necessary to adhere to target cells because they can recognize oligosaccharide units on the surface of these target cells. Other fimbriae bind to components of the extracellular matrix. Fimbriae found in Gram-negative have the pilin subunits covalently linked.

Some aerobic bacteria form a thin layer at the surface of a broth culture. This layer, called a pellicle, consists of many aerobic bacteria that adhere to the surface by their fimbriae or "attachment pili". Thus, fimbriae allow the aerobic bacteria to remain on the broth, from which they take nutrients, while they congregate near the air.

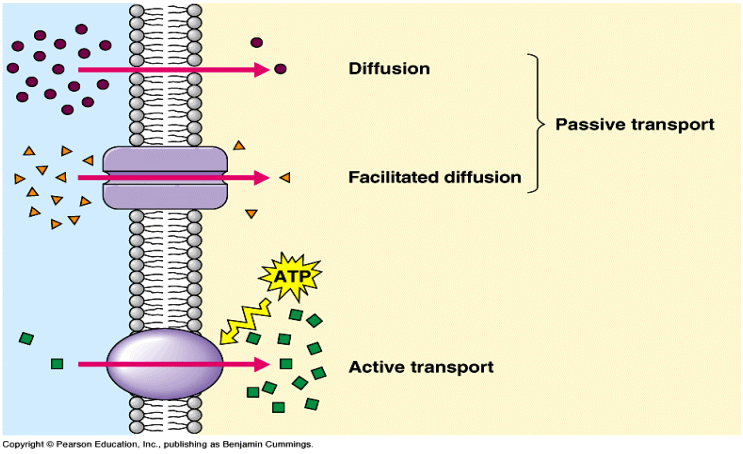
Virulence

Pili are responsible for virulence in the pathogenic strains of many bacteria, including *E. coli*, *Vibrio cholerae*, and many strains of *Streptococcus*.

This is because the presence of pili greatly enhances bacteria's ability to bind to body tissues, which then increases replication rates and ability to interact with the host organism. If a species of bacteria has multiple strains but only some are pathogenic, it is likely that the pathogenic strains will have pili while the nonpathogenic strains won't.

The development of attachment pili may then result in the development of further virulence traits. Nonpathogenic strains of *V. cholerae*first evolved pili, allowing them to bind to human tissues and form microcolonies. These pili then served as binding sites for the lysogenic bacteriophage that carries the disease-causing toxin. The gene for this toxin, once incorporated into the bacterium's genome, is expressed when the gene coding for the pilus is expressed (hence the name "toxin mediated pilus").

Diffusion, Osmosis, Active Transport



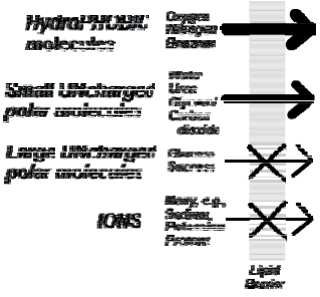
There are **two** ways in which substances can enter or leave a cell:

1) Passive
- a) Simple Diffusion
- b) Facilitated Diffusion
- c) Osmosis (**water** only)

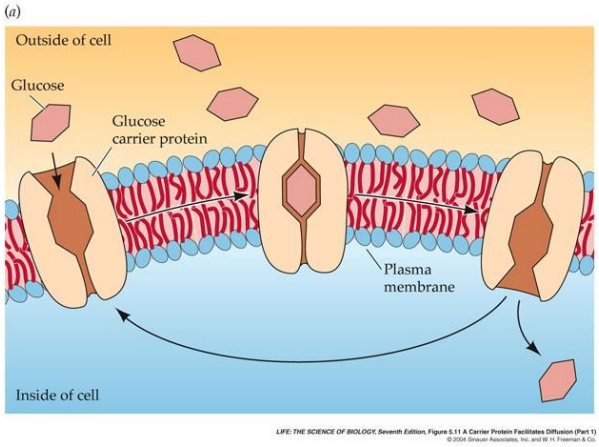
2) Active
- a) Molecules
- b) Particles

Diffusion

Diffusion is the net passive movement of particles (atoms, ions or molecules) from a region in which they are in higher concentration to regions of lower concentration. It continues until the concentration of substances is uniform throughout.



Some major examples of diffusion in biology:



- Gas exchange at the alveoli — oxygen from air to blood, carbon dioxide from blood to air.
- Gas exchange for photosynthesis — carbon dioxide from air to leaf, oxygen from leaf to air.
- Gas exchange for respiration — oxygen from blood to tissue cells, carbon dioxide in opposite direction.
- Transfer of transmitter substance — acetylcholine from presynaptic to postsynaptic membrane at a synapse.
- Osmosis — diffusion of water through a semipermeable membrane.

High Diffusion Rate: short distance, large surface area, big concentration difference (Fick's Law). High temperatures **increase** diffusion; large molecules **slow**diffusion.

Facilitated Diffusion

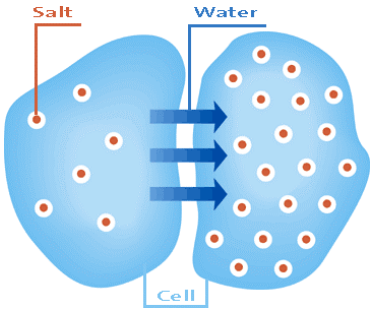
This is the movement of **specific** molecules **down a concentration gradient**, passing through the membrane *via* a **specific carrier protein.** Thus, rather like

enzymes, each carrier has its own shape and only allows one molecule (or one group of closely related molecules) to pass through.
Selection is by size; shape; charge.
Common molecules entering/leaving cells this way include glucose and amino-acids.
It is **passive** and requires no energy from the cell.

If the molecule is changed on entering the cell (glucose + ATP → glucose phosphate + ADP), then the **concentration gradient of glucose** will be kept high, and there will a steady one-way traffic.

Osmosis

Osmosis is a special example of diffusion. It is the diffusion of water through a partially permeable membrane from a more dilute solution to a more concentrated solution – **down the water potential gradient)**



Note: diffusion and osmosis are both passive, i.e. energy from ATP is **not** used.

A partially permeable membrane is a barrier that permits the passage of some substances but not others; it allows the passage of the solvent molecules but not some of the larger solute molecules.

Cell membranes are described as selectively permeable because not only do they allow the passage of water but also allow the passage of certain solutes. The presence of particular solutes stimulates the membrane to open specific channels or trigger active transport mechanisms to allow the passage of those chemicals across the membrane.

Some major examples of osmosis

- Absorption of water by plant roots.
- Re-absorption of water by the proximal and distal convoluted tubules of the nephron.
- Re-absorption of tissue fluid into the venule ends of the blood capillaries.
- Absorption of water by the alimentary canal — stomach, small intestine and the colon.

Osmoregulation

Osmoregulation is keeping the concentration of cell cytoplasm or blood at a suitable concentration.

(a) *Amoeba*, living in freshwater, uses a contractile vacuole to expel

the excess water from its cytoplasm (thus need more respiration/O_2/ATP than isotonic (marine) *Amoebae).*

(b) The kidneys maintain the blood (thus, whole body) at the correct concentration.

- he plant cells gain water by osmosis.
- the vacuole and cytoplasm increase in volume.
- the cell membrane is pushed harder Turgor

Cellulose cell wall Plant Cell

Turgor is the pressure of the swollen cell contents against the cell wall when the external solution more dilute than the cell sap of the vacuole.

Role of Turgor in Plants

- Mechanical support for soft non-woody tissue, e.g., leaves.
- Change in shape of guard cells forming the stomatal opening between them.
- Enlargement of young immature plant cells to mature size.

Water Potential

- This is the tendency of water to move from one place to another.
- Values are always negative!
- Water always **flows downhill** i.e. towards the more negative number.
- Units are pressure (kPa)
- Calculations are **not** set, but this formula may be:

$$\text{Water Potential } (\psi) = \text{Pressure Potential } (\psi_p) + \text{Solute Potential } (\psi_s)$$

- Pressure Potential = **the force of the cell wall on the contents**,
- so for animal cells, this is zero, thus, in animals:

$$\text{Water Potential } (\psi) = \text{Solute Potential } (\psi_s)$$

Active Transport

Active transport is the energy-demanding transfer of a substance across a cell membrane **against** its concentration gradient, i.e., from lower

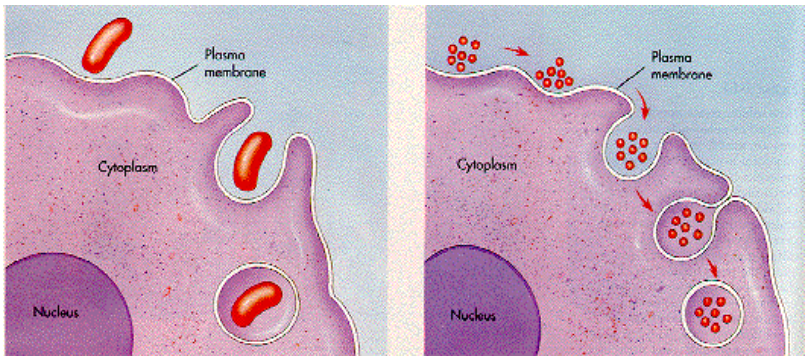
concentration to higher concentration.
Special proteins within the cell membrane act as specific protein 'carriers'.
The energy for active transport comes from ATP generated by respiration (in mitochondria).

Major examples of Active Transport

Re-absorption of glucose, amino acids and salts by the proximal convoluted tubule of the nephron in the kidney.
Sodium/potassium pump in cell membranes (especially nerve cells)

Endo/exocytosis

This is the movement of **very large** molecules (or particles, bacteria or other organisms) across the cell membrane. It involves the fusion of vesicles (containing the target/victim) with the cell membrane e.g. bacteria entering **macrophages**.
Substances destined for secretion are packaged in the **Golgi body** first.



Pinocytosis *('cell drinking')*

This is the uptake of large molecules (DNA, protein) from **solution**, by a form of endocytosis – the vesicles formed are minute and short-lived.
Phagocytosis *('cell eating')*

This is the uptake of **solid particles** by a cell e.g. *Amoeba* feeding, phagocytes engulfing bacteria.

Passive transport and active transport across a cell membrane

Passive Transport: Osmosis

Osmosis is the diffusion of water through a semipermeable membrane according to the concentration gradient of water across the membrane. Whereas diffusion transports material across membranes and within cells, osmosis transports *only water* across a membrane and the membrane limits the diffusion of solutes in the water. Osmosis is a special case of diffusion. Water, like other substances, moves from an area of higher concentration to one of lower concentration. Imagine a beaker with a semipermeable membrane, separating the two sides or halves **(Figure 3**). On both sides of the membrane, the water level is the same, but there are different concentrations on each side of a dissolved substance, or **solute**, that cannot cross the membrane. If the volume of the water is the same, but the concentrations of solute are different, then there are also different concentrations of water, the **solvent,** on either side of the membrane.

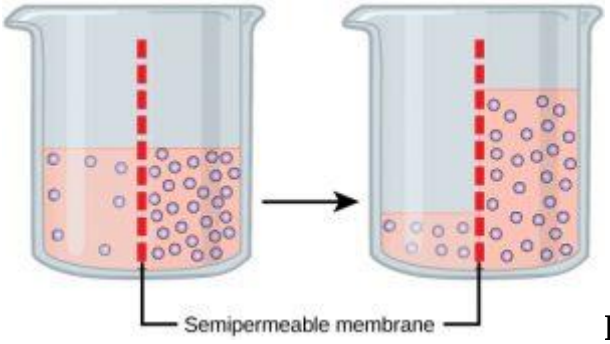


Figure 3 In osmosis, water always moves from an area of higher concentration (of water) to one of lower concentration (of water). In this system, the solute cannot pass through the selectively permeable membrane.

A principle of diffusion is that the molecules move around and will spread evenly throughout the medium if they can. However, only the material capable of getting through the membrane will diffuse through it. In this example, the solute cannot diffuse through the membrane, but the water can. Water has a concentration gradient in this system. Therefore, water will diffuse down its concentration gradient, crossing the membrane to the side where it is less concentrated. This diffusion of water through the membrane— **osmosis** —will continue until the concentration gradient of water goes to zero. Osmosis proceeds constantly in living systems.

TONICITY

Tonicity describes the amount of solute in a solution. The measure of the tonicity of a solution, or the total amount of solutes dissolved in a specific amount of solution, is called its **osmolarity**. Three terms—hypotonic, isotonic, and hypertonic—are used to relate the osmolarity of a cell to the osmolarity of the extracellular fluid that contains the cells. All three of these terms are a *comparison* between two different solutions (for example, inside a cell compared to outside the cell).

In a **hypotonic** solution, such as tap water, the extracellular fluid has a lower concentration of solutes than the fluid inside the cell, and water enters the cell. (In living systems, the point of reference is always the cytoplasm, so the prefix *hypo–* means that the extracellular fluid has a lower concentration of solutes, or a lower osmolarity, than the cell cytoplasm.) It also means that the extracellular fluid has a higher concentration of water than does the cell. In this situation, water will follow its concentration gradient and enter the cell. This may cause an animal cell to burst, or **lyse.**

In a **hypertonic** solution (the prefix *hyper–* refers to the extracellular fluid having a higher concentration of solutes than the cell's cytoplasm), the fluid contains less water than the cell does, such as seawater. Because the cell has a lower concentration of solutes, the water will leave the cell. In effect, the solute is drawing the water out of the cell. This may cause an animal cell to shrivel, or **crenate.**

In an **isotonic** solution, the extracellular fluid has the same osmolarity as the cell. If the concentration of solutes of the cell matches that of the extracellular fluid, there will be no net movement of water into or out of the cell. The cell will retain its "normal" appearance. Blood cells in hypertonic, isotonic, and hypotonic solutions take on characteristic appearances (**Figure 4**).

Remember that all three of these terms are *comparisons* between two solutions (i.e. inside and outside the cell). A solution can't be hypotonic, that would be like saying that Bob is taller. That doesn't make sense – you need to say that Bob is taller than Mike. You can say that the solution inside the cell is hypotonic to the solution outside the cell. That also means that the solution outside is hypertonic to the solution inside (just like Mike would be shorter than Bob).

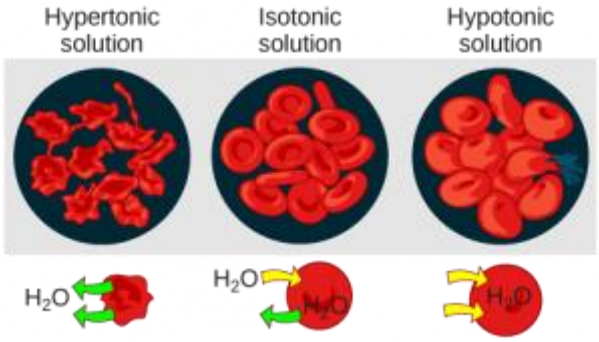
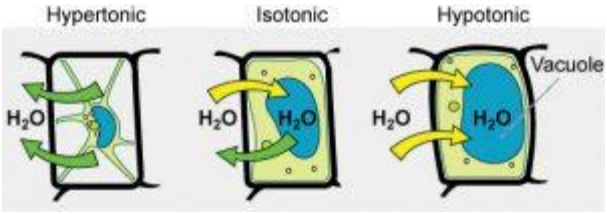


Figure 4 Osmotic pressure changes the shape of red blood cells in hypertonic, isotonic, and hypotonic solutions. (credit: modification of work by Mariana Ruiz Villarreal)

Some organisms, such as plants, fungi, bacteria, and some protists, have **cell walls** that surround the plasma membrane and prevent cell lysis. The plasma membrane can only expand to the limit of the cell wall, so the cell will not lyse. In fact, the cytoplasm in plants is always slightly hypertonic compared to the cellular environment, and water will always enter the plant cell if water is available. This influx of water produces **turgor pressure**, which stiffens the cell walls of the plant (**Figure 5**). In nonwoody plants, turgor pressure supports the plant. If the plant cells become hypertonic, as occurs in drought or if a plant is not watered adequately, water will leave the cell. Plants lose turgor pressure in this condition and wilt.



Unit 2

Microbial Growth

Provided with the right conditions (food, correct temperature, etc) microbes can grow very quickly. Depending on the situation, this could be a good thing for humans (yeast growing in wort to make beer) or a bad thing (bacteria growing in your throat causing strep throat). It's important to have knowledge of their growth, so we can predict or control their growth under particular conditions. While growth for muticelluar organisms is typically measured in terms of the increase in size of a single organism, microbial growth is measured by the increase in population, either by measuring the increase in cell number or the increase in overall mass.

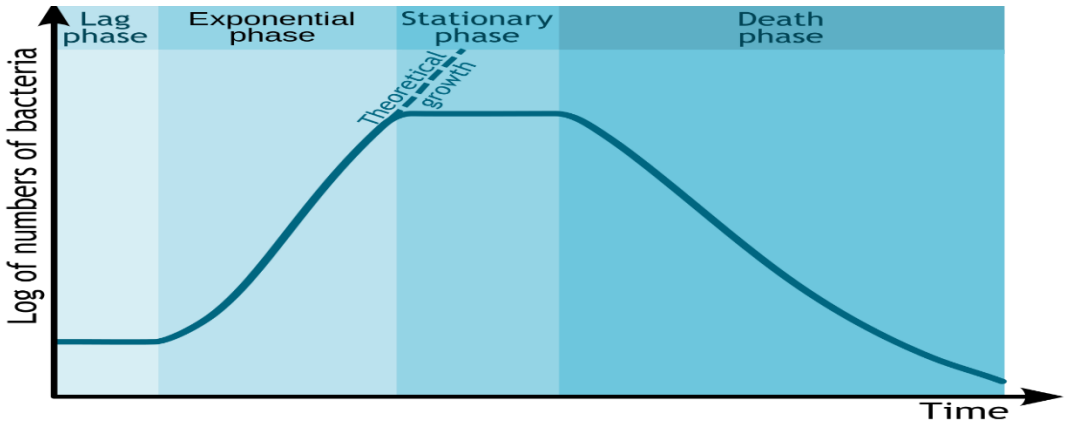
Bacterial Division

Bacteria and archaea reproduce asexually only, while eukartyotic microbes can engage in either sexual or asexual reproduction. Bacteria and archaea most commonly engage in a process known as **binary fission**, where a single cell splits into two equally sized cells. Other, less common processes can include **multiple fission**, **budding**, and the production of **spores**.

The process begins with cell elongation, which requires careful enlargement of the cell membrane and the cell wall, in addition to an increase in cell volume. The cell starts to replicate its DNA, in preparation for having two copies of its chromosome, one for each newly formed cell. The protein FtsZ is essential for the formation of a septum, which initially manifests as a ring in the middle of the elongated cell. After the nucleoids are segregated to each end of the elongated cell, septum formation is completed, dividing the elongated cell into two equally sized daughter cells. The entire process or **cell cycle**can take as little as 20 minutes for an active culture of *E. coli* bacteria.

Growth Curve

Since bacteria are easy to grow in the lab, their growth has been studied extensively. It has been determined that in a **closed system** or **batch culture** (no food added, no wastes removed) bacteria will grow in a predictable pattern, resulting in a **growth curve** composed of four distinct phases of growth: the lag phase, the exponential or log phase, the stationary phase, and the death or decline phase. Additionally, this growth curve can yield generation time for a particular organism – the amount of time it takes for the population to double.



Bacterial Growth Curve.

The details associated with each growth curve (number of cells, length of each phase, rapidness of growth or death, overall amount of time) will vary from organism to organism or even with different conditions for the same organism. But the pattern of four distinct phases of growth will typically remain.

Lag phase

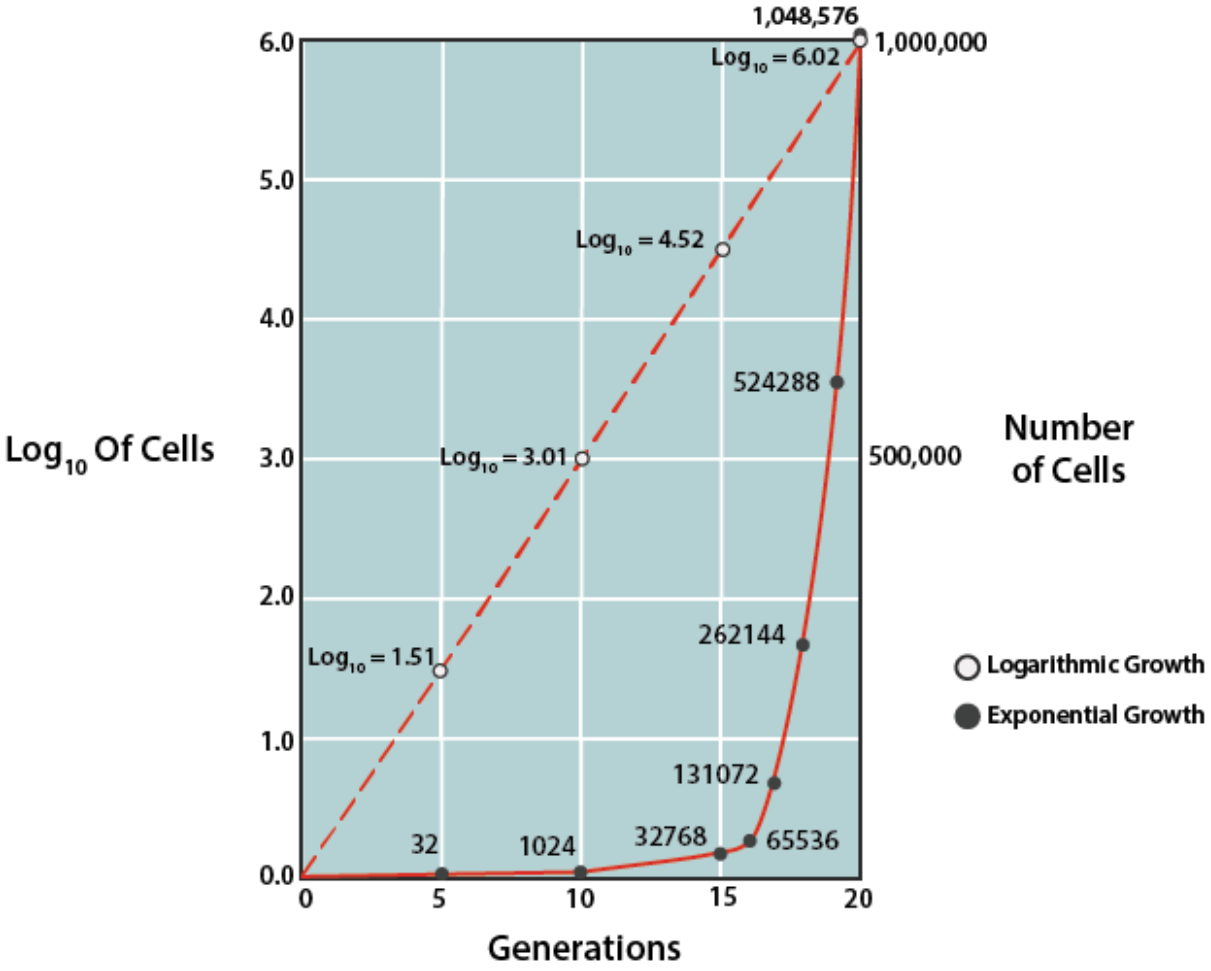
The **lag phase** is an adaptation period, where the bacteria are adjusting to their new conditions. The length of the lag phase can vary considerably, based on how different the conditions are from the conditions that the bacteria came from, as well as the condition of the bacterial cells themselves. Actively growing cells transferred from one type of media into the same type of media, with the same environmental conditions, will have the shortest lag period. Damaged cells will have a long lag period, since they must repair themselves before they can engage in reproduction.

Typically cells in the lag period are synthesizing RNA, enzymes, and essential metabolites that might be missing from their new environment (such as growth factors or macromolecules), as well as adjusting to environmental changes such as changes in temperature, pH, or oxygen availability. They can also be undertaking any necessary repair of injured cells.

Exponential or Log phase

Once cells have accumulated all that they need for growth, they proceed into cell division. The **exponential or log phase** of growth is marked by predictable doublings of the population, where 1 cell become 2 cells, becomes 4, becomes 8

etc. Conditions that are optimal for the cells will result in very rapid growth (and a steeper slope on the growth curve), while less than ideal conditions will result in slower growth. Cells in the exponential phase of growth are the healthiest and most uniform, which explains why most experiments utilize cells from this phase.



Bacterial Growth Rates.

Due to the predictability of growth in this phase, this phase can be used to mathematically calculate the time it takes for the bacterial population to double in number, known as the **generation time (*g*)**. This information is used by microbiologists in basic research, as well as in industry. In order to determine generation time, the natural logarithm of cell number can be plotted against time (where the units can vary, depending upon speed of growth for the particular population), using a semilogarithmic graph to generate a line with a predictable slope.

The slope of the line is equal to 0.301/*g*. Alternatively one can rely on the fixed relationship between the initial number of cells at the start of the exponential phase and the number of cells after some period of time, which can be expressed by:

N=N02n(9.1)(9.1)N=N02n

where NN is the final cell concentration, N0N0 is the initial cell concentration, and nn is the number of generations that occurred between the specified period of time.

Generation time (*g*) can be represented by *t/n*, with ***t*** being the specified period of time in minutes, hours, days, or months. Thus, if one knows the cell concentration at the start of the exponential phase of growth and the cell concentration after some period of time of exponential growth, the number of generations can be calculated. Then, using the amount of time that growth was allowed to proceed (*t*), one can calculate *g*.

Stationary Phase

All good things must come to an end (otherwise bacteria would equal the mass of the Earth in 7 days!). At some point the bacterial population runs out of an essential nutrient/chemical or its growth is inhibited by its own waste products (it is a closed container, remember?) or lack of physical space, causing the cells to enter into the **stationary phase**. At this point the number of new cells being produced is equal to the number of cells dying off or growth has entirely ceased, resulting in a flattening out of growth on the growth curve.

Physiologically the cells become quite different at this stage, as they try to adapt to their new starvation conditions. The few new cells that are produced are smaller in size, with bacilli becoming almost spherical in shape. Their plasma membrane becomes less fluid and permeable, with more hydrophobic molecules on the surface that promote cell adhesion and aggregation. The nucleoid condenses and the DNA becomes bound with **DNA-binding proteins from starved cells (DPS)**, to protect the DNA from damage. The changes are designed to allow the cell to survive for a longer period of time in adverse conditions, while waiting for more optimal conditions (such as an infusion of nutrients) to occur. These same strategies are used by cells in **oligotrophic** or

low-nutrient environments. It has been hypothesized that cells in the natural world (i.e. outside of the laboratory) typically exist for long periods of time in oligotrophic environments, with only sporadic infusions of nutrients that return them to exponential growth for very brief periods of time.

During the stationary phase cells are also prone to producing **secondary metabolites**, or metabolites produced after active growth, such as antibiotics. Cells that are capable of making an endospore will activate the necessary genes during this stage, in order to initiate the sporulation process.

Death or Decline phase

In the last phase of the growth curve, the **death or decline phase**, the number of viable cells decreases in a predictable (or exponential) fashion. The steepness of the slope corresponds to how fast cells are losing viability. It is thought that the culture conditions have deteriorated to a point where the cells are irreparably harmed, since cells collected from this phase fail to show growth when transferred to fresh medium. It is important to note that if the turbidity of a culture is being measured as a way to determine cell density, measurements might not decrease during this phase, since cells could still be intact.

It has been suggested that the cells thought to be dead might be revived under specific conditions, a condition described as **viable but nonculturable (VBNC)**. This state might be of importance for pathogens, where they enter a state of very low metabolism and lack of cellular division, only to resume growth at a later time, when conditions improve.

It has also been shown that 100% cell death is unlikely, for any cell population, as the cells mutate to adapt to their environmental conditions, however harsh. Often there is a tailing effect observed, where a small population of the cells cannot be killed off. In addition, these cells might benefit from their death of their fellow cells, which provide nutrients to the environment as they lyse and release their cellular contents.

Methods of measuring bacterial growth

Estimating the growth of bacteria is extremely important. Environmental health officers regularly inspect food premises and take sample for analysis. Water boards check water supplies daily. Many products are produced using bacteria

grown in fermenters. Measuring their growth is an important part of the process.

There are several different methods of measuring growth:

• Rough estimates of growth rates can be made by regularly measuring the diameter of a bacterial or fungal colony as it spreads from a central point to cover the surface of a **solid growth medium** (such as an **agar plate**)

• The size of a population of microorganisms in **liquid culture** may be measured by **counting cells directly or by first diluting the original sample and then counting cell numbers (see below)**, or by **taking some indirect method** such as the **turbidity (cloudiness)** of the culture.

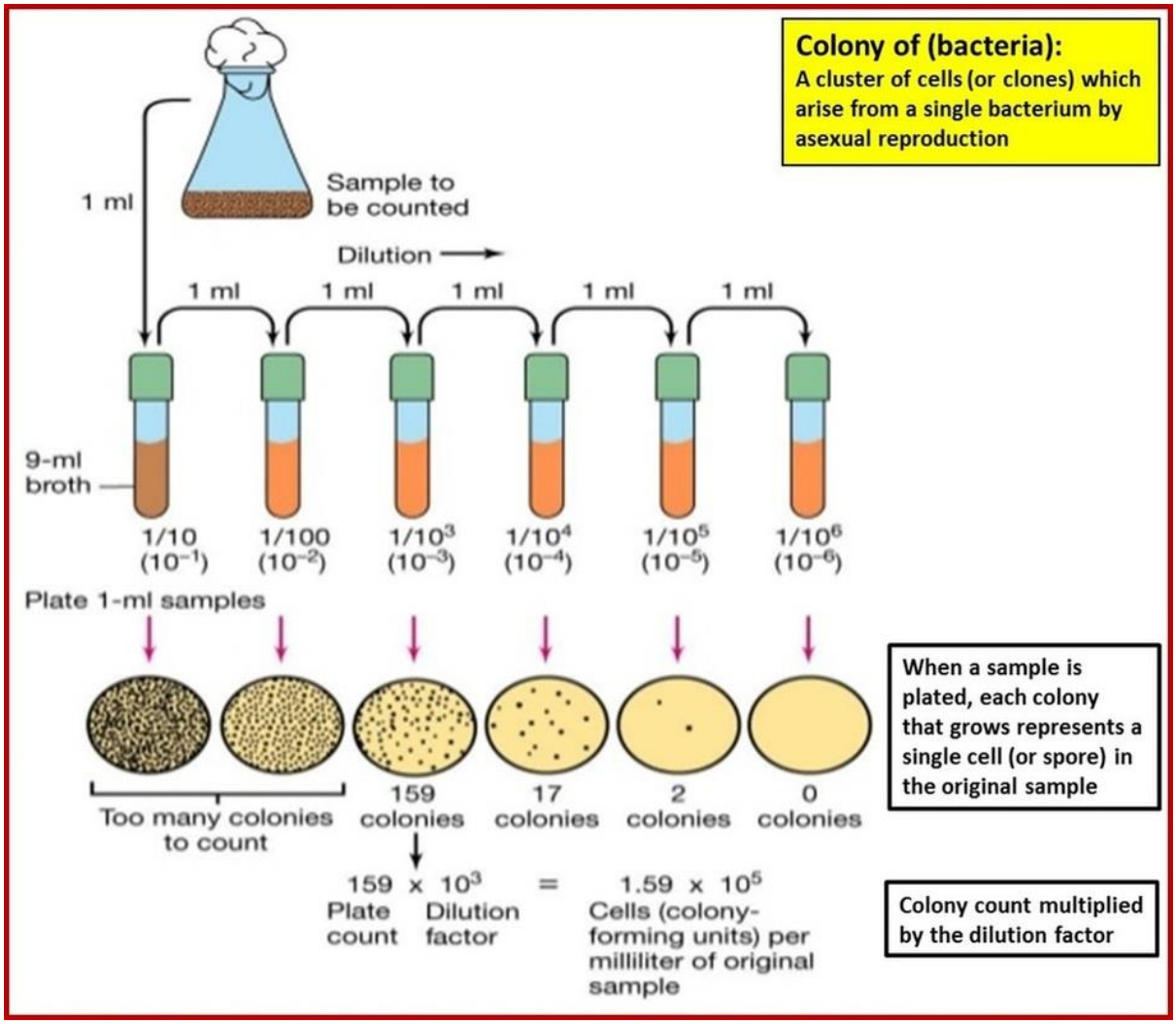
Direct cell counts may be divided into:

1. Total counts = which include **both living cells** and **dead cells**
2. Viable counts = which count **living cells only**

In practice, it is **never possible** to count **whole populations** of microorganisms. Instead, the cells in a **very small sample** of culture are counted, and the **result multiplied** up to give a **population density** in **organisms per cm3 of culture**. Even then, the population density is likely to be so high that **cell counts** are usually **made in known dilutions of the culture**, usually in **10-fold steps**. This is known as **serial dilution.**

The dilution plating technique is as follows:

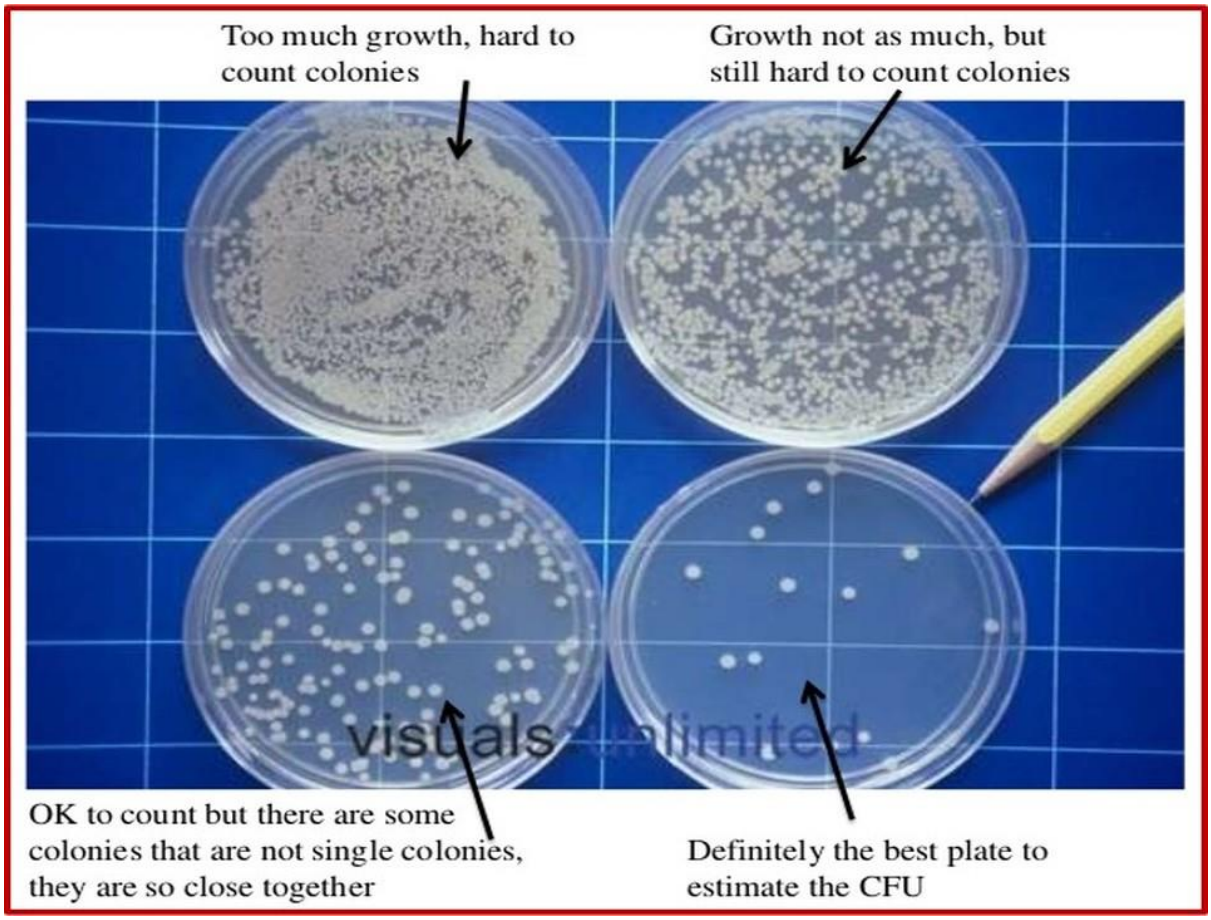
A culture medium, such as milk or a water sample, is made into a **series of dilutions** using the **serial dilution technique**.



1cm3 (the same as saying 1ml) of each of the **diluted samples** (see diagram above), are individually streaked onto **sterile agar plates**, which are then placed in an incubator at **25oC** for **two days** to allow time for the **bacteria to grow**. After all the streaks have been allowed to grow, the dilution at which the **colonies** are **distinct and separate** is noted down (this might be the 1/1000 dilution from the example above). If the dilution is insufficient then colonies will merge, referred to as 'clumping', and counting is inaccurate (the example above would be the 1/10 and the 1/100 dilution). The **distinct and separate** colonies of bacteria from the agar plate you have chosen are counted with the assumption being made that **each colony** has **arisen from a single cell**, which has **divided asexually**, from the **original culture medium**. To find the **total viable cell count** the **number of colonies** you count is **multiplied** by the **appropriate dilution factor**. So in the example above this would be:

159 colonies counted on the 1/1000 dilution agar plate
So 159 x 1000 = **1.59 x 10 to the power 5** bacteria in the **original sample.**

However, if the dilution is insufficient then colonies will merge (see 1/10 and 1/100 dilution agar plates above), referred to as 'clumping', and counting is inaccurate, resulting in an underestimate of numbers



Using a Haemocytometer

A more accurate method involves using a **haemocytometer**. This is a **specialised microscope slide** originally used to count **red blood cells**. Using the **haemocytometer** gives **total cell counts** as it is **not possible to distinguish between living and dead cells** (you are not required to describe or use a haemocytometer.)

Using Turbidimetry

A third method, known as **turbidimetry**, involves using a **colorimeter** to measure the **cloudiness** or turbidity of the culture as**cell numbers increase**. Results are derived by comparison with a **standard graph** of **light**

absorbance plotted against **known cell numbers** (You are not required to describe or use a calorimeter).

Growth Rate and Generation Time

As mentioned above, bacterial growth rates during the phase of exponential growth, under standard nutritional conditions (culture medium, temperature, pH, etc.), define the bacterium's generation time. Generation times for bacteria vary from about 12 minutes to 24 hours or more. The generation time for *E. coli* in the laboratory is 15-20 minutes, but in the intestinal tract, the coliform's generation time is estimated to be 12-24 hours. For most known bacteria that can be cultured, generation times range from about 15 minutes to 1 hour. Symbionts such as *Rhizobium* tend to have longer generation times. Many lithotrophs, such as the nitrifying bacteria, also have long generation times. Some bacteria that are pathogens, such as *Mycobacterium tuberculosis* and *Treponema pallidum*, have especially long generation times, and this is thought to be an advantage in their virulence. Generation times for a few bacteria are are shown in Table 2.

Table 2. Generation times for some common bacteria under optimal conditions of growth.

Bacterium	**Medium**	**Generation Time (minutes)**
Escherichia coli	Glucose-salts	17
Bacillus megaterium	Sucrose-salts	25
Streptococcus lactis	Milk	26
Streptococcus lactis	Lactose broth	48
Staphylococcus aureus	Heart infusion broth	27-30
Lactobacillus acidophilus	Milk	66-87
Rhizobium japonicum	Mannitol-salts-yeast extract	344-461
Mycobacterium tuberculosis	Synthetic	792-932
Treponema pallidum	Rabbit testes	1980

Calculation of Generation Time

When growing exponentially by binary fission, the increase in a bacterial population is by geometric progression. If we start with one cell, when it divides, there are 2 cells in the first generation, 4 cells in the second generation, 8 cells in the third generation, and so on. The **generation time** is the time interval required for the cells (or population) to divide.

G (generation time) = (time, in minutes or hours)/n(number of generations)

$G = t/n$

t = time interval in hours or minutes

B = number of bacteria at the beginning of a time interval

b = number of bacteria at the end of the time interval

n = number of generations (number of times the cell population doubles during the time interval)

$b = B \times 2^n$ (This equation is an expression of growth by binary fission)

Solve for n:

$\log b = \log B + n \log 2$

$$n = \frac{\log b - \log B}{\log 2}$$

$$n = \frac{\log b - \log B}{.301}$$

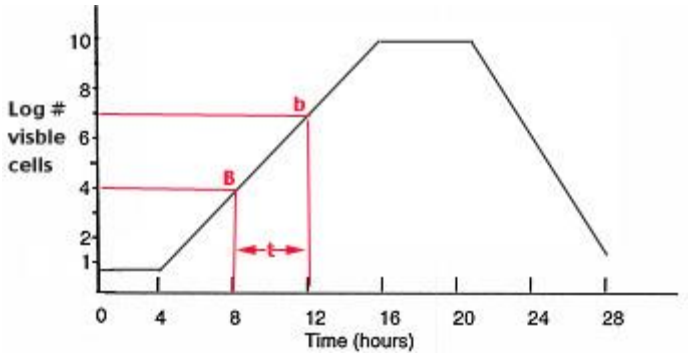
$n = 3.3 \log b/B$

$G = t/n$

Solve for G

$$G = \frac{t}{3.3 \log b/B}$$

Example: What is the generation time of a bacterial population that increases from 10,000 cells to 10,000,000 cells in four hours of growth?



$$G = \frac{t}{3.3 \log b/B}$$

$$G = \frac{240 \text{ minutes}}{3.3 \log 10^7/10^4}$$

$$G = \frac{240 \text{ minutes}}{3.3 \times 3}$$

G = 24 minutes

The Growth of Bacterial Populations

Continuous Culture of Bacteria

The cultures so far discussed for growth of bacterial populations are called **batch cultures**. Since the nutrients are not renewed, exponential growth is limited to a few generations. Bacterial cultures can be maintained in a state of exponential growth over long periods of time using a system of **continuous culture** (Figure 4), designed to relieve the conditions that stop exponential growth in batch cultures. Continuous culture, in a device called a **chemostat**, can be used to maintain a bacterial population at a constant density, a situation that is, in many ways, more similar to bacterial growth in natural environments.

In a chemostat, the growth chamber is connected to a reservoir of sterile medium. Once growth is initiated, fresh medium is continuously supplied from

the reservoir. The volume of fluid in the growth chamber is maintained at a constant level by some sort of overflow drain. Fresh medium is allowed to enter into the growth chamber at a rate that limits the growth of the bacteria. The bacteria grow (cells are formed) at the same rate that bacterial cells (and spent medium) are removed by the overflow. The rate of addition of the fresh medium determines the rate of growth because the fresh medium always contains a limiting amount of an essential nutrient. Thus, the chemostat relieves the insufficiency of nutrients, the accumulation of toxic substances, and the accumulation of excess cells in the culture, which are the parameters that initiate the stationary phase of the growth cycle. The bacterial culture can be grown and maintained at relatively constant conditions, depending on the flow rate of the nutrients.

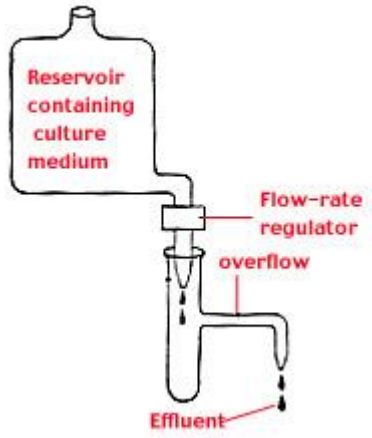


Figure 4. Schematic diagram of a chemostat, a device for the continuous culture of bacteria. The chemostat relieves the environmental conditions that restrict growth by continuously supplying nutrients to cells and removing waste substances and spent cells from the culture medium.

Synchronous Growth of Bacteria

Studying the growth of bacterial populations in batch or continuous cultures does not permit any conclusions about the growth behavior of individual cells, because the distribution of cell size (and hence cell age) among the members of the population is completely random. Information about the growth behavior of individual bacteria can, however, be obtained by the study of **synchronous cultures**. Synchronized cultures must be composed of cells which are all at the same stage of the **bacterial cell cycle**. Measurements made on synchronized cultures are equivalent to measurements made on individual cells.

A number of clever techniques have been devised to obtain bacterial populations at the same stage in the cell cycle. Some techniques involve manipulation of environmental parameters which induces the population to start or stop growth at the same point in the cell cycle, while others are physical methods for selection of cells that have just completed the process of binary fission. Theoretically, the smallest cells in a bacterial population are those that have just completed the process of cell division. Synchronous growth of a population of bacterial cells is illustrated in Figure 5. Synchronous cultures rapidly lose synchrony because not all cells in the population divide at exactly the same size, age or time.

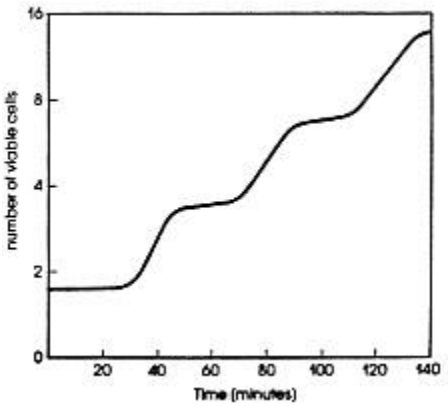


Figure 5. The synchronous growth of a bacterial population. By careful selection of cells that have just divided, a bacterial population can be synchronized in the bacterial cell division cycle. Synchrony can be maintained for only a few generations.

Synchronous Cultures of Micro-organisms

Introduction

Synchronous culture technique enables separation of the smallest cells from an exponentially growing culture. This separation is achieved by passing the culture through a continuous flow centrifuge rotor under controlled conditions. The controlled conditions represent the seed of the rotor and flow rate. The separated smallest-sized class of organisms, which is suspended in their growth medium, remains in the effluent. The growth of these cells is not disturbed throughout the procedure. This culture exhibit synchronous cell division. This technique has been successfully applied to budding and fission yeasts, amoeboid, ciliated protozoa. This application of this procedure can be extended

to any non-filamentous, non-aggregating, unicellular organism or to cells of higher plants or animals growing in liquid tissue-cultures.

Preparation of synchronous cultures can be broadly classified into two types: The first type induces all the cells of the culture to divide synchronously by some physical or chemical treatment. This type is known as induction synchrony The other type selects cells at a particular stage of the cycle. The selected cycles are allowed to grow through their natural cycle. It is termed as selection synchrony. If a study needs to study of them in normal cell cycle selection synchrony is preferred as induction synchrony involves metabolic stress or interference.

Differential sedimentation of the exponentially growing cell population through a sucrose density gradient is the widely used method for selection of the smallest cells of a culture. This method is seriously limited by yield. Scale up of this process with better yield is achieved using zonal rotors which generate greater gradient capacity. The time taken for this separation is usefully high around 1 hour. During this The organism is exposed different growing conditions such as sub-optimal temperature and anaerobic conditions.

The high osmotic pressure became a limiting factor in this modification. Separation of osmotically fragile organisms cannot be separated by this method. Even the substitution of high-molecular-weight gradient media to eliminate high osmotic pressures does not prevent distortion of the first subsequent cell-cycle. Rapid motile organisms cannot be separated or size selected using velocity sedimentation. Maintaining aseptic conditions are almost impossible. Even the alternative approach of cell-cycle fractionation also had similar problems but the cell yield is high during the use of large-volume zonal rotors. The difficulties discussed above are eliminated in the below-described method. The applied preparation has been successfully carried on for a period of two years.

Methods & Organisms

Maintenance, growth, and harvesting of the organism

S. pombe 972^{h-} was maintained and grown on a defined medium containing 1 % (w/v) glucose. Batch cultures (4-6 litres) were grown in a 6-liter capacity. Forced aeration was at 1 liter of air/min per liter of culture, stirring rate 400 rev./min and the growth temperature was 30°C. The exponentially growing culture was harvested during the phase of glucose repression when the population reached 2.0×10^{7}– 3.0 x 10^{7} cells/ml, by centrifugation at 2000rev./min in the 4xl-liter rotor of an International centrifuge at 4°C. All subsequent operations were performed at this temperature.

Candida utilis

Stock cultures of T. utilis were maintained on slopes of Saboraud's agar at 20°C. At about 2-monthly intervals subcultures were made, incubated at 30°C for 48 h and then stored at 20°C. To prepare an inoculum for initiating a continuous culture, a recent slope culture was recultured on a plate of Saboraud's agar at 30°C for 48h. A suitable colony was then removed with a sterile platinum loop and used to inoculate 100 ml of glycerol medium with or without added $FeCl_3$, as appropriate.

Tetrahymena pyriformis strain ST

Tetrahymena pyriformis strain ST was used throughout because, unlike strains T, w and GL, it stores little glycogen and isolated mitochondria did not show the instability of oxidative and phosphorylating activities previous reported for other strains. The organism was maintained in 75 ml. growth medium (250 ml. flasks) at room temperature and was transferred every 10 days to fresh medium. The growth medium contained 2 % (w/v) protease peptone , 0.1% (w/v) liver digest and 0.05 %(w/v) silicone MS antifoam RD. The first two components were dissolved in a quarter of the final volume of distilled water, heated to 1200 for 15 min., cooled and centrifuged at 200g for 20 min. to remove suspended solids. The supernatant fluid was decanted, diluted by adding three volumes of distilled water, the antifoam added and the pH adjusted to 7.2 with KOH. The medium was then sterilized by autoclaving at 15 lb/in^2 for 20 min. Growth of the organism. Cultures were grown at 29° under forced aeration through a glass tube without a sparger. There was approximately a 5 h. lag phase on inoculation of fresh growth medium with exponentially growing cells (1 day old), and this was followed by a phase of logarithmic growth to give a population of 70,000 to 80,000 cells/ml. at a rate of 0.44 h/l: the growth rate then became slower and the population finally reached 3 to 5 x 10^5 cells/ml.

Acanthamoeba castellanii

Cysts produced by the replacement technique were stored as suspensions in distilled water at 4°. The cysts were collected by centrifuging at 2000 g for 10 min. at laboratory temperature and were washed three times with sterile distilled water. Experiments were normally carried out in 50 ml. or 250 ml. conical flasks containing 10 ml. and 50 ml. of culture, respectively. The flasks were incubated in a reciprocal shaking water bath at a shaking rate of 100 cycles/min. at 30°. Oxygen uptake was measured on a Gilson differential respirometer. Respirometer flasks contained 5 mg. dry wt cysts in 2.8 ml. 4 % (w/v) mycological peptone. The gas phase was air, and CO_2 was adsorbed by 0.2 ml.

20% (w/v) KOH. Flasks were shaken at 150 cycles/min. at 30°. Acid phosphatase activity was assayed. Turbidimetric measurements were carried out on a Unicam SP-600 spectrophotometer.

Crithidia fasciculata

The growth medium contained 2 % (w/v) proteose peptone, 0.1 % liver digest, 1 % (w/v) glycerol, 0.5 % Tween 80 and 0.6 % triethanolamine. Half the triethanolamine and a11 the other components were dissolved in a sixth of the final volume of distilled water, heated to 100 °C, and centrifuged at 2000 g for 15 min to remove suspended solids. The supernatant was decanted and 25 mg haemin/l dissolved in the rest of the triethanolamine (made up as a 50 %, v/v, solution) were added. Folk acid dissolved in the minimum volume of 1.0 M-KOH was added to give 2-5 mg/l. The medium was adjusted to pH 8.0 to 8-2 with 2.0 M-HCI, made up to the final volume with distilled water and autoclaved (103 kNm^{-2} for 20 min) in 200 ml amounts in 1 L conical flasks or in 6 L amounts in a 14 L. The organism was maintained in tubes containing 10 ml of this medium or on slopes of medium containing 2 % agar. The growth of organisms was measured by counting in a haemacytometer slide. Cultures were inoculated with organisms from the late exponential phase of growth; the initial population was 2 x 10^6 organisms/ml. Cultures were grown at 29°C in a rotary orbital shaker for 48 h at 150 rev./min, or in the Fermentor with a stirring rate of 90 rev./min under forced aeration at 1 L- air/l medium/min. Sterile silicone MS antifoam RD (0.05 %) was added to prevent foaming. The population at the stationary phase of growth was 2 to 2.5 x 10^5 organisms/ml; the doubling time in the exponential phase of growth was 5 to 5.5 h. Except where otherwise stated, cultures were harvested in the late exponential phase of growth when the population was 10^5 organisms/ml. Harvesting was by centrifugation at 4 °C for 10 min at 1500 g in the 6 x 250 ml rotor of an MSE centrifuge or at 1500 g in the 6 x 11 rotor of an MSE Mistral centrifuge. Organisms were washed in 20 mM-potassium phosphate buffer (pH 8-0) and finally resuspended to a known density in this buffer.

The major carbon source in the yeast cultures was glucose. Cell numbers. Cells were counted in a Thoma hemocytometer slide or, for T. pyriformis in a Sedgewick-Rafter cell.

Centrifugation

The culture (mid- to a late-exponential phase of growth) was siphoned from the growth vessel through a ‘continuous action rotor’ fitted with a high-efficiency polypropylene insert running in a High Speed 18 centrifuge. This rotor achieves

rate-separation of suspended particles during flow through the main compartment and through the vertical holes in the insert. The maximum flow rate is 2 l/min, and maximum rotor speed 18000 rev./min; suspended particles may be collected until a packed cell volume of 300 ml has accumulated in the rotor. Suitable flow rates (in the range 300 to 2000 ml/min) were provided by inclusion of one of a series of calibrated tapered glass tubes in the inlet gravity feed. Accurate control of rotor speed at less than 1800 rev./min was by means of a 'low-speed zonal control' ancillary circuit fitted to the centrifuge. Preliminary experiments established optimum conditions for the retention of about 90 % of the cells of a growing culture; those for a range of eukaryotic micro-organisms are given. Rotor effluent (containing the smallest cells of the culture) was aerated at the temperature of growth and provided the starting material for synchronous growth. The whole procedure could be carried out aseptically by autoclaving the rotor after plugging its ports with cotton wool and using a liquid seal in the rotor lid.

Assessment of synchrony.

The degree of synchrony was assessed by the synchrony index, F, calculated from the equation:

equation synchronous theory where F has a maximum value of 1.00 in a culture exhibiting theoretically perfect synchrony, N is the number of organisms at time t, No the number of organisms at zero time, and g the mean generation time.

In the presentation of results, vertical lines indicate the mid-points of doublings in cell numbers, and Fl and F2 denote the synchrony indices of the first and second doublings in cell numbers, respectively.

Discussion

For each of the first four organisms, cell counts doubled in numbers over a time interval which was short compared with the mean generation time. The achieved synchrony of cell division was satisfactory. The mean generation time of exponentially growing cultures and their duration of the cell cycle is similar for all cultures. For C. fasciculata the degree of synchrony was not good; only 45 % of the organisms underwent division because many of the smallest cells were not viable.

Characteristics

- Changes in temperature do not impact growth.
- Fall in oxygen tension does not impact growth, as the culture was only in the rotor for a few seconds.
- As organisms are not removed from the medium, nutrient status is preserved.

- Establishment of synchronous cultures is achieved within a few minutes.
- This method can be applied to large volumes; for large cultures.
- Rapid flow rates (up to 2 l/min) are necessary to reduce the total time of collection to a minimum, especially for organisms with short cell-cycle times. The above-described method was successfully applied to a culture volume of 20 liters.
- Also, this procedure can be carried out under strictly aseptic conditions. This technical characteristic can be used in the separation of slow-growing organisms in complex growth media.
- The growth can be carried out uncontaminated for long periods having an upper limit of 24 h.
- A Lowering the shear stress as low as 60 Nm-2 have been achieved to disrupt amoebae exposed to mechanical stress for periods of the order of 1 ms. This property makes this procedure useful for an easily disrupted organism as A . castellanii. This suggests the liquid shearing forces generated are not sufficient to damage even the most fragile cell-types.
- This procedure can be used with any centrifuges given they are fitted with a variable speed-control rheostat.
- Increasing the path length of a continuous flow zonal rotor does not improve the synchrony indices. Also, the problem of contamination increases with long path length.
- Size variation of the cells of the same stage of the cycle limits the maximum useful size resolution.
- The application of this method, to any non-filamentous, non-aggregating organism, is not restricted to size, shape or motility given smallest cells of the culture is viable.
- This procedure is even applied to mammalian cells in tissue culture.
- A maximum rotor speed (18000 rev./min) is adequate for the size-selection of bacteria.
- Limitation of this method is, defined in terms of cell density, sufficient limiting nutrient must remain to support 10 % of the population through one further cycle.
- The election must be carried out before the original culture attains a population one generation before the stationary phase of growth.

Applications

Physical Methods For Obtaining Synchronous Culture

According to the organism employed methods used for synchronous culture techniques purpose differ. A rhythmic growth is achieved in an early phase of growth resulting from inoculation of aged cells of Protozoa into a fresh medium. The same characteristic is shown in case of bacteria and for yeast. Cycling temperature in the culture has also resulted in the synchronous growth of bacteria and of Protozoa. Also, synchronization of cell division is induced by proper regulation of the growth medium using wild-type and a deficient Escherichia coli strain B. The achievement of synchronous growth is obtained by intermittent illumination in case of an autotrophic alga, Chlorella.

The above-mentioned methods are based on the physiological conditioning of the microbial cells, This can lead to the possibility of synchronous cells obtained may have some abnormality in their physiological pattern. Fractional sedimentation and fractional filtration were tested for the separation of larger (mature) and smaller (immature) cells present in a logarithmic phase culture of E. coli are the methods tested to counter the physiological abnormalities formation. Methodology and inference of this procedure is discussed in this application part.

Materials & Methods

E.coli strain B was cultured in a medium containing NH4Cl, 1 g; Na2HPO4 12H20, 18 g; KH2PO4, 1 g; MgSO4.H20, 0.2 g; glucose, 1 g; and 0.01 g of “tween 80” in 1 L of distilled water adjusted to pH 7.4. Cells grown in this medium were inoculated into a fresh medium and when the number reached 108 cells per ml at 37 C, the culture was centrifuged at 4,000 rpm for 12 min. The sedimented cells were used for the experiments to be described. Viable counts were carried out by the capillary tube method. The standard error of this method was 11 per cent under the present experimental conditions.

Factors that Influence Bacterial Growth

Physical requirements

a. Temperature

Bacteria have a minimum, optimum, and maximum temperature for growth and can be divided into 3 groups based on their optimum growth temperature:

1. Psychrophiles are cold-loving bacteria. Their optimum growth temperature is between -5C and 15C. They are usually found in the Arctic and Antarctic regions and in streams fed by glaciers.

2. Mesophiles are bacteria that grow best at moderate temperatures. Their optimum growth temperature is between 25C and 45C. Most bacteria are mesophilic and include common soil bacteria and bacteria that live in and on the body.

3. Thermophiles are heat-loving bacteria. Their optimum growth temperature is between 45C and 70C and are commonly found in hot springs and in compost heaps.

4. Hyperthermophiles are bacteria that grow at very high temperatures. Their optimum growth temperature is between 70C and 110C. They are usually members of the Archaea and are found growing near hydrothermal vents at great depths in the ocean.

b. Oxygen requirements

Bacteria show a great deal of variation in their requirements for gaseous oxygen. Most can be placed in one of the following groups:

1. Obligate aerobes are organisms that grow only in the presence of oxygen. They obtain their energy through aerobic respiration .

2. Microaerophils are organisms that require a low concentration of oxygen (2% to 10%) for growth, but higher concentrations are inhibitory. They obtain their energy through aerobic respiration .

3. Obligate anaerobes are organisms that grow only in the absence of oxygen and, in fact, are often inhibited or killed by its presence. They obtain their energy through anaerobic respiration or fermentation .

4. Aerotolerant anaerobes , like obligate anaerobes, cannot use oxygen to transform energy but can grow in its presence. They obtain energy only by fermentation and are known as obligate fermenters.

5. Facultative anaerobes are organisms that grow with or without oxygen, but generally better with oxygen. They obtain their energy through aerobic

respiration if oxygen is present, but use fermentation or anaerobic respiration if it is absent. Most bacteria are facultative anaerobes.

c. pH

Microorganisms can be placed in one of the following groups based on their optimum pH requirements:

1. Neutrophiles grow best at a pH range of 5 to 8.

2. Acidophiles grow best at a pH below 5.5.

3. Alkaliphiles grow best at a pH above 8.5.

d. Osmosis

Osmosis is the diffusion of water across a membrane from an area of higher water concentration (lower solute concentration) to lower water concentration (higher solute concentration). Osmosis is powered by the potential energy of a concentration gradient and does not require the expenditure of metabolic energy. While water molecules are small enough to pass between the phospholipids in the cytoplasmic membrane, their transport can be enhanced by water transporting transport proteins known as aquaporins . The aquaporins form channels that span the cytoplasmic membrane and transport water in and out of the cytoplasm.

To understand osmosis, one must understand what is meant by a solution . A solution consists of a solute dissolved in a solvent . In terms of osmosis, solute refers to all the molecules or ions dissolved in the water (the solvent). When a solute such as sugar dissolves in water, it forms weak hydrogen bonds with water molecules. While free, unbound water molecules are small enough to pass through membrane pores, water molecules bound to solute are not.Therefore, the higher the solute concentration, the lower the concentration of free water molecules capable of passing through the membrane.

A cell can find itself in one of three environments: isotonic , hypertonic , or hypotonic . (The prefixes iso-, hyper-, and hypo- refer to the solute concentration).

- In an isotonic environment , both the water and solute concentration are the same inside and outside the cell and water goes into and out of the cell at an equal rate.
- If the environment is hypertonic , the water concentration is greater inside the cell while the solute concentration is higher outside (the interior of the cell is hypotonic to the surrounding hypertonic environment). Water goes out of the cell.
- In an environment that is hypotonic , the water concentration is greater outside the cell and the solute concentration is higher inside (the interior of the cell is hypertonic to the hypotonic surroundings). Water goes into the cell.
- Most bacteria require an isotonic environment or a hypotonic environment for optimum growth. Organisms that can grow at relatively high salt concentration (up to 10%) are said to be osmotolerant . Those that require relatively high salt concentrations for growth, like some of the Archaea that require sodium chloride concentrations of 20 % or higher halophiles .
- Nutritional requirements
- In addition to a proper physical environment, microorganisms also depend on an available source of chemical nutrients. Microorganisms are often grouped according to their energy source and their source of carbon.
- ***a. Energy source***
- 1. Phototrophs use radiant energy (light) as their primary energy source.
- 2. Chemotrophs use the oxidation and reduction of chemical compounds as their primary energy source.
- ***b. Carbon source***
- Carbon is the structural backbone of the organic compounds that make up a living cell. Based on their source of carbon bacteria can be classified as autotrophs or heterotrophs.
- 1. Autotrophs : require only carbon dioxide as a carbon source. An autotroph can synthesize organic molecules from inorganic nutrients.
- 2. Heterotrophs : require organic forms of carbon. A Heterotroph cannot synthesize organic molecules from inorganic nutrients.
- Combining their nutritional patterns, all organisms in nature can be placed into one of four separate groups: photoautotrophs, photoheterotrophs, chemoautotrophs, and chemoheterotrophs.

- 1. Photoautotrophs use light as an energy source and carbon dioxide as their main carbon source. They include photosynthetic bacteria (green sulfur bacteria, purple sulfur bacteria, and cyanobacteria), algae, and green plants. Photoautotrophs transform carbon dioxide and water into carbohydrates and oxygen gas through photosynthesis .
- Cyanobacteria, as well as algae and green plants, use hydrogen atoms from water to reduce carbon dioxide to form carbohydrates, and during this process oxygen gas is given off (an oxygenic process). Other photosynthetic bacteria (the green sulfur bacteria and purple sulfur bacteria) carry out an anoxygenic process, using sulfur, sulfur compounds or hydrogen gas to reduce carbon dioxide and form organic compounds.
- 2. Photoheterotrophs use light as an energy source but cannot convert carbon dioxide into energy. Instead they use organic compounds as a carbon source. They include the green nonsulfur bacteria and the purple nonsulfur bacteria.
- 3. Chemolithoautotrophs use inorganic compounds such as hydrogen sulfide, sulfur, ammonia, nitrites, hydrogen gas, or iron as an energy source and carbon dioxide as their main carbon source.
- 4. Chemooganoheterotrophs use organic compounds as both an energy source and a carbon source. Saprophytes live on dead organic matter while parasites get their nutrients from a living host. Most bacteria, and all protozoans, fungi, and animals are chemoorganoheterotrophs.
- ***c. Nitrogen source***
- Nitrogen is needed for the synthesis of such molecules as amino acids, DNA, RNA and ATP . Depending on the organism, nitrogen, nitrates, ammonia, or organic nitrogen compounds may be used as a nitrogen source.
- ***d. Minerals***
- 1. Sulfur
- Sulfur is needed to synthesizes sulfur-containing amino acids and certain vitamins. Depending on the organism, sulfates, hydrogen sulfide, or sulfur-containing amino acids may be used as a sulfur source.
- 2. Phosphorus
- Phosphorus is needed to synthesize phospholipids , DNA, RNA, and ATP . Phosphate ions are the primary source of phosphorus.
- 3. Potassium, magnesium, and calcium

- These are required for certain enzymes to function as well as additional functions.
- 4. Iron
- Iron is a part of certain enzymes.
- 5. Trace elements
- Trace elements are elements required in very minute amounts, and like potassium, magnesium, calcium, and iron, they usually function as cofactors in enzyme reactions. They include sodium, zinc, copper,molybdenum, manganese, and cobalt ions. Cofactors usually function as electron donors or electron acceptors during enzyme reactions.
- ***e. Water***
- ***f. Growth factors***
- Growth factors are organic compounds such as amino acids , purines , pyrimidines , and vitamins that a cell must have for growth but cannot synthesize itself. Organisms having complex nutritional requirements and needing many growth factors are said to be fastidious .

Factor affecting bacterial growth

- Growth of bacteria is affected by many factors such as nutrition concentration and other environmental factors.

Some of the important factors affecting bacterial growth are:

1. Nutrition concentration
2. Temperature
3. Gaseous concentration
4. pH
5. Ions and salt concentration
6. Available water

1. Nutrient concentration:

- If culture media is rich in growth promoting substance, growth of bacteria occurs faster. Decrease in nutrient concentration decreases the growth rate.
- Different bacteria have different nutritional requirement.

The relationship between substrate concentration (nutrition) and growth rate is shown in figure.

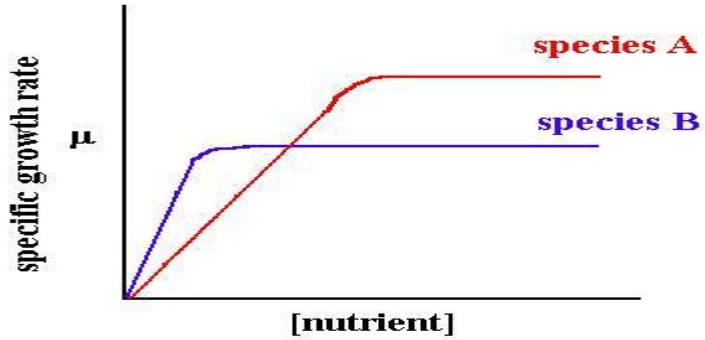


figure: nutrient vs growth rate

- With increase in concentration nutrition, growth rate of bacteria increases up to certain level and then growth rate remains constant irrespective of nutrition addition.

2. Temperature:

- Temperature affects the growth of bacteria by various ways.
- The lowest temperature that allows the growth is called minimum temperature and the highest temperature that allows growth is called maximum temperature.
- There is no growth below minimum and above maximum temperature.
- Below minimum temperature cell membrane solidifies and become stiff to transport nutrients in to the cell, hence no growth occurs.
- Above maximum temperature, cellular proteins and enzymes denatures, so the bacterial growth ceases.

The relationship between temperature and growth rate is shown in figure below.

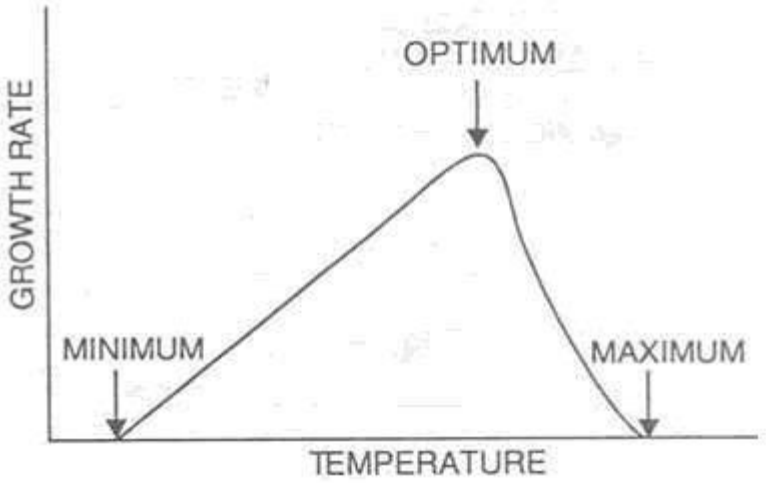


figure: temperature vs growth rate

- When temperature is increases continuously from its minimum, growth rate of bacteria increases because the rate of metabolic reaction increases with increase in temperature.
- At certain temperature the growth rate become maximum, this temperature is known as optimal temperature.
- On further increasing the temperature above optimal, growth rate decreases abruptly and completely ceases with reaching maximum temperature.

3. pH:

- pH affects the ionic properties of bacterial cell so it affects the growth of bacteria.
- Most of the bacteria grow at neutral pH (60.5-7.5). However there are certain bacteria that grow best at acidic or basic pH.
- relationship between pH and bacterial growth is given in figure below.

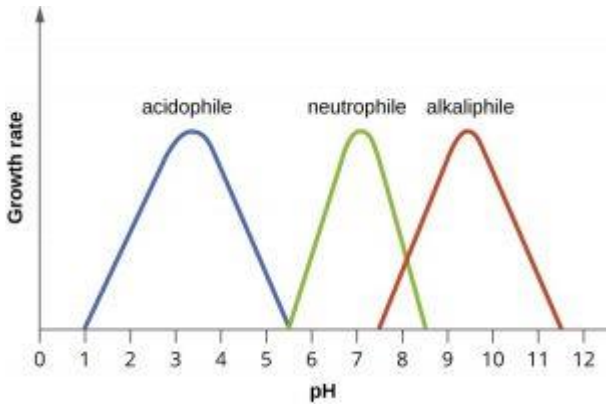


figure: pH vs growth rate

4. Ions and salt:

- All bacteria requires metal ions such as K+, Ca ++, Mg++, Fe++, Zn++, Cu++,Mn++ etc to synthesize enzymes and proteins.
- Most bacteria do not require NaCl in media however they can tolerate very low concentration of salt.
- There is some halophilic bacteria such as *Archeobacteria* that require high concentration of salt in media.

5. Gaseous requirement:

- Oxygen and carbon-dioxide are important gases that affects the growth of bacteria.
- Oxygen is required for aerobic respiration and obligate aerobic bacteria must require O2 for growth. Eg. *Mycobacterium, Bacillus*
- For obligate anaerobes Oxygen is harmful or sometime lethal. However facultative anaerobes can tolerate low concentration of O2.
- Carbon-dioxide is needed for capnophilic bacteria. Such as *Campylobacter*, *Helicobacter pylori*

6. Available water:

- Water is the most essential factor for bacterial growth.
- Available water in the culture media determines the rate of metabolic and physiological activities of bacteria.
- Sugar, salts and other substances are dissolved in water and are made available for bacteria.

Thermophile

Thermophiles produce some of the bright colors of Grand Prismatic Spring, Yellowstone National Park

A **thermophile** is an organism—a type of extremophile—that thrives at relatively high temperatures, between 41 and 122 °C (106 and 252 °F). Many thermophiles are archaea. Thermophilic eubacteria are suggested to have been among the earliest bacteria.

Thermophiles are found in various geothermally heated regions of the Earth, such as hot springs like those in Yellowstone National Park (see image) and deep seahydrothermal vents, as well as decaying plant matter, such as peat bogs and compost.

Thermophiles can survive at high temperatures, whereas other bacteria would be damaged and sometimes killed if exposed to the same temperatures.

The enzymes in thermophiles function at high temperatures. Some of these enzymes are used in molecular biology, for example the taq polymerase used in PCR. "Thermophile" is derived from the Greek: θερμότητα (*thermotita*), meaning heat, and Greek: φίλια (*philia*), love.

Classification

Thermophiles can be classified in various ways. One classification sorts these organisms according to their optimal growth temperatures:

1. Simply thermophiles: 50–64 °C
2. Extreme thermophiles 65–79 °C
3. Hyperthermophiles 80 °C and beyond, but not < 50 °C.

In a related classification, thermophiles are sorted as follows:

1. Facultative thermophiles (also called moderate thermophiles) can thrive at high temperatures, but also at lower temperatures (below 50 °C (122 °F)), whereas
2. Obligate thermophiles (also called extreme thermophiles) require such high temperatures for growth.
3. Hyperthermophiles are particularly extreme thermophiles for which the optimal temperatures are above 80 °C (176 °F).

A colony of thermophiles in the outflow of Mickey Hot Springs, Oregon, the water temperature is approximately 60 °C (140 °F).

Many of the hyperthermophilic Archaea require elemental sulfur for growth. Some are anaerobesthat use the sulfur instead of oxygen as an electron acceptor during cellular respiration. Some are lithotrophs that oxidize sulphur to create sulfuric acid as an energy source, thus requiring the microorganism to be adapted to very low pH (i.e., it is an acidophile as well as thermophile). These organisms are inhabitants of hot, sulfur-rich environments usually associated with volcanism, such as hot springs, geysers, and fumaroles. In these places, especially in Yellowstone National Park, zonation of microorganisms according to their temperature optima occurs. Often, these organisms are colored, due to the presence of photosynthetic pigments.

Thermophile versus mesophile

Thermophiles can be discriminated from mesophiles from genomic features. For example, the GC-content levels in the coding regions of some signatures genes were consistently identified as correlated with the temperature range condition when the association analysis was applied to mesophilic and thermophilic organisms regardless of their phylogeny, oxygen requirement, salinity, or habitat conditions**.**

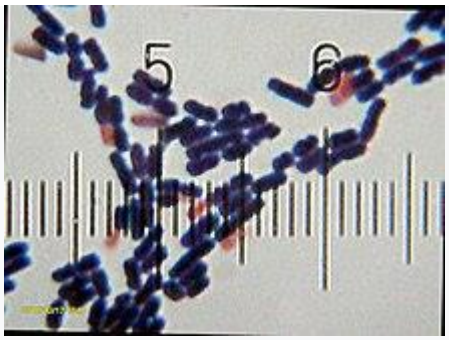
Gene transfer and genetic exchange

Sulfolobus solfataricus and *Sulfolobus acidocaldarius* are hyperthermophilic archaea. When these organisms are exposed to the DNA damaging agents UV irradiation, bleomycin or mitomycin C, species-specific cellular aggregation is induced.[6][7] In *S. acidocaldarius*, UV-induced cellular aggregation mediates chromosomal marker exchange with high frequency. Recombination rates exceed those of uninduced cultures by up to three orders of magnitude. Frols et al.[6][8] and Ajon et al.[7](2011) hypothesized that cellular aggregation enhances species-specific DNA transfer between *Sulfolobus* cells in order to provide

increased repair of damaged DNA by means of homologous recombination. Van Wolferen et al., in discussing DNA exchange in the hyperthermophiles under extreme conditions, noted that DNA exchange likely plays a role in repair of DNA via homologous recombination. They suggested that this process is crucial under DNA damaging conditions such as high temperature. Also it has been suggested that DNA transfer in *Sulfolobus* may be a primitive form of sexual interaction similar to the more well-studied bacterial transformation systems that are associated with species-specific DNA transfer between cells leading to homologous recombinational repair of DNA damage [see Transformation (genetics)].

Alkaliphile

Alkaliphiles are a class of extremophilic microbes capable of survival in alkaline (pH roughly 8.5–11) environments, growing optimally around a pH of 10. These bacteria can be further categorized as obligate alkaliphiles (those that require high pH to survive), facultative alkaliphiles (those able to survive in high pH, but also grow under normal conditions) and haloalkaliphiles (those that require high salt content to survive).



A typical bacillus culture. Many alkaliphiles possess a bacillus morphology.

Background information

Microbial growth in alkaline conditions presents several complications to normal biochemical activity and reproduction, as high pH is detrimental to normal cellular processes. For example, alkalinity can lead to denaturation of DNA, instability of the plasma membrane and inactivation of cytosolic enzymes, as well as other unfavorable physiological changes.

Thus, to adequately circumvent these obstacles, alkaliphiles must either possess specific cellular machinery that works best in the alkaline range, or they must have methods of acidifying the cytosol in relation to the extracellular

environment. To determine which of the above possibilities an alkaliphile uses, experimentation has demonstrated that alkaliphilic enzymes possess relatively normal pH optimums. The determination that these enzymes function most efficiently near physiologically neutral pH ranges (about 7.5–8.5) was one of the primary steps in elucidating how alkaliphiles survive intensely basic environments. Since the cytosolic pH must remain nearly neutral, alkaliphiles must have one or more mechanisms of acidifying the cytosol when in the presence of a highly alkaline environment.

Mechanisms of cytosolic acidification

Alkaliphiles maintain cytosolic acidification through both passive and active means. In passive acidification, it has been proposed that cell walls contain acidic polymers composed of residues such as galacturonic acid, gluconic acid, glutamic acid, aspartic acid, and phosphoric acid. Together, these residues form an acidic matrix that helps protect the plasma membrane from alkaline conditions by preventing the entry of hydroxide ions, and allowing for the uptake of sodium and hydronium ions. In addition, the peptidoglycan in alkaliphilic *B. subtilis*has been observed to contain higher levels of hexosamines and amino acids as compared to its neutrophilic counterpart. When alkaliphiles lose these acidic residues in the form of induced mutations, it has been shown that their ability to grow in alkaline conditions is severely hindered. However, it is generally agreed upon that passive methods of cytosolic acidification are not sufficient to maintain an internal pH 2-2.3 levels below that of external pH; there must also be active forms of acidification. The most characterized method of active acidification is in the form of Na+/H+ antiporters. In this model, H+ ions are first extruded through the electron transport chain in respiring cells and to some extent through an ATPase in fermentative cells. This proton extrusion establishes a proton gradient that drives electrogenic antiporters—which drive intracellular Na+ out of the cell in exchange for a greater number of H+ ions, leading to the net accumulation of internal protons. This proton accumulation leads to a lowering of cytosolic pH. The extruded Na+ can be used for solute symport, which are necessary for cellular processes. It has been noted that Na+/H+ antiport is required for alkaliphilic growth, whereas either K+/H+ antiporters or Na+/H+ antiporters can be utilized by neutrophilic bacteria. If Na+/H+ antiporters are disabled through mutation or another means, the bacteria are rendered neutrophilic. The sodium required for this antiport system is the reason some alkaliphiles can only grow in saline environments.

Differences in alkaliphilic ATP production

In addition to the method of proton extrusion discussed above, it is believed that the general method of cellular respiration is different in obligate alkaliphiles as compared to neutrophiles. Generally, ATP production operates by establishing a proton gradient (greater H+ concentration outside the membrane) and a transmembrane electrical potential (with a positive charge outside the membrane). However, since alkaliphiles have a reversed pH gradient, it would seem that ATP production—which is based on a strong proton motive force—would be severely reduced. However, the opposite is true. It has been proposed that while the pH gradient has been reversed, the transmembrane electrical potential is greatly increased. This increase in charge causes the production of greater amounts of ATP by each translocated proton when driven through an ATPase.[2][4] Research in this area is ongoing.

Applications and future research

Alkaliphiles promise several interesting uses for biotechnology and future research. Alkaliphilic methods of regulating pH and producing ATP are of interest in the scientific community. However, perhaps the greatest area of interest from alkaliphiles lies in their enzymes: alkaline proteases; starch-degrading enzymes; cellulases; lipases; xylanases; pectinases; chitinases and their metabolites, including: 2-phenylamine; carotenoids; siderophores; cholic acid derivatives and organic acids. It is hoped that further research into alkaliphilic enzymes will allow scientists to harvest alkaliphiles' enzymes for use in basic conditions. Research aimed at discovering alkaliphile-produced antibiotics showed some success, yet has been held at bay by the fact that some products produced at high pH are unstable and unusable at a physiological pH range.[1]

Examples

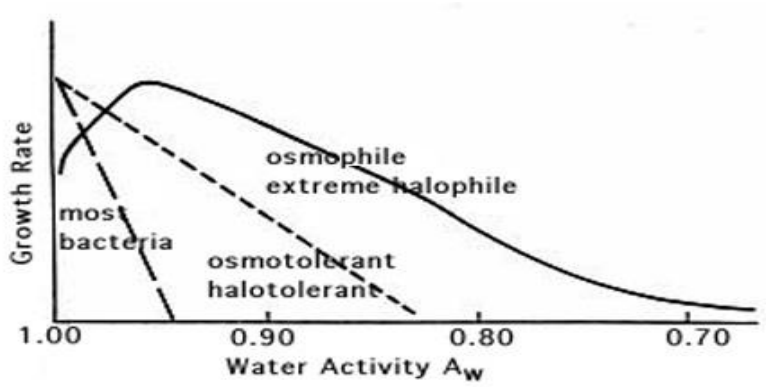
Examples of alkaliphiles include *Halorhodospira halochloris*, *Natronomonas pharaonis*, and *Thiohalospira alkaliphila*.[

Osmophile

Osmophilic organisms are microorganisms adapted to environments with high osmotic pressures, such as high sugar concentrations. Osmophiles are similar to halophilic (salt-loving) organisms because a critical aspect of both types of environment is their low water activity, a_W. High sugar concentrations represent a growth-limiting factor for many microorganisms, yet osmophiles protect themselves against this high osmotic pressure by the synthesis of osmoprotectants such as alcohols and amino acids. Many osmophilic microorganisms are yeasts; a variety of bacteria are also osmophilic.

Osmophile yeasts are important because they cause spoilage in the sugar and sweet goods industry, with products such as fruit juices, fruit juice concentrates, liquid sugars (such as golden syrup), honey and in some cases marzipan. Among the most osmophillic are:

Organism	**Minimum a_W**
Saccharomyces rouxii	0.62
Saccharomyces bailii	0.80
Debaryomyces	0.83
Wallemia sebi	0.87
Saccharomyces cerevisiae	0.90



Psychrophile

Psychrophiles or **cryophiles** (adj. psychrophilic or cryophilic) are extremophilic organisms that are capable of growth and reproductionin low temperatures, ranging from −20 °C to +10 °C. They are found in places that are permanently cold, such as the polar regions and the deep sea. They can be contrasted with thermophiles, which are organisms that thrive at unusually high temperatures. Psychrophile is Greek for 'cold-loving'.

Many such organisms are bacteria or archaea, but some eukaryotes such as lichens, snow algae, fungi, and wingless midges, are also classified as psychrophiles.

Biology

The lichen *Xanthoria elegans* can continue to photosynthesize at −24 °C.

Habitat

The cold environments that psychrophiles inhabit are ubiquitous on Earth, as a large fraction of our planetary surface experiences temperatures lower than 15 °C. They are present in permafrost, polar ice, glaciers, snowfields and deep ocean waters. These organisms can also be found in pockets of sea ice with high salinity content. Microbial activity has been measured in soils frozen below −39 °C. In addition to their temperature limit, psychrophiles must also adapt to other extreme environmental constraints that may arise as a result of their

habitat. These constraints include high pressure in the deep sea, and high salt concentration on some sea ice.

Adaptations

Psychrophiles are protected from freezing and the expansion of ice by ice-induced desiccation and vitrification (glass transition), as long as they cool slowly. Free living cells desiccate and vitrify between −10 °C and −26 °C. Cells of multicellular organisms may vitrify at temperatures below −50 °C. The cells may continue to have some metabolic activity in the extracellular fluid down to these temperatures, and they remain viable once restored to normal temperatures.

They must also overcome the stiffening of their lipid cell membrane, as this is important for the survival and functionality of these organisms. To accomplish this, psychrophiles adapt lipid membrane structures that have a high content of short, unsaturated fatty acids. Compared to longer saturated fatty acids, incorporating this type of fatty acid allows for the lipid cell membrane to have a lower melting point, which increases the fluidity of the membranes.[6] In addition, carotenoids are present in the membrane, which help modulate the fluidity of it.

Antifreeze proteins are also synthesized to keep psychrophiles' internal space liquid, and to protect their DNA when temperatures drop below water's freezing point. By doing so, the protein prevents any ice formation or recrystallization process from occurring.

The enzymes of these organisms have been hypothesized to engage in an activity-stability-flexibility relationship as a method for adapting to the cold; the flexibility of their enzyme structure will increase as a way to compensate for the freezing effect of their environment.

Certain cryophiles, such as Gram-negative bacteria *Vibrio* and *Aeromonas* spp., can transition into a viable but nonculturable (VBNC)state.[8] During VBNC, a micro-organism can respirate and use substrates for metabolism – however, it cannot replicate. An advantage of this state is that it is highly reversible. It has been debated whether VBNC is an active survival strategy or if eventually the organism's cells will no longer be able to be revived. There is proof however it may be very effective – Gram positive bacteria Actinobacteria have been shown to have lived about 500,000 years in the permafrost conditions of Antarctica, Canada, and Siberia.

Taxonomic range

The wingless midge (Chironomidae) *Belgica antarctica*.

Psychrophiles include bacteria, lichens, fungi, and insects. Among the bacteria that can tolerate extreme cold are *Arthrobacter* sp., *Psychrobacter* sp. And members of the genera *Halomonas*, *Pseudomonas*, *Hyphomonas*, and *Sphingomonas*. Another example is *Chryseobacterium greenlandensis*, a psychrophile that was found in 120,000-year-old ice.

Umbilicaria antarctica and *Xanthoria elegans* are lichens that have been recorded photosynthesizing at temperatures ranging down to −24 °C, and they can grow down to around −10 °C. Some multicellular eukaryotes can also be metabolically active at sub-zero temperatures, such as some conifers;[13] those in the *Chironomidae* family are still active at −16 °C.

Penicillium is a genus of fungi found in a wide range of environments including extreme cold.

Among the psychrophile insects, the Grylloblattidae or icebugs, found on mountaintops, have optimal temperatures between 1-4 °C. The wingless midge (Chironomidae) *Belgica antarctica* can tolerate salt, being frozen and strong ultraviolet, and has the smallest known genome of any insect. The small genome, of 99 million base pairs, is thought to be adaptive to extreme environments.

Psychrotrophic bacteria

Psychrotrophic microbes are able to grow at temperatures below 7 °C (44.6 °F), but have better growth rates at higher temperatures. Psychrotrophic bacteria and fungi are able to grow at refrigeration temperatures, and can be responsible for

food spoilage. They provide an estimation of the product's shelf life, but also they can be found in soils, in surface and deep sea waters, in Antarctic ecosystems, and in foods.

Psychrotrophic bacteria are of particular concern to the dairy industry. Most are killed by pasteurization; however, they can be present in milk as post-pasteurization contaminants due to less than adequate sanitation practices. According to the Food Science Department at Cornell University, psychrotrophs are bacteria capable of growth at temperatures at or less than 7 °C (44.6 °F). At freezing temperatures, growth of psychrotrophic bacteria becomes negligible or virtually stops.

All three subunits of the RecBCD enzyme are essential for physiological activities of the enzyme in the Antarctic *Pseudomonas syringae*, namely, repairing of DNA damage and supporting the growth at low temperature. The RecBCD enzymes are exchangeable between the psychrophilic *P. syringae* and the mesophilic *E. coli* when provided with the entire protein complex from same species. However, the RecBC proteins (RecBCPs and RecBCEc) of the two bacteria are not equivalent; the RecBCEc is proficient in DNA recombination and repair, and supports the growth of *P. syringae* at low temperature, while RecBCPs is insufficient for these functions. Finally, both helicase and nuclease activity of the RecBCDPs are although important for DNA repair and growth of *P. syringae* at low temperature, the RecB-nuclease activity is not essential in vivo.

Versus psychrotroph

In 1940, ZoBell and Conn stated that they had never encountered "true psychrophiles" or organisms that grow best at relatively low temperatures. In 1958, J. L. Ingraham supported this by concluding that there are very few or possibly no bacteria that fit the textbook definitions of psychrophiles. Richard Y. Morita emphasizes this by using the term *psychrotroph* to describe organisms that do not meet the definition of psychrophiles. The confusion between the terms *psychrotrophs* and *psychrophiles* was started because investigators were unaware of the thermolability of psychrophilic organisms at the laboratory temperatures. Due to this, early investigators did not determine the cardinal temperatures for their isolates.

The similarity between these two is that they are both capable of growing at zero, but optimum and upper temperature limits for the growth are lower for psychrophiles compared to psychrotrophs. Psychrophiles are also more often isolated from permanently cold habitats compared to psychrotrophs. Although psychrophilic enzymes remain under-used because the cost of production and processing at low temperatures is higher than for the commercial enzymes that are presently in use, the attention and resurgence of research interest in psychrophiles and psychrotrophs will be a contributor to the betterment of the environment and the desire to conserve energy.

UNIT 3

Autotrophs are organisms that can produce their own food from the substances available in their surroundings using light (photosynthesis) or chemical energy (chemosynthesis).

Heterotrophs cannot synthesize their own food and rely on other organisms — both plants and animals — for nutrition. Technically, the definition is that autotrophs obtain carbon from inorganic sources like carbon dioxide (CO_2) while heterotrophs get their reduced carbon from other organisms. Autotrophs are usually plants; they are also called "self feeders" or "primary producers".

Comparisson chart

Autotroph versus Heterotroph comparison chart		
	Autotroph	**Heterotroph**
Produce own food	Yes	No
Food chain level	Primary	Secondary and tertiary
Types	Photoautotroph, Chemoautotroph	Photoheterotroph, Chemoheterotroph
Examples	Plants, algae, and some bacteria	Herbivores, omnivores, and carnivores
Definition	An organism that is able to form nutritional organic substances from simple inorganic substances such as carbon dioxide.	Heterotrophs cannot produce organic compounds from inorganic sources and therefore rely on consuming other organisms in the food chain.

Autotroph versus Heterotroph comparison chart		
	Autotroph	**Heterotroph**
What or How they eat ?	Produce their own food for energy.	They eat other organisms to get proteins and energy.

Monotropastrum humile, a myco-heterotroph dependent on fungi throughout its lifetime

Energy Production

Autotrophs produce their own energy by one of the following two methods:

- Photosynthesis - Photoautotrophs use energy from sun to convert water from the soil and carbon dioxide from the air into glucose. Glucose provides energy to plants and is used to make cellulose which is used to build cell walls. E.g. Plants, algae, phytoplankton and some bacteria. Carnivorous plants like pitcher plant use photosynthesis for energy production but depend on other organisms for other nutrients like nitrogen, potassium and phosphorous. Hence, these plants are basically autotrophs.

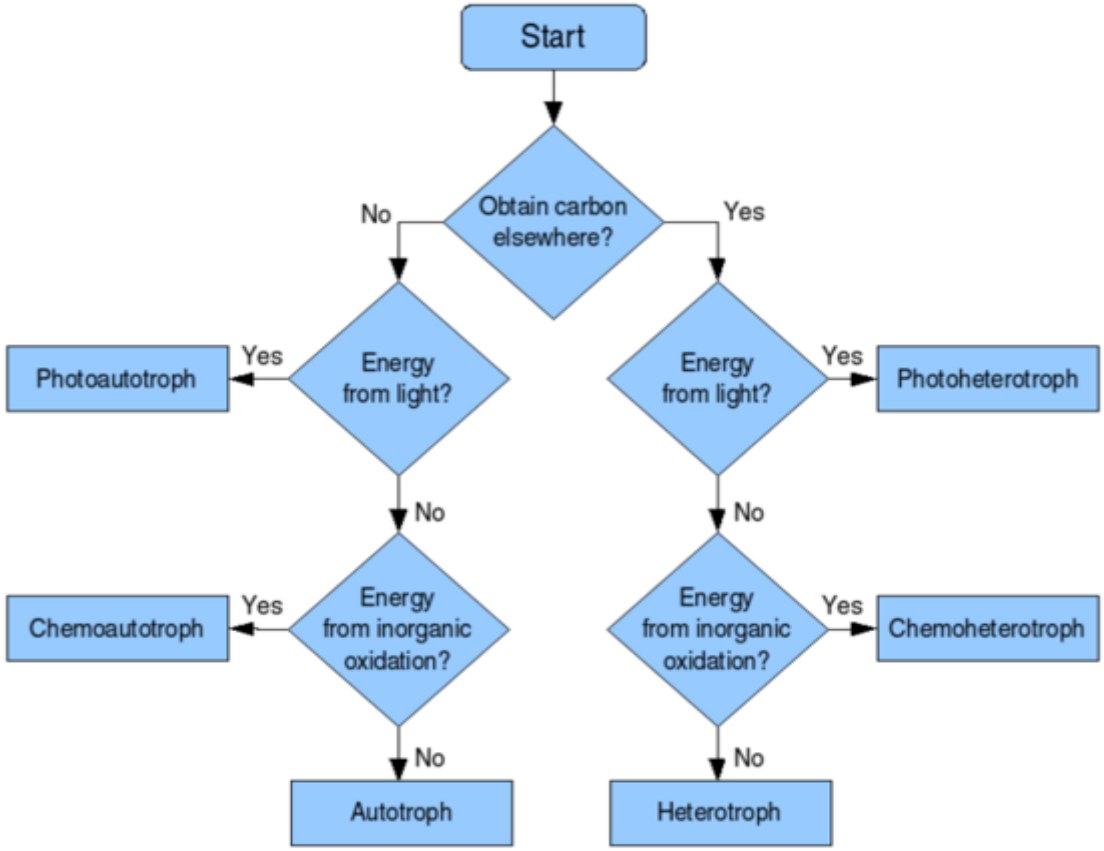
- Chemosynthesis - Chemoautotrophs use energy from chemical reactions to make food. The chemical reactions are usually between hydrogen sulfide/methane with oxygen. Carbon dioxide is the main source of carbon for Chemoautotrophs. E.g. Bacteria found inside active volcano, hydrothermal vents in sea floor, hot watersprings.

Heterotrophs survive by feeding on organic matter produced by or available in other organisms. There are two types of heterotrophs:

- Photoheterotroph – These heterotrophs use light for energy but cannot use carbon dioxide as their carbon source. They get their carbon from compounds such as carbohydrates, fatty acids and alcohol. E.g. purple non-sulfur bacteria, green-non sulfur bacteria and heliobacteria.

- Chemoheterotroph – Heterotrophs that get their energy by oxidation of preformed organic compounds, i.e. by eating other organisms either dead or alive. E.g. animals, fungi, bacteria and almost all pathogens.

Type of organism	**Energy source**	**Carbon source**
Photoautotroph	Light	Carbon dioxide
Chemoautotroph	Chemicals	Carbon dioxide
Photoheterotroph	Light	Carbon from other organisms
Chemoheterotroph	Other organisms	Other organisms

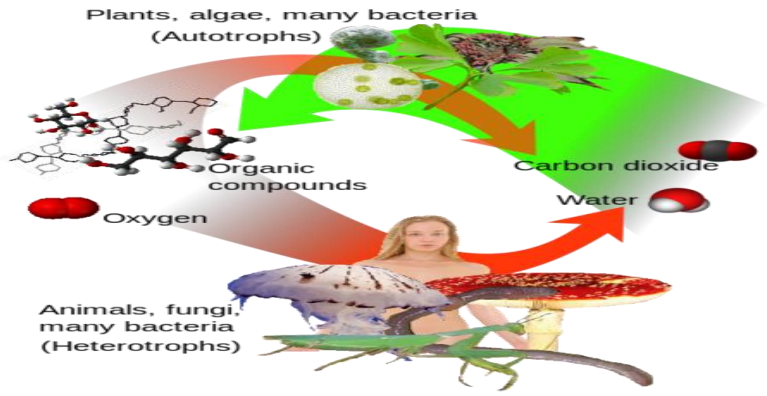


A flowchart explaining the various types of trophs

Food Chain

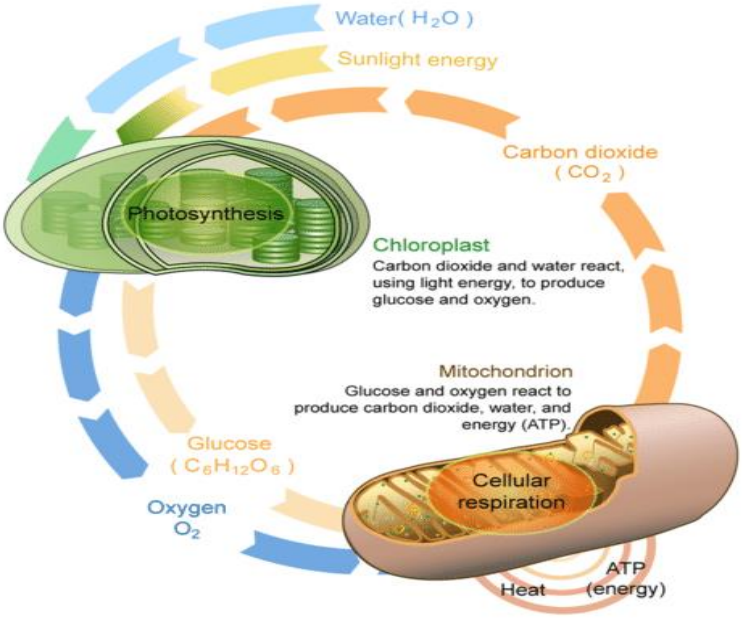
Autotrophs do not depend on other organism for their food. They are the primary producer and are placed first in the food chain. Heterotrophs that depend on autotrophs and other heterotrophs for their energy level are placed next on the food chain.

Herbivores that feed on autotrophs are placed in the second trophic level. Carnivores that eat meat and omnivores that eat all types of organisms are placed next in the trophic level.



Making and Using Food

The flow of energy through living organisms begins with photosynthesis. This process stores energy from sunlight in the chemical bonds of glucose. By breaking the chemical bonds in glucose, cells release the stored energy and make the ATP they need. The process in which glucose is broken down and ATP is made is called **cellular respiration**.



Photosynthesis and cellular respiration are like two sides of the same coin. The products of one process are the reactants of the other. Together, the two processes store and release energy in living organisms. The two processes also work together to recycle oxygen in Earth's atmosphere.

This diagram compares and contrasts photosynthesis and cellular respiration. It also shows how the two processes are related.

Photosynthesis

Photosynthesis is often considered to be the single most important life process on Earth. It changes light energy into chemical energy and also releases oxygen. Without photosynthesis, there would be no oxygen in the atmosphere. Photosynthesis involves many chemical reactions, but they can be summed up in a single chemical equation:

$6CO_2 + 6H_2O + \text{Light Energy} \rightarrow C_6H_{12}O_6 + 6O_2$.

Photosynthetic autotrophs capture light energy from the sun and absorb carbon dioxide and water from their environment. Using the light energy, they combine the reactants to produce glucose and oxygen, which is a waste product. They store the glucose, usually as starch, and they release the oxygen into the atmosphere.

Cellular Respiration

Cellular respiration actually “burns” glucose for energy. However, it doezsn’t produce light or intense heat as some other types of burning do. This is because it releases the energy in glucose slowly, in many small steps. It uses the energy that is released to form molecules of ATP. Cellular respiration involves many chemical reactions, which can be summed up with this chemical equation:

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ + Chemical Energy (in ATP)

Cellular respiration occurs in the cells of all living things. It takes place in the cells of both autotrophs and heterotrophs. All of them burn glucose to form ATP.

Summary

- Autotrophs store chemical energy in carbohydrate food molecules they build themselves. Most autotrophs make their "food" through photosynthesis using the energy of the sun.
- Heterotrophs cannot make their own food, so they must eat or absorb it.
- Chemosynthesis is used to produce food using the chemical energy stored in inorganic molecules.

Heterotrophic bacteria (Heterotrophs):
They are most abundant in nature. They do not synthesize their own food but depend on other organisms or on dead organic matter for food. They may be parasites, saprophytes or symbiontes.

(a) Parasites:
They live on other organisms called the host, from which they obtain food. e.g. Streptococcus, Clostridium, Mycobacterium tuberculosis etc. Disease causing parasites are called pathogens.

(b) Saprophytes:
They are the major decomposers in nature. They obtain their food from detritus i.e. dead organic matters by Fermentation (anaerobic breakdown of carbohydrates), putrefaction (anaerobic breakdown of proteins) and decay (aerobic breakdown of organic compounds).

(c) Symbionts:
They live in symbiotic association with other plants for mutual benefit. A few species of Rhizobium, such as R. leguminosarum, live in the root nodules of leguminous plants forming intimate symbiotic relationship. Bacteria fix atmospheric nitrogen for the plant and in return get protection. Escherichia coli live in human intestine as commensal. It feeds on undigested matter, checks the growth of putrefying bacteria and produces vitamins B and K. The presence of E. coli in water supply indicates contamination by sewage.

Autotrophic Bacteria (Autotrophs):
They synthesize their own organic food from inorganic substances (CO_2 and hydrogen donor) Autotrophic bacteria are of two types:
(i) photoautotrophic (photosynthetic) which make use of light energy and

(ii) chemoautotrophic (chemosynthetic) which utilize chemical energy.

(i) Photoautotrophic bacteria (Photoautotrophs):
They use solar energy for the synthesis of their own food. These bacteria are anaerobic, which could be purple or green. The purple bacteria possess pigment bacteriochlorophyll located in the membranes of thylakoids while green bacteria possess bacteriophaeophytin (chlorobium chlorophyll) located inside small sacs called chlorosomes.

Photoautotrophs carry out anoxygenic photosynthesis in which water is not used as reducing power. Instead sulphur compounds like hydrogen sulphide (H_2S), hydrogen gas (H_2), thiosulphates ($Na_2S_20_3$) or some organic compounds are used to obtain reducing power. Most photoautotrophs live near the bottoms of ponds and lakes where reduced sulphur or other compounds are in plenty and oxygen content is very low.
(ii) Chemoautotrophic bacteria (chemoautotrophs):
They do not have photosynthetic pigment and hence utilize chemical energy to reduce CO_2 to organic food. The chemical energy is obtained from the oxidation

of certain chemicals such as ammonia, nitrites, methane, carbon monoxide, molecular hydrogen, iron salts, sulphur and sulphur compounds (e.g., nitrifying bacteria Nitrosomonas, Nitrobacter, denitrifying bacteria Bacillus denitrificans, sulphur bacteria Thiobacillus thiooxidans, iron bacteria, hydrogen bacteria).

Cyanobacteria

Overview

Also referred to as blue-green algae and blue-green bacteria, **cyanobacteria** is a bacteria phylum that obtain their energy through a process known as photosynthesis. Because they require the basic environmental conditions, this bacteria can be found in a variety of environments ranging from marine to terrestrial habitats.

Cyanobacteria is also composed of a wide variety of bacteria species of different shapes are sizes that can be found in different habitats in the environment. These are spread across the 150 genera that have been identified so far and play various important roles in nature.

Examples include:

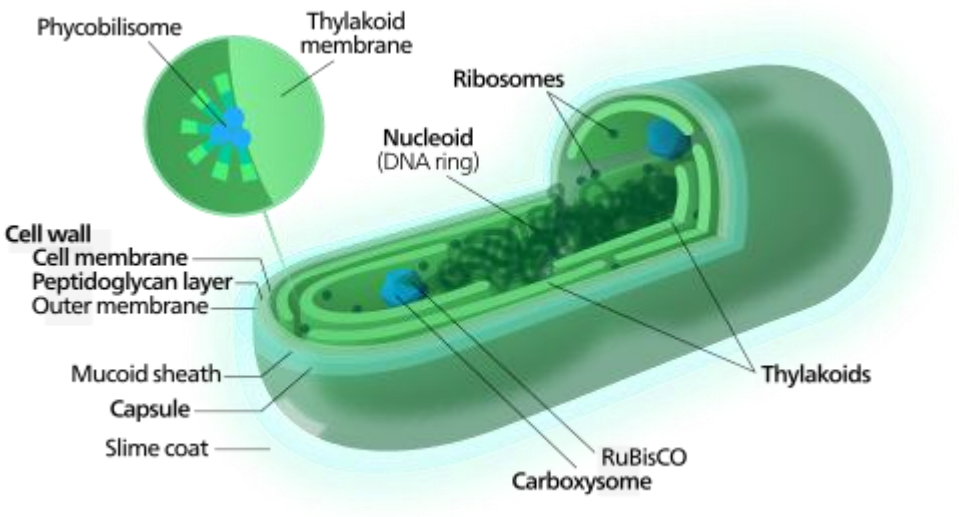
- Microcystis aeruginosa
- Cylindrospermopsis raciborskii
- Anabaena circinalis
- Cyanophora paradoxa
- Nostoc commune

Taxonomy/Classification

In 1985, the proposed classification of cyanobacteria took into account the Bacteriological factor. The proposal identified four Orders of the bacteria which included Chroococcales, Nostocales, Oscillatoriales and Stigonematales. However, other orders of the phylum that have been discovered include Chroococcales, Gloeobacterales, and Pleurocapsales.

The bacteria also falls under Kingdom Monera and Division Eubacteria. Further classification has however resulted in significant debate at higher taxonomic levels.

***** Initially, they were classified as blue-green algae because they possess chlorophyll and algal-like appearance. However, further studies showed that they are prokaryotic, which helped re-classify them appropriately.



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Characteristics Based on their Order

Its structural diversity has been shown to be considerable. For this reason, species had to be grouped into categories that have similar characteristics. This section will focus on the major Orders under the phylum to highlight their respective traits.

Chroococcales

This Order is composed of two main classes (Chroococcaceae and Entophysalidaceae). Species in these classes are coccoid or rod-shaped (with Entophysalidaceae being largely composed of coccoid organisms).

Some of the other characteristics among these organisms include the fact that they reproduce through binary fission and they can create colonies to form dense masses that can be seen on such surfaces as moist rocks.

Members of Order Chroococcales also have the following characteristics:

A spherical, ovoid or cylindrical cell shape structure

- They may occur singly following cell division
- As they mature, they aggregate to form colonies that are held together by a slimy matrix

Other members of Order Chroococcales include:

- Pleurocapsa
- Aphanocapsa
- Merismopedia
- Gloeocapsa
- Microcystis

Pleurocapsales

Some of the unifying characteristics of Order Pleurocapsales are that they all reproduce through multiple fission in addition to releasing endospores. Compared to other organisms that also form endospores, members of Pleurocapsales divide through binary and multiple fission, which is the main distinguishing factor.

Here, the enlargement of the spores is followed by additional binary fission to produce a mass of vegetative cells. Some of the cells in the mass go through multiple fission to release more endospores.

The order is also composed of a wide range of organisms that can be found in an array of habitats ranging from terrestrial and marine environments. As they develop, some members of this order have been shown to develop as epiphytes on algae and as epiliths on such surfaces as moist rocks.

***** They can also produce pseudo-filaments that can reproduce through baeocytes.

Members of Order Pleurocapsales include:

Chroococcidiopsis

Pleurocapsa

Dermocarpella

Xenococcus

Oscillatoriales

The Order Oscillatoriales is largely composed of filamentous cyanobacteria (having uniseriate filaments). However, members of this group lack true branching, akinetes and heterocysts.

Members of this Order can be found in a variety of environments from fresh water and saline water bodies to terrestrial habitats.

Some of the other traits of this Order include:

They tend to form multicellular elongated structures

- They are trichal/filamentous

Members of Oscillatoriales include:

- Phormidium
- Microcoleus
- Lyngbya
- Planktothrix

Nostocales

Like Stigonematales, members of Order Nostocales have heterogenus cellular composition in their trichomes. The vegetative cells in this order are also divided into heterocysts that have a thick hyaline protoplast and are involved in nitrogen fixation as well as akinetes that have in place thick cell walls that allow them to survive when conditions are unfavourable.

Some of the other traits include: False branching in some species

Some members of this Order include:

Cylindrospermopsis

- Calothrix
- Anabaena
- Nostoc

Stigonematales

Members of Order Stigonematales share several similar traits to those of Order Nostocales. These include such traits as trichomes with heterogeneous cellular composition as well as heterocysts and akinetes vegetative cells.

Some of the other traits associated with the order Stigonematales include multiseriated filaments with true branching.

Members of this Order include:

Stigonema

- Mastigocladus

Gloebacterales

Consisting of such organisms such as the members of Gloeobacter, Gloeobacterales possess phycobilisomes which are light harvesting complexes. As a result, Gloeobacterales like Gloeobacter lack thylakoids that are found in other members of cyanobacteria. However, like other cyanobacteria, they can be found in a range of environments like limestone rocks and other aquatic environments.

Occurrence of Cyanobacteria in Nature

While the Phylum cyanobacteria is composed of diverse members, there are a number of traits that can be found in the majority, if not all cyanobacteria species.

For instance, a majority of the species are aerobic photoautotrophs. As such, they are heavily reliant on water, carbon dioxide, light as well as various inorganic substances for their life processes.

Given that photosynthesis is the primary mode of energy production among these organisms, they are heavily dependent on sunlight, carbon dioxide and water for this mechanism to be successful.

For their survival, a good number have been shown to be able to adapt through a number of mechanisms. A good example of this includes ultraviolet absorbing sheath pigments that allows some of the species to be able to survive exposed environments.

They are particularly prominent in cold/hot springs; marine bodies as well as others areas where microalgae are incapable of surviving. This is made possible by the fact that they are capable of adapting to different environments.

Cyanobacteria play an important role within the microbial community; for this reason, they can be found almost everywhere on our planet. However, some of these environments are unfavourable which makes it difficult for many other live organisms to survive in them. These include such environments as deserts, very hot springs and Antarctic ice shelves among others.

For this reason, they need to adapt in various ways in order to survive these conditions. In addition to the ultraviolet absorbing sheath pigments found in some species, many planktonic cyanobacteria have been shown to be able to survive Ultra Violet Rays (UVR) by either floating or sinking in their environment by using gas vacuoles.

For others like Oscillatoriales, survival is enhanced by actively moving from microbial mat surfaces into the matrix sediments which provide more favorable conditions. This allows the organisms to survive instances of UVR. In Antarctic Lakes, Cyanobacteria have been shown to significantly lower their oxygen consumption.

The presence of chlorophyll is one of the characteristics identified. However, it is worth noting that they also have other accessory pigments that include allophycocyanin, phycoerythrin and phycocyanin. These pigments play an important role given that they help effectively utilize the light spectrum in a given region/environment.

In the event of varying light spectrums, the organisms can still carry on their photosynthetic process which in turn enhance their survival. In addition, members of cyanobacteria are capable of storing various important nutrients and metabolites within thier cytoplasm.

This, in addition to dinitrogen fixations, provides them with the necessary nutrition they require to survive. Among other mechanisms, this helps the bacteria survive in deep and dark environments for a long period of time. Through such adaptations, members have been able to survive in different environments, both favorable and extreme, on earth.

Some of the other survival strategies used include:

- The production of mucopolysaccharides - this slows down liquid flow during freeze and thaw
- Production of compatible solutes (sucrose, glucosylglycerol etc) - produced by water-stressed organisms
- The ability to fix carbon dioxide at very low water potentials

***** Are the only bacteria capable of oxygenic photosynthesis.

Symbioses

Because of their ability to fix nitrogen, heterocystous cyanobacteria develop symbiotic relationships with a variety of eukaryotic plant species such as algae, liverworts, angiospores and ferns among others.

As studies have shown, development of symbiotic relationships between free-living cyanobacteria and other eukaryotic plants results in morphological, physiological and biochemical modification thus causing a change to the original morphology, physiology and biochemical form of the bacteria.

During nitrogen fixation, an enzyme known as nitrogenase transfers several pairs (three pairs) of electrons and protons to the nitrogen molecule to create two ammonia molecules. This is then added to glutamic acid to form glutamine (in the plant's cells) that in turn provides amide nitrogen used for the synthesis of amino acids as well as components of DNA and RNA. In this relationship, cyanobacteria, which are nitrogen-fixers benefit from carbon dioxide that is produced by the host (algae etc)

***** With fungi, cyanobacteria symbiosis produces an association known as lichens. The symbiotic relationship (lichenization) between fungi and cyanobacteria only accounts for 10 percent of all lichens.

Given that fungi are incapable of photosynthesis; this relationship is beneficial to the fungi given that they can receive various nutrients and thus energy from the photosynthetic activities of the cyanobacteria.

Gram Negative

Cyanobacteria have a gram-negative structure, which means that they cannot retain the primary stain during gram-staining. However, microscopic observation of the species has shown that they have a thicker peptidoglycan layer compared to other gram-negative bacteria.

Studies on the peptidoglycan layer has also revealed a more complex structure (with the cross-link between the peptidoglycan chains being higher compared to other organisms).

Toxins and Treatment

In their environment, cyanobacteria produce a wide range of compounds such as 2-methylisoborneol and geosmin. When released in water, these compounds can result in a change in the taste and odor.

Apart from these compounds, they have also been shown to produce such toxins as hepatotoxins, neurotoxins and dermatoxins. These toxins, often released by planktonic species can have negative impacts on human beings in causing infections of the liver (hepatotoxins), the nervous system (neurotoxins) and skin irritation which is caused by dermatotoxins.

Cyanobacteria are also responsible for microcystins (cyclic peptides that may either contain seven amino acids or other constituents that resemble amino-acid). These compounds are synthesized by enzyme complexes. The toxin produced through this process can cause serious health issues and death of metazoa.

Because of these toxins, the presence of cyanobacteria in drinking reservoirs is of great concern since they can have serious health impacts on all animals including human beings.

Whereas some of the toxins are released in water, some of these bacteria have been shown to release potent toxins such as anatoxin-a which is produced by Anabaena flosaquae that can cause death within 30 minutes.

Some of the toxins (capable of causing paralysis e.g. neosaxitoxin produced by Aphanizornenon flosaquae) have been identified in some blooms and can also result in death.

Following contact with algal blooms, these toxins can cause allergic rhinitis and dermatitis among other symptoms. Bloom forming species are often found in environments with favorable conditions (warm, stable with high nutrient levels). Some of the organisms identified here include members of Anabaena, Microcystis and Aphanizomenon.

Some of the signs/symptoms in human beings include (impacts of the peptide toxins)

- Diarrhea
- Pilo-erection
- Vomiting
- General weakness
- Cold
- Death

Effective treatment is yet to be developed and thus prevention is the best means of avoiding such infections. Here, prevention involves not drinking water from lakes and rivers (particularly those with blooms and scum).

Such water should also not be used for such purposes as cooking or preparing refreshments given that they may contain the toxins. While some may think of boiling such water for safe consumption, this is also not recommended because doing so may increase the effects of the toxic substances.

Depending on the infection and the type of cyanobacteria, some of the methods used in treatment include:

Intravenous electrolytes

- Oxygen therapy
- Antibiotics
- Vitamins
- Use of drugs such as cholestyramine

Mixotroph

A **mixotroph** is an organism that can use a mix of different sources of energy and carbon, instead of having a single trophic mode on the continuum from complete autotrophy at one end to heterotrophy at the other. It is estimated that mixotrophs comprise more than half of all microscopic plankton. There are two types of eukaryotic mixotrophs: those with their own chloroplasts, and those with endosymbionts—and those that acquire them through kleptoplasty or by enslaving the entire phototrophic cell.

Possible combinations
are photo- and chemotrophy, litho- and organotrophy (osmotrophy, phagotrophy and myzocytosis), auto-and heterotrophy or other combinations of these. Mixotrophs can be either eukaryotic or prokaryotic. They can take advantage of different environmental conditions.

If a trophic mode is obligate, then it is always necessary for sustaining growth and maintenance; if facultative, it can be used as a supplemental source. Some organisms have incomplete Calvin cycles, so they are incapable of fixing carbon dioxide and must use organic carbon sources.

Types of mixotrophy

Organisms may employ mixotrophy **obligately** or **facultatively**.

- Obligate mixotrophy: To support growth and maintenance, an organism must utilize both heterotrophic and autotrophic means.
- Obligate autotrophy with facultative heterotrophy: Autotrophy alone is sufficient for growth and maintenance, but heterotrophy may be used as a supplementary strategy when autotrophic energy is not enough, for example, when light intensity is low.
- Facultative autotrophy with obligate heterotrophy: Heterotrophy is sufficient for growth and maintenance, but autotrophy may be used to supplement, for example, when prey availability is very low.
- Facultative mixotrophy: Maintenance and growth may be obtained by heterotrophic or autotrophic means alone, and mixotrophy is used only when necessary.

To characterize the sub-domains within mixotrophy, several very similar categorization schemes have been suggested.

Consider the example of a marine protist with heterotrophic and photosynthetic capabilities: In the breakdown put forward by Jones, there are four mixotrophic groups based on relative roles of phagotrophy and phototrophy.

- A: Heterotrophy (phagotrophy) is the norm, and phototrophy is only used when prey concentrations are limiting.
- B: Phototrophy is the dominant strategy, and phagotrophy is employed as a supplement when light is limiting.
- C: Phototrophy results in substances for both growth and ingestion, phagotrophy is employed when light is limiting.
- D: Phototrophy is most common nutrition type, phagotrophy only used during prolonged dark periods, when light is extremely limiting.

An alternative scheme by Stoeker also takes into account the role of nutrients and growth factors, and includes mixotrophs that have a photosynthetic

symbiont or who retain chloroplasts from their prey. This scheme characterizes mixotrophs by their efficiency.

- Type 1: "Ideal mixotrophs" that use prey and sunlight equally well
- Type 2: Supplement phototrophic activity with food consumption
- Type 3: Primarily heterotrophic, use phototrophic activity during times of very low prey abundance

Microorganisms

Examples

- *Paracoccus pantotrophus* is a bacterium that can live chemoorganoheterotrophically, whereby a large variety of organic compounds can be metabolized. Also a facultative chemolithoautotrophic metabolism is possible, as seen in colorless sulfur bacteria (some *Thiobacillus*), whereby sulfur compounds such as hydrogen sulfide, elemental sulfur, or thiosulfate are oxidized to sulfate. The sulfur compounds serve as electron donors and are consumed to produce ATP. The carbon source for these organisms can be carbon dioxide (autotrophy) or organic carbon (heterotrophy). Organoheterotrophy can occur under aerobic or under anaerobic conditions; lithoautotrophy takes place aerobically.

Pigments

Pigments are colorful compounds. Pigments are chemical compounds which reflect only certain wavelengths of visible light. This makes them appear "colorful". Flowers, corals, and even animal skin contain pigments which give them their colors. More important than their reflection of light is the ability of pigments to absorb certain wavelengths.

Because they interact with light to absorb only certain wavelengths, pigments are useful to plants and other **autotrophs** --organisms which make their own food using **photosynthesis**. In plants, algae, and cyanobacteria, pigments are the means by which the energy of sunlight is captured for photosynthesis. However, since each pigment reacts with only a narrow range of the spectrum, there is usually a need to produce several kinds of pigments, each of a different color, to capture more of the sun's energy.

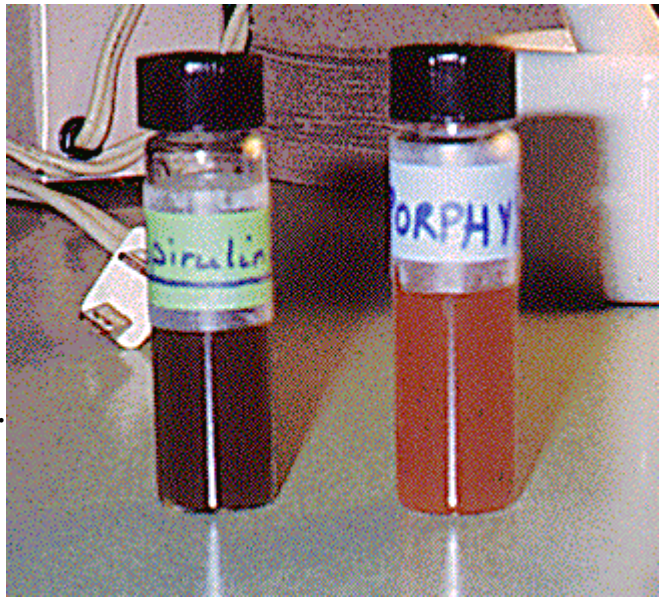
There are three basic classes of pigments.

 Chlorophylls are greenish pigments which contain a **porphyrin ring**. This is a stable ring-shaped molecule around which electrons are free to migrate. Because the electrons move freely, the ring has the potential to gain or lose electrons easily, and thus the potential to provide energized electrons to other molecules. This is the fundamental process by which chlorophyll "captures" the energy of sunlight.

There are several kinds of chlorophyll, the most important being chlorophyll "a". This is the molecule which makes photosynthesis possible, by passing its energized electrons on to molecules which will manufacture sugars. All plants, algae, and cyanobacteria which photosynthesize contain chlorophyll "a". A second kind of chlorophyll is chlorophyll "b", which occurs only in "green algae" and in the plants. A third form of chlorophyll which is common is (not surprisingly) called chlorophyll "c", and is found only in the photosynthetic members of the Chromista as well as the dinoflagellates. The differences between the chlorophylls of these major groups was one of the first clues that they were not as closely related as previously thought.

 Carotenoids are usually red, orange, or yellow pigments, and include the familiar compound carotene, which gives carrots their color. These compounds are composed of two small six-carbon rings connected by a "chain" of carbon atoms. As a result, they do not dissolve in water, and must be attached to membranes within the cell. Carotenoids cannot transfer sunlight energy directly to the photosynthetic pathway, but must pass their absorbed energy to chlorophyll. For this reason, they are called **accessory pigments**. One very visible accessory pigment is **fucoxanthin** the brown pigment which colors kelps and other brown algae as well as the diatoms.

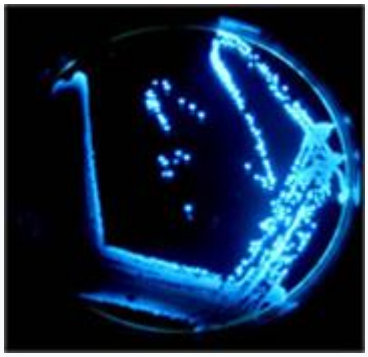
 Phycobilins are water-soluble pigments, and are therefore found in the cytoplasm, or in the stroma of the chloroplast. They occur only in Cyanobacteria and Rhodophyta.



The picture at the right shows the two classes of phycobilins which may be extracted from these "algae". The vial on the left contains the bluish pigment **phycocyanin**, which gives the Cyanobacteria their name. The vial on the right contains the reddish pigment **phycoerythrin**, which gives the red algae their common name.

Phycobilins are not only useful to the organisms which use them for soaking up light energy; they have also found use as research tools. Both pycocyanin and phycoerythrin **fluoresce** at a particular wavelength. That is, when they are exposed to strong light, they absorb the light energy, and release it by emitting light of a very narrow range of wavelengths. The light produced by this fluorescence is so distinctive and reliable, that phycobilins may be used as chemical "tags". The pigments are chemically bonded to antibodies, which are then put into a solution of cells. When the solution is sprayed as a stream of fine droplets past a laser and computer sensor, a machine can identify whether the cells in the droplets have been "tagged" by the antibodies. This has found extensive use in cancer research, for "tagging" tumor cells.

Bioluminescent bacteria



Bioluminescent plate

Bioluminescent bacteria are light-producing bacteria that are predominantly present in sea water, marine sediments, the surface of decomposing fish and in the gut of marine animals. While not as common, bacterial bioluminescence is also found in terrestrial and freshwater bacteria. These bacteria may be free living (such as *Vibrio harveyi*) or in symbiosis with animals such as the Hawaiian Bobtail squid (*Aliivibrio fischeri*) or terrestrial nematodes(*Photorhabdus luminescens*). The host organisms provide these bacteria a safe home and sufficient nutrition. In exchange, the hosts use the light produced by the bacteria for camouflage, prey and/or mate attraction. Bioluminescent bacteria have evolved symbiotic relationships with other organisms in which both participants benefit close to equally. Another possible reason bacteria use luminescence reaction is for quorum sensing, an ability to regulate gene expression in response to bacterial cell density.

History

Records of bioluminescent bacteria existed for thousands of years. They appear in the folklore of many regions, including Scandinavia and the Indian subcontinent. Both Aristotle and Charles Darwin have described the phenomenon of the oceans glowing. Since its discovery less than 30 years ago, the enzyme luciferase and its regulatory gene, *lux*, have led to major advances in molecular biology, through use as a reporter gene. Luciferase was first purified by McElroy and Green in 1955. It was later discovered that there were two subunits to luciferase, called subunits α and β. The genes encoding these enzymes, *luxA* and *luxB,* respectively, were first isolated in the *lux* operon of *Aliivibrio fisheri* .

Purpose of bio-luminescence

The wide-ranged biological purposes of bio-luminescence include but are not limited to attraction of mates, defense against predators, and warning signals. In the case of bioluminescent bacteria, bio-luminescence mainly serves as a form of dispersal. It has been hypothesized that enteric bacteria (bacteria that survive in the guts of other organisms) - especially those prevalent in the depths of the ocean - employ bio-luminescence as an effective form of distribution. After making their way into the digestive tracts of fish and other marine organisms and being excreted in fecal pellets, bioluminescent bacteria are able to utilize their bio-luminescent capabilities to lure in other organisms and prompt ingestion of these bacterial-containing fecal pellets. The bio-luminescence of

bacteria thereby ensures their survival, persistence, and dispersal as they are able to enter and inhabit other organisms.

Regulation of bio-luminescence

The regulation of bio-luminescence in bacteria is achieved through the regulation of the oxidative enzyme called luciferase. It is important that bio-luminescent bacteria decrease production rates of luciferase when the population is sparse in number in order to conserve energy. Thus, bacterial bioluminescence is regulated by means of chemical communication referred to as quorum sensing. Essentially, certain signaling molecules named autoinducers with specific bacterial receptors become activated when the population density of bacteria is high enough. The activation of these receptors leads to a coordinated induction of luciferase production that ultimately yields visible luminescence.

Biochemistry of bio-luminescence

The chemical reaction that is responsible for bio-luminescence is catalyzed by the enzyme luciferase. In the presence of oxygen, luciferase catalyzes the oxidation of an organic molecule called luciferin.[13] Though bio-luminescence across a diverse range of organisms such as bacteria, insects, and dinoflagellates function in this general manner (utilizing luciferase and luciferin), there are different types of luciferin-luciferase systems. For bacterial bio-luminescence specifically, the biochemical reaction involves the oxidation of an aliphatic aldehyde by a reduced flavin mononucleotide. The products of this oxidation reaction include an oxidized flavin mononucleotide, a fatty acid chain, and energy in the form of a blue-green visible light.

Reaction: $FMNH2 + O2 + RCHO \rightarrow FMN + RCOOH + H2O + light$

Bioelectromagnetics of bio-luminescence

It has been proposed that bioelectromagnetics may be involved in biological processes behind bio-luminescence, which may function as a pump. This pump may involve mm and sub-mm wave coupling of bio-luminescence radiation for quorum sensing regulation. This proposal arises from the observation that mm-wave radiation exposure has been reported to induce changes in DNA conformation and possibly gene expression. Agents that change DNA conformation also increase light emission in bio-luminescence of bacteria.

Evolution of bio-luminescence

Of all light emitters in the ocean, bio-luminescent bacteria is the most abundant and diverse. However, the distribution of bio-luminescent bacteria is uneven, which suggests evolutionary adaptations. The bacterial species in terrestrial genera such as Photorhabdus are bio-luminescent. On the other hand, marine genera with bio-luminescent species such as Vibrio and Shewanella oneidensis have different closely related species that are not light emitters. Nevertheless, all bio-luminescent bacteria share a common gene sequence: the enzymatic oxidation of Aldehyde and reduced Flavin mononucleotide by luciferasewhich are contained in the lux operon. Bacteria from distinct ecological niches contain this gene sequence; therefore, the identical gene sequence evidently suggests that bio-luminescence bacteria result from evolutionary adaptations.

Use as laboratory tool

After the discovery of the lux operon, the use of bioluminescent bacteria as a laboratory tool is claimed to have revolutionized the area of environmental microbiology. The applications of bioluminescent bacteria include biosensors for detection of contaminants, measurement of pollutant toxicity and monitoring of genetically engineered bacteria released into the environment. Biosensors, created by placing a *lux* gene construct under the control of an inducible promoter, can be used to determine the concentration of specific pollutants. Biosensors are also able to distinguish between pollutants that are bioavailable and those that are inert and unavailable. For example, *Pseudomonas fluorescens* has been genetically engineered to be capable of degrading salicylate and naphthalene, and is used as a biosensor to assess the bioavailability of salicylate and naphthalene. Biosensors can also be used as an indicator of cellular metabolic activity and to detect the presence of pathogens.

Evolution

The light-producing chemistry behind bioluminescence varies across the lineages of bioluminescent organisms. Based on this observation, bioluminescence is believed to have evolved independently at least 40 times. In bioluminescent bacteria, the reclassification of the members of*Vibrio fischeri* species group as a new genus, *Aliivibrio,* has led to increased interest in the evolutionary origins of bioluminescence. Among bacteria, the distribution of

bioluminescent species is polyphyletic. For instance, while all species in the terrestrial genus *Photorhabdus* are luminescent, the genera *Aliivibrio, Photobacterium, Shewanella* and *Vibrio* contain both luminous and non-luminous species. Despite bioluminescence in bacteria not sharing a common origin, they all share a gene sequence in common. The appearance of the highly conserved lux operon in bacteria from very different ecological niches suggests a strong selective advantage despite the high energetic costs of producing light. DNA repair is thought to be the initial selective advantage for light production in bacteria. Consequently, the lux operon may have been lost in bacteria that evolved more efficient DNA repair systems but retained in those where visible light became a selective advantage. The evolution of quorum sensing is believed to have afforded further selective advantage for light production. Quorum sensing allows bacteria to conserve energy by ensuring that they do not synthesize light-producing chemicals unless a sufficient concentration are present to be visible.

Bacterial groups that exhibit bioluminescence

All bacterial species that have been reported to possess bioluminescence belong within the families *Vibrionaceae*, *Shewanellaceae*, or *Enterobacteriaceae*, all of which are assigned to the class Gammaproteobacteria.

Family	Genus	Species
Enterobacteriaceae	*Photorhabdus*	*Photorhabdus asymbiotica* *Photorhabdus luminescens* *Photorhabdus temperata*
Shewanellaceae	*Shewanella*	*Shewanella woodyi* *Shewanella hanedai*
Vibrionaceae	*Aliivibrio*	*Aliivibrio fischeri* *Aliivibrio logei* *Aliivibrio salmonicida* *Aliivibrio sifiae* *Aliivibrio "thorii"* *Aliivibrio wodanis*

	Photobacterium	*Photobacterium aquimaris* *Photobacterium damselae* *Photobacterium kishitanii* *Photobacterium leiognathi* *Photobacterium mandapamensis* *Photobacterium phosphoreum*
	Vibrio	*Vibrio azureus* *Vibrio "beijerinckii"* *Vibrio campbellii* *Vibrio chagasii* *Vibrio cholerae* *Vibrio harveyi* *Vibrio mediterranea* *Vibrio orientalis* *Vibrio sagamiensis* *Vibrio splendidus* *Vibrio vulnicus*
	"*Candidatus Photodesmus*"	"*Candidatus Photodesmus katoptron*"

Distribution

Bioluminescent bacteria are most abundant in marine environments during spring blooms when there are high nutrient concentrations. These light-emitting organisms are found mainly in coastal waters near the outflow of rivers, such as the northern Adriatic Sea, Gulf of Trieste, northwestern part of the Caspian Sea, coast of Africa and many more. These are known as milky seas. Bioluminescent bacteria are also found in freshwater and terrestrial environments but are less wide spread than in seawater environments. They are found globally, as free-living, symbiotic or parasitic forms and possibly as opportunistic pathogens. Factors that affect the distribution of bioluminescent bacteria include temperature, salinity, nutrient concentration, pH level and solar radiation. For example, *Aliivibrio fischeri* grows favourably in environments that have temperatures between 5 and 30 °C and a pH that is less than 6.8; whereas, *Photobacterium phosphoreum* thrives in conditions that have temperatures between 5 and 25 °C and a pH that is less than 7.0.

Genetic diversity

All bioluminescent bacteria share a common gene sequence: the *lux* operon characterized by the *luxCDABE* gene organization. *LuxAB* codes for luciferase while *luxCDE* codes for a fatty-acid reductase complex that is responsible for synthesizing aldehydes for the bioluminescent reaction. Despite this common gene organization, variations, such as the presence of other lux genes, can be observed among species. Based on similarities in gene content and organization, the *lux*operon can be organized into the following four distinct types: the *Aliivibrio*/*Shewanella* type, the *Photobacterium* type, the*Vibrio*/*Candidatus* Photodesmus type, and the *Photorhabdus* type. While this organization follows the genera classification level for members of *Vibrionaceae* (*Aliivibrio*, *Photobacterium*, and *Vibrio*), its evolutionary history is not known.

With the exception of the *Photorhabdus* operon type, all variants of the *lux* operon contain the flavin reductase-encoding *luxG*gene. Most of the *Aliivibrio*/*Shewanella* type operons contain additional *luxI/luxR* regulatory genes that are used for autoinduction during quorum sensing. The *Photobacterum* operon type is characterized by the presence of *rib* genes that code for riboflavin, and forms the *lux-rib* operon. The*Vibrio*/*Candidatus* Photodesmus operon type differs from both the *Aliivibrio*/*Shewanella* and the *Photobacterium* operon types in that the operon has no regulatory genes directly associated with it.

Mechanism

All bacterial luciferases are approximately 80 KDa heterodimers containing two subunits: α and β. The α subunit is responsible for light emission. The *luxA* and *luxB* genes encode for the α and β subunits, respectively. In most bioluminescent bacteria, the *luxA* and *luxB* genes are flanked upstream by *luxC* and *luxD* and downstream by *luxE*.

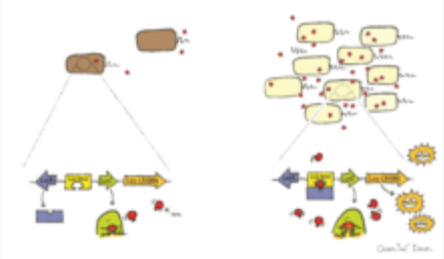
The bioluminescent reaction is as follows:

$FMNH_2 + O_2 + R\text{-}CHO \rightarrow FMN + H_2O + R\text{-}COOH + Light\ (\sim 495\ nm)$

Molecular oxygen reacts with FMNH2 (reduced flavin mononucleotide) and a long-chain aldehyde to produce FMN (flavin mononucleotide), water and a corresponding fatty acid. The blue-green light emission of bioluminescence, such as that produced by *Photobacterium phosphoreum* and *Vibro harveyi*, results from this reaction.[4] Because light emission involves expending six ATP

molecules for each photon, it is an energetically expensive process. For this reason, light emission is not constitutively expressed in bioluminescent bacteria; it is expressed only when physiologically necessary.

Quorum sensing



Bacterial quorum sensing

Bioluminescence in bacteria can be regulated through a phenomenon known as autoinduction or quorum sensing. Quorom sensing is a form of cell-to-cell communication that alters gene expression in response to cell density. Autoinducer is a diffusible pheromone produced constitutively by bioluminescent bacteria and serves as an extracellular signalling molecule. When the concentration of autoinducer secreted by bioluminescent cells in the environment reaches a threshold (above 10^7 cells per mL), it induces the expression of luciferase and other enzymes involved in bioluminescence. Bacteria are able to estimate their density by sensing the level of autoinducer in the environment and regulate their bioluminescence such that it is expressed only when there is a sufficiently high cell population. A sufficiently high cell population ensures that the bioluminescence produced by the cells will be visible in the environment.

A well known example of quorum sensing is that which occurs between *Aliivibrio fischeri* and its host. This process is regulated by LuxI and LuxR, encoded by *luxI* and *luxR* respectively. LuxI is autoinducer synthase that produces autoinducer (AI) while LuxR functions as both a receptor and transcription factor for the lux operon.[4] When LuxR binds AI, LuxR-AI complex activates transcription of the lux operon and induces the expression of luciferase. Using this system, *A. fischeri* has shown that bioluminescence is expressed only when the bacteria are host-associated and have reached sufficient cell densities.

Another example of quorum sensing by bioluminescent bacteria is by *Vibrio harveyi*, which are known to be free-living. Unlike *Aliivibrio fischeri*, *V. harveyi* do not possess the *luxI/luxR* regulatory genes and therefore have a

different mechanism of quorum sensing regulation. Instead, they use the system known as three-channel quorum sensing system.

Role

The uses of bioluminescence and its biological and ecological significance for animals, including host organisms for bacteria symbiosis, have been widely studied. Its benefits for bacteria, however, still remain unclear.

One explanation for the role of bacterial bioluminescence is from the biochemical aspect. Several studies have shown the biochemical roles of the luminescence pathway. It can function as an alternate pathway for electron flow under low oxygen concentration, which can be advantageous when no fermentable substrate is available. In this process, light emission is a side product of the metabolism.

Evidence also suggests that bacterial luciferase contributes to the resistance of oxidative stress. In laboratory culture, *luxA* and *luxB* mutants of *Vibrio harveyi*, which lacked luciferase activity, showed impairment of growth under high oxidative stress compared to wild type. The *luxD* mutants, which had an unaffected luciferase but were unable to produce luminescence, showed little or no difference. This suggests that luciferase mediates the detoxification of reactive oxygen.

Bacterial bioluminescence has also been proposed to be a source of internal light in photoreactivation, a DNA repair process carried out by photolyase. Experiments have shown that non-luminescent *V. harveyi* mutants are more sensitive to UV irradiation, suggesting the existence of a bioluminescent-mediated DNA repair system.

Another hypothesis, called the "bait hypothesis", is that bacterial bioluminescence attracts predators who will assist in their dispersal. They are either directly ingested by fish or indirectly ingested by zooplankton that will eventually be consumed by higher trophic levels. Ultimately, this may allow passage into the fish gut, a nutrient-rich environment where the bacteria can divide, be excreted, and continue their cycle. Experiments using luminescent *Photobacterium leiognathi* and non-luminescent mutants have shown that luminescence attracts zooplankton and fish, thus supporting this hypothesis.

Symbiosis with other organisms

The symbiotic relationship between the Hawaiian bobtail squid *Euprymna scolopes* and the marine gram-negative bacterium *Aliivibrio fischeri* has been well studied. The two organisms exhibit a mutualistic relationship in which bioluminescence produced by *A. fischeri* helps to attract pray to the squid host, which provides nutrient-rich tissues and a protected environment for*A. fischeri*. Bioluminescence provided by *A. fischeri* also aids in the defense of the squid *E. scolopes* by providing camouflage during its nighttime foraging activity. Following bacterial colonization, the specialized organs of the squid undergo developmental changes and a relationship becomes established. The squid expels 90% of the bacterial population each morning, because it no longer needs to produce bioluminescence in the daylight. This expulsion benefits the bacteria by aiding in their dissemination. A single expulsion by one bobtail squid produces enough bacterial symbionts to fill 10,000m^3 of seawater at a concentration that is comparable to what is found in coastal waters. Thus, in at least some habitats, the symbiotic relationship between *A. fischeri* and *E. scolopes* plays a key role in determining the abundance and distribution of *E. scolopes*. There is a higher abundance of *A. fischeri* in the vicinity of a population of *E. scolopes* and this abundance markedly decreases with increasing distance from the host's habitat.

Bioluminescent *Photobacterium* species also engage in mutually beneficial associations with fish and squid. Dense populations of *P. kishitanii, P. leiogathi,* and *P. mandapamensis* can live in the light organs of marine fish and squid, and are provided with nutrients and oxygen for reproduction in return for providing bioluminescence to their hosts, which can aid in sex-specific signaling, predator avoidance, locating or attracting prey, and schooling.

Phosphorescence

Phosphorescence is a type of photoluminescence related to fluorescence. Unlike fluorescence, a phosphorescent material does not immediately re-emit the radiation it absorbs. The slower time scales of the re-emission are associated with "forbidden" energy state transitions in quantum mechanics. As these transitions occur very slowly in certain materials, absorbed radiation is re-emitted at a lower intensity for up to several hours after the original excitation.

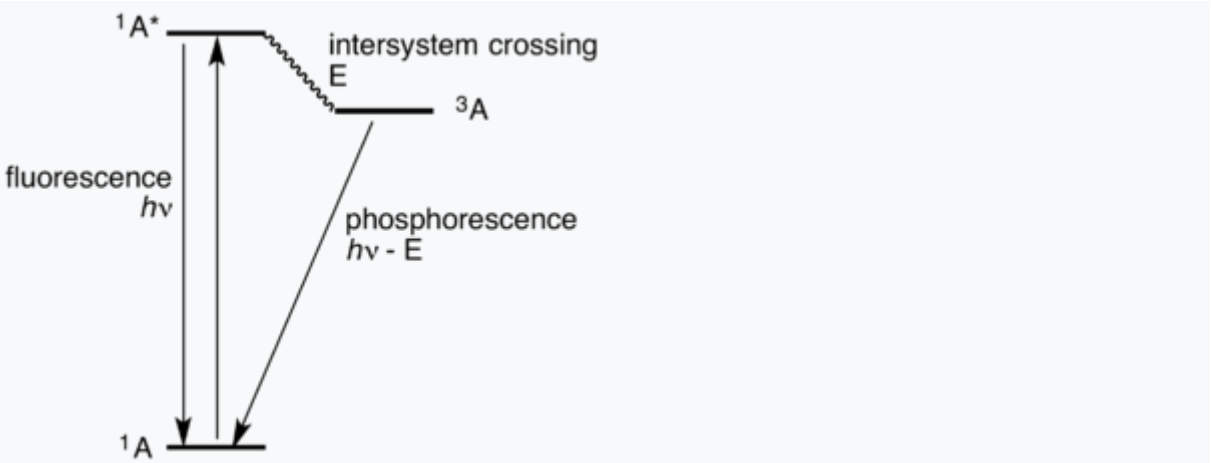
Everyday examples of phosphorescent materials are the glow-in-the-dark toys, stickers, paint, wristwatch and clock dials that glow after being charged with a bright light such as in any normal reading or room light. Typically, the glow

slowly fades out, sometimes within a few minutes or up to a few hours in a dark room.[1]

Around 1604, Vincenzo Casciarolo discovered a "lapis solaris" near Bologna, Italy. Once heated in an oxygen-rich furnace, it thereafter absorbed sunlight and glowed in the dark. The study of phosphorescent materials led to the discovery of radioactivityin 1896.

Explanations

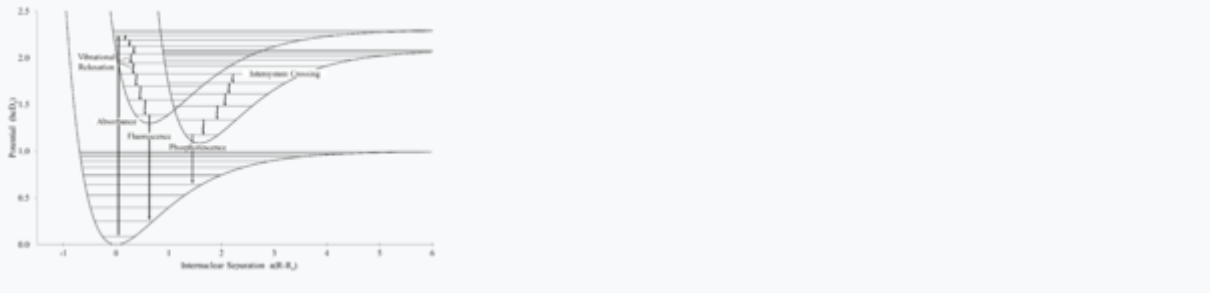
Simple



Jablonski diagram of an energy scheme used to explain the difference between fluorescence and phosphorescence. The excitation of molecule A to its singlet excited state ($^1A^*$)is followed by intersystem crossing to the triplet state (^3A) that relaxes to the ground state by phosphorescence.

In simple terms, phosphorescence is a process in which energy absorbed by a substance is released relatively slowly in the form of light. This is in some cases the mechanism used for "glow-in-the-dark" materials which are "charged" by exposure to light. Unlike the relatively swift reactions in fluorescence, such as those seen in a common fluorescent tube, phosphorescent materials "store" absorbed energy for a longer time, as the processes required to re-emit energy occur less often.

Quantum mechanical



After an electron absorbs a photon of high energy, it may undergo vibrational relaxations and intersystem crossing to another spin state. Again the system relaxes vibrationally in the new spin state and eventually emits light by phosphorescence.

Most photoluminescent events, in which a chemical substrate absorbs and then re-emits a photon of light, are fast, in the order of 10 nanoseconds. Light is absorbed and emitted at these fast time scales in cases where the energy of the photons involved matches the available energy states and allowed transitions of the substrate. In the special case of phosphorescence, the electron which absorbed the photon (energy) undergoes an unusual intersystem crossing into an energy state of higher *spin multiplicity* (*see term symbol*), usually a triplet state. As a result, the excited electron can become trapped in the triplet state with only "forbidden" transitionsavailable to return to the lower energy singlet state. These transitions, although "forbidden", will still occur in quantum mechanics but are kinetically unfavored and thus progress at significantly slower time scales. Most phosphorescent compounds are still relatively fast emitters, with triplet lifetimes in the order of milliseconds. However, some compounds have triplet lifetimes up to minutes or even hours, allowing these substances to effectively store light energy in the form of very slowly degrading excited electron states. If the phosphorescent quantum yield is high, these substances will release significant amounts of light over long time scales, creating so-called "glow-in-the-dark" materials.

Chemiluminescence

Some examples of glow-in-the-dark materials do not glow by phosphorescence. For example, glow sticks glow due to a chemiluminescent process which is commonly mistaken for phosphorescence. In chemiluminescence, an excited state is created via a chemical reaction. The light emission tracks the kinetic progress of the underlying chemical reaction. The excited state will then transfer to a dye molecule, also known as a sensitizer or fluorophor, and subsequently fluoresce back to the ground state.

Materials

Common pigments used in phosphorescent materials include zinc sulfide and strontium aluminate. Use of zinc sulfide for safety related products dates back to the 1930s. However, the development of strontium

aluminate, with a luminance approximately 10 times greater than zinc sulfide, has relegated most zinc sulfide based products to the novelty category. Strontium aluminate based pigments are now used in exit signs, pathway marking, and other safety related signage.[2]

- **Phosphorescent pigments – zinc sulfide vs. strontium aluminate**
- 

Left: zinc sulfide
Right: strontium aluminate

- 

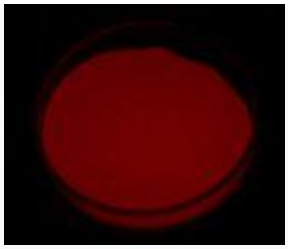
Pigments in the dark

- 

Pigments in the dark after 4 min

- **Phosphorescent**
- 

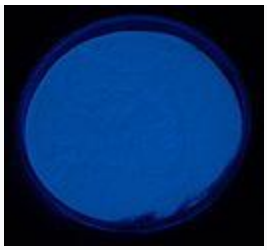
Phosphorescent pigment red (calcium sulfide)

- 

Phosphorescent pigment red in the dark

- 

Phosphorescent pigment blue (alkaline earth metal silicate)

- 

Phosphorescent pigment blue in the dark

Pigments in Photosynthetic Bacteria

presence of carotenoids in all photosynthetic tissues their role is anticipated in photosynthesis. The tissue/cells rich in carotenoids devoid of chlorophyll do not photosynthesize. Light energy absorbed by carotenoids appears to be transferred to chlorophyll a or bacteriochlorophyll a and utilized in the photosynthesis.

It was observed if light is absorbed directly by chlorophyll a, generally found to be less efficient in photosynthesis than that of light absorbed by accessory pigments such as phycocyanin or phycoerythrin.

All the photosynthetic bacteria contain chromatophores which have β-carotene, xanthophyll (carotenoids), and phycobilisomes (phycocyanin and phycoerythrin). Most of the carotenes are present in photosystem I while phycobilisomes are present in photosystem II.

Bacteriochlorophylls:

Photosynthetic bacteria appear in different colour suspension. They are green, purple, violet, red, blue-green, brown coloured due to the presence of photosynthetic pigments in their photosynthetic apparatus. The pigments can be measured by their absorption spectra. Generally chlorophyll absorbs maximally at < 450 nm, carotenoid at 400-550 nm and phycobiliproteins of cyanobacteria at 550-650 nm.

Sometimes chlorophyll pigments absorb maximum in infra red region of 650-1000 nm. There are several types of chlorophyll molecules present in bacteria called bacteriochlorophyll (BChl). They differ from each other mainly by the presence or absence of a double bond between carbon atoms 3 and 4 and by the substituents on the porphyrin ring.

The main absorption maxima of chlorophyll a in cyanobacteria is between 680 and 685 nm, whereas bacteriochlorophylls c, d and e present in green sulphur bacteria show absorption maxima between 715-755 nm.

Most of the purple bacteria contains BChl a of 850-890 nm. BChl b shows absorption maxima at 1020 to 1035 nm (Table 13.3). They are found in Rhodopseudomonas viridis, Ectothiorhodospira halochloris and Thiocapsa pfeningii.

Table 13.3 : Absorption maxima (long wavelength) of bacteriochlorophyll molecules.

Pigment	*Adsorption maxima (nm)*	*In methanol*
Bacteriochlorophyll *a*	830-890	771
Bacteriochlorophyll *b*	835-850	794
	1020-1040	
Bacteriochlorophyll *c*	744-755	660-66
Bacteriochlorophyll *cs*	750	667
Bacteriochlorophyll *d*	705-740	654
Bacteriochlorophyll *e*	719-726	646
Bacteriochlorophyll *g*	670,788	765

Sometimes, type of binding and position of the BChl molecule in the pigment protein complex in photosynthetic apparatus gives rise to different spectral forms to the individual chlorophyll molecule. For instance, BChl a in purple bacteria has four spectral froins: BChl 800, BChl 820, BChl 850 and BChl 870-890 (in Chloroflexus), however, BChl a has two distinct absorption maxima at 808 and 868 nm (Table 13.3).

(i) Carotenoids:

These accessory pigments (400-500 nm, absorption spectrum) are found in phototrophs. They are yellow, green, red-brown in colour and absorb light in blue region. They are insoluble in water and embedded in membrane.

The presence of an oxo or aldehyde group can give them a deep colouration. They have long hydrocarbon chains of C_{40} compounds (tetraterpenoids) with tertiary hydroxyl or methoxy groups with alternating C-C and C=C bonds (conjugate bonds system).

Fig. 13.1 : Structure of β- carotene, a carotenoid.

(Fig. 13.1). The carotenoids do not function directly in ATP synthesis however, transfer energy to reaction center. They are photo-protective and quench singlet oxygen. The members of Chromatiaceae bear okenone, while iso-renceratene is present in Chlorobiaceae. Carotenoids are also reported to be present in certain airborne pigmented bacteria and in halophiles (Halobacter sp.). They have a defensive role in such bacteria.

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These accessory pigments (400-500 nm, absorption spectrum) are found in phototrophs. They are yellow, green, red-brown in colour and absorb light in blue region. They are insoluble in water and embedded in membrane.

The presence of an oxo or aldehyde group can give them a deep colouration. They have long hydrocarbon chains of C_{40} compounds (tetraterpenoids) with tertiary hydroxyl or methoxy groups with alternating C-C and C=C bonds (conjugate bonds system).

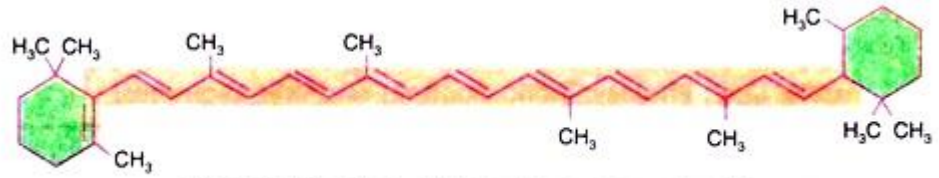


Fig. 13.1 : Structure of β- carotene, a carotenoid.

(Fig. 13.1). The carotenoids do not function directly in ATP synthesis however, transfer energy to reaction center. They are photo-protective and quench singlet oxygen. The members of Chromatiaceae bear okenone, while iso-renceratene is present in Chlorobiaceae. Carotenoids are also reported to be present in certain airborne pigmented bacteria and in halophiles (Halobacter sp.). They have a defensive role in such bacteria.

Phycobilins are water soluble proteins which contain covalently bound linear tetra pyrroles (bilins) as chromophores present inside the granules. They occurs on outer surface of thylakoid membrane of cyanobacteria.

Phycobiliproteins are red or blue in colour, contains tetrapyrroles coupled to proteins. Some of the phycobiliproteins are phycocyanin and allophycocyanins. On aggregation,these proteins form a structure called phycobilisomes which are attached to photosynthetic membranes.

Unit 4

Carbohydrate Anabolism

Anabolism is the process by which the body utilizes the energy released by catabolism to synthesize complex molecules. These complex molecules are then utilized to form cellular structures that are formed from small and simple precursors that act as building blocks.

Stages of anabolism

There are three basic stages of anabolism.

- Stage 1 involves production of precursors such as amino acids, monosaccharides, isoprenoids and nucleotides.
- Stage 2 involves activation of these precursors into reactive forms using energy from ATP
- Stage 3 involves the assembly of these precursors into complex molecules such as proteins, polysaccharides, lipids and nucleic acids.

Sources of energy for anabolic processes

Different species of organisms depend on different sources of energy. Autotrophs such as plants can construct the complex organic molecules in cells such as polysaccharides and proteins from simple molecules like carbon dioxide and water using sunlight as energy.

Heterotrophs, on the other hand, require a source of more complex substances, such as monosaccharides and amino acids, to produce these complex molecules. Photoautotrophs and photoheterotrophs obtain energy from light while chemoautotrophs and chemoheterotrophs obtain energy from inorganic oxidation reactions.

Anabolism of carbohydrates

In these steps simple organic acids can be converted into monosaccharides such as glucose and then used to assemble polysaccharides such as starch. Glucose is made from pyruvate, lactate, glycerol, glycerate 3-phosphate and amino acids and the process is called gluconeogenesis. Gluconeogenesis converts pyruvate

to glucose-6-phosphate through a series of intermediates, many of which are shared with glycolysis.

Usually fatty acids stored as adipose tissues cannot be converted to glucose through gluconeogenesis as these organisms cannot convert acetyl-CoA into pyruvate. This is the reason why when there is long term starvation, humans and other animals need to produce ketone bodies from fatty acids to replace glucose in tissues such as the brain that cannot metabolize fatty acids.

Plants and bacteria can convert fatty acids into glucose and they utilize the glyoxylate cycle, which bypasses the decarboxylation step in the citric acid cycle and allows the transformation of acetyl-CoA to oxaloacetate. From this glucose is formed.

Glycans and polysaccharides are complexes of simple sugars. These additions are made possible by glycosyltransferase from a reactive sugar-phosphate donor, such as uridine diphosphate glucose (UDP-glucose), to an acceptor hydroxyl group on the growing polysaccharide. The hydroxyl groups on the ring of the substrate can be acceptors and thus polysaccharides produced can have straight or branched structures. These polysaccharides so formed may be transferred to lipids and proteins by enzymes called oligosaccharyltransferases.

Photosynthesis

Heterotrophic organisms ranging from *E. coli* to humans rely on the chemical energy found mainly in carbohydrate molecules. Many of these carbohydrates are produced by **photosynthesis**, the biochemical process by which phototrophic organisms convert solar energy (sunlight) into chemical energy. Although photosynthesis is most commonly associated with plants, microbial photosynthesis is also a significant supplier of chemical energy, fueling many diverse ecosystems. In this section, we will focus on microbial photosynthesis.

Photosynthesis takes place in two sequential stages: the light-dependent reactions and the light-independent reactions (Figure 1). In the **light-dependent reactions**, energy from sunlight is absorbed by pigment molecules in photosynthetic membranes and converted into stored chemical energy. In the **light-independent reactions**, the chemical energy produced by the light-dependent reactions is used to drive the assembly of sugar molecules using CO_2;

however, these reactions are still light dependent because the products of the light-dependent reactions necessary for driving them are short-lived. The light-dependent reactions produce ATP and either NADPH or NADH to temporarily store energy. These energy carriers are used in the light-independent reactions to drive the energetically unfavorable process of "fixing" inorganic CO_2 in an organic form, sugar.

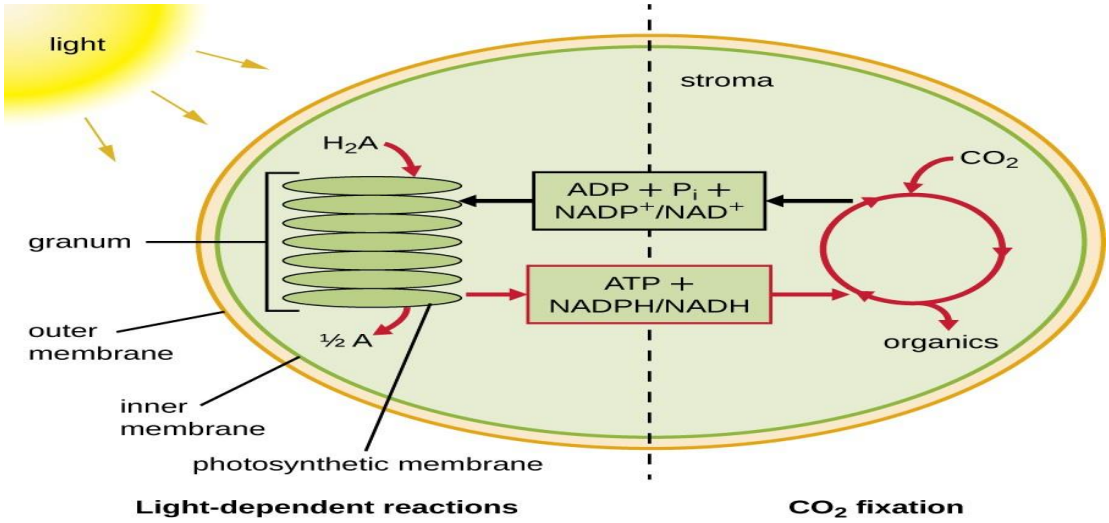


Figure 1. The light-dependent reactions of photosynthesis (left) convert light energy into chemical energy, forming ATP and NADPH. These products are used by the light-independent reactions to fix CO_2, producing organic carbon molecules.

Photosynthetic Structures in Eukaryotes and Prokaryotes

In all **phototrophic eukaryotes**, photosynthesis takes place inside a **chloroplast**, an organelle that arose in eukaryotes by endosymbiosis of a photosynthetic bacterium (see **Unique Characteristics of Eukaryotic Cells**). These chloroplasts are enclosed by a double membrane with inner and outer layers. Within the chloroplast is a third membrane that forms stacked, disc-shaped photosynthetic structures called **thylakoids** (Figure 2). A stack of thylakoids is called a **granum**, and the space surrounding the granum within the chloroplast is called **stroma**.

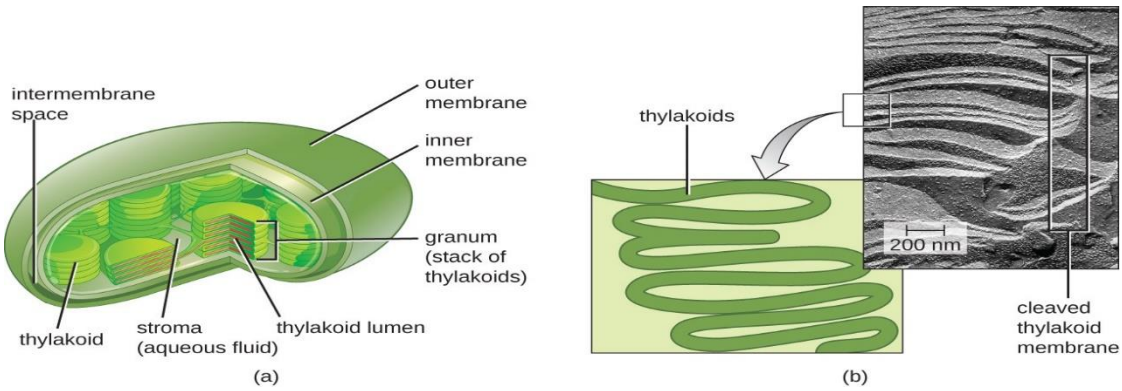


Figure 2. (a) Photosynthesis in eukaryotes takes place in chloroplasts, which contain thylakoids stacked into grana. (b) A photosynthetic prokaryote has infolded regions of the plasma membrane that function like thylakoids. (credit: scale bar data from Matt Russell.)

Photosynthetic membranes in prokaryotes, by contrast, are not organized into distinct membrane-enclosed organelles; rather, they are infolded regions of the plasma membrane. In cyanobacteria, for example, these infolded regions are also referred to as thylakoids. In either case, embedded within the thylakoid membranes or other photosynthetic bacterial membranes are **photosynthetic pigment**molecules organized into one or more photosystems, where light energy is actually converted into chemical energy.

Photosynthetic pigments within the photosynthetic membranes are organized into **photosystems**, each of which is composed of a light-harvesting (antennae) complex and a reaction center. The **light-harvesting complex** consists of multiple proteins and associated pigments that each may absorb light energy and, thus, become excited. This energy is transferred from one pigment molecule to another until eventually (after about a millionth of a second) it is delivered to the reaction center. Up to this point, only energy—not electrons—has been transferred between molecules. The **reaction center** contains a pigment molecule that can undergo oxidation upon excitation, actually giving up an electron. It is at this step in **photosynthesis** that light energy is converted into an excited electron.

Different kinds of light-harvesting pigments absorb unique patterns of wavelengths (colors) of visible light. Pigments reflect or transmit the wavelengths they cannot absorb, making them appear the corresponding color. Examples of photosynthetic pigments (molecules used to absorb solar energy) are **bacteriochlorophylls** (green, purple, or red), **carotenoids** (orange, red, or

yellow), **chlorophylls** (green), **phycocyanins** (blue), and **phycoerythrins** (red). By having mixtures of pigments, an organism can absorb energy from more wavelengths. Because photosynthetic bacteria commonly grow in competition for sunlight, each type of photosynthetic bacteria is optimized for harvesting the wavelengths of light to which it is commonly exposed, leading to stratification of microbial communities in aquatic and soil ecosystems by light quality and penetration.

Once the light harvesting complex transfers the energy to the reaction center, the reaction center delivers its high-energy electrons, one by one, to an electron carrier in an **electron transport system**, and electron transfer through the **ETS** is initiated. The ETS is similar to that used in **cellular respiration** and is embedded within the photosynthetic membrane. Ultimately, the electron is used to produce **NADH** or **NADPH**. The **electrochemical gradient** that forms across the photosynthetic membrane is used to generate **ATP** by chemiosmosis through the process of **photophosphorylation**, another example of **oxidative phosphorylation** (Figure 3).

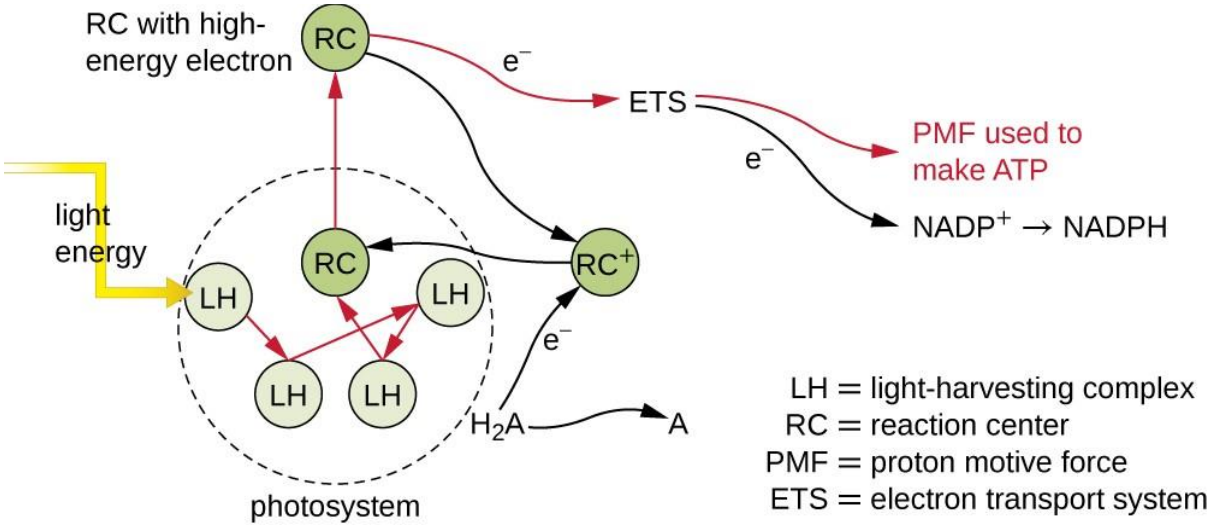


Figure 3. This figure summarizes how a photosystem works. Light harvesting (LH) pigments absorb light energy, converting it to chemical energy. The energy is passed from one LH pigment to another until it reaches a reaction center (RC) pigment, exciting an electron. This high-energy electron is lost from the RC pigment and passed through an electron transport system (ETS), ultimately producing NADH or NADPH and ATP. A reduced molecule (H_2A) donates an electron, replacing electrons to the electron-deficient RC pigment.

Oxygenic and Anoxygenic Photosynthesis

For photosynthesis to continue, the electron lost from the reaction center pigment must be replaced. The source of this electron (H_2A) differentiates the **oxygenic photosynthesis** of plants and cyanobacteria from **anoxygenic photosynthesis** carried out by other types of bacterial phototrophs (Figure 4). In oxygenic photosynthesis, H_2O is split and supplies the electron to the reaction center. Because oxygen is generated as a byproduct and is released, this type of photosynthesis is referred to as oxygenic photosynthesis. However, when other reduced compounds serve as the electron donor, oxygen is not generated; these types of photosynthesis are called anoxygenic photosynthesis. Hydrogen sulfide (H_2S) or thiosulfate ($S2O2-3$)($S2O32-$) can serve as the electron donor, generating elemental sulfur and sulfate ($SO2-4$)($SO42-$) ions, respectively, as a result.

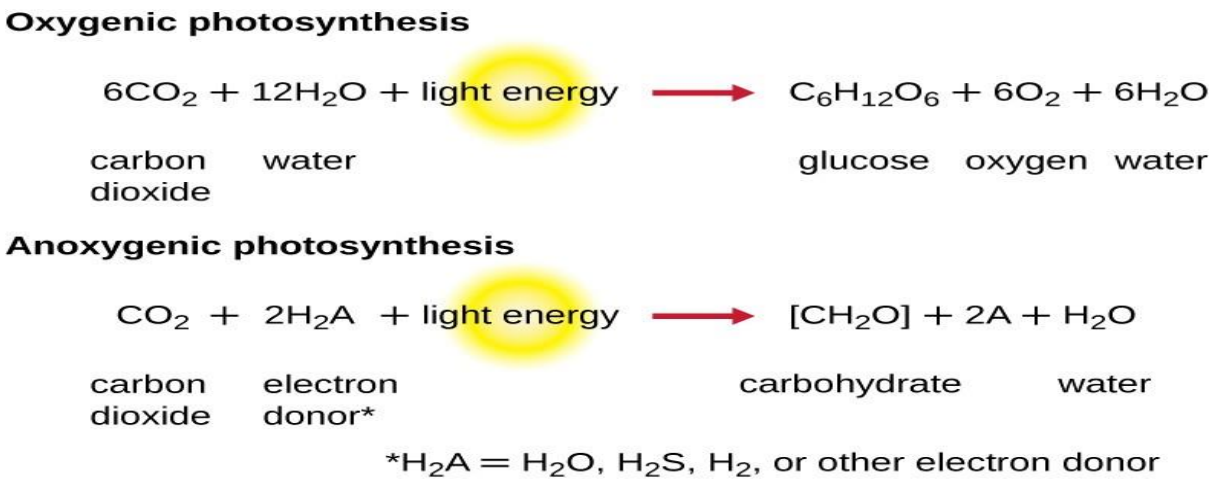


Figure 4. Eukaryotes and cyanobacteria carry out oxygenic photosynthesis, producing oxygen, whereas other bacteria carry out anoxygenic photosynthesis, which does not produce oxygen.

Photosystems have been classified into two types: **photosystem I (PSI)** and **photosystem II (PSII)** (Figure 5). Cyanobacteria and plant chloroplasts have both photosystems, whereas anoxygenic photosynthetic bacteria use only one of the photosystems. Both photosystems are excited by light energy simultaneously. If the cell requires both ATP and NADPH for biosynthesis, then it will carry out **noncyclic photophosphorylation**. Upon passing of the PSII reaction center electron to the ETS that connects PSII and PSI, the lost electron from the PSII reaction center is replaced by the splitting of water. The excited PSI reaction center electron is used to reduce $NADP^+$ to

NADPH and is replaced by the electron exiting the ETS. The flow of electrons in this way is called the **Z-scheme**.

If a cell's need for ATP is significantly greater than its need for NADPH, it may bypass the production of reducing power through **cyclic photophosphorylation**. Only PSI is used during cyclic photophosphorylation; the high-energy electron of the PSI reaction center is passed to an ETS carrier and then ultimately returns to the oxidized PSI reaction center pigment, thereby reducing it.

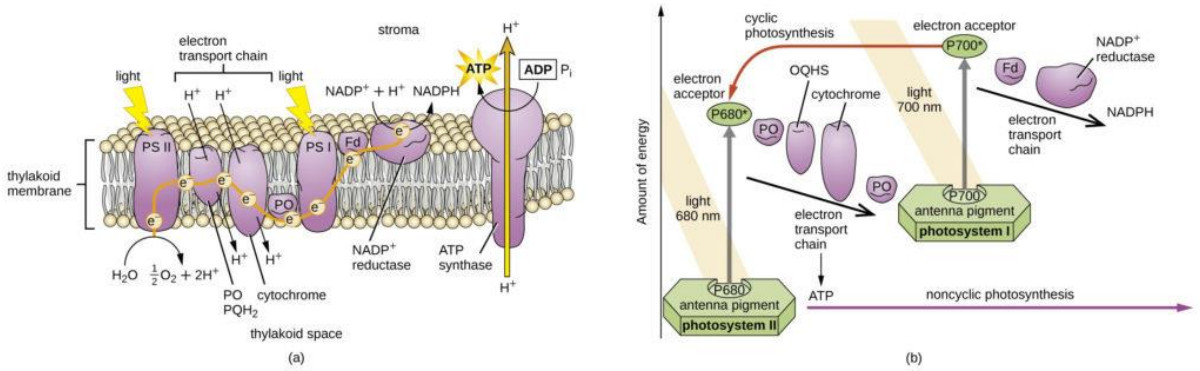


Figure 6.. (a) PSI and PSII are found on the thylakoid membrane. The high-energy electron from PSII is passed to an ETS, which generates a proton motive force for ATP synthesis by chemiosmosis, and ultimately replaces the electron lost by the PSI reaction center. The PSI reaction center electron is used to make NADPH. (b) When both ATP and NADPH are required, noncyclic photophosphorylation (in cyanobacteria and plants) provides both. The electron flow described here is referred to as the Z-scheme (shown in yellow in [a]). When the cell's ATP needs outweigh those for NADPH, cyanobacteria and plants will use only PSI, and its reaction center electron is passed to the ETS to generate a proton motive force used for ATP synthesis.

Light-Independent Reactions

After the energy from the sun is converted into chemical energy and temporarily stored in ATP and NADPH molecules (having lifespans of millionths of a second), photoautotrophs have the fuel needed to build multicarbon carbohydrate molecules, which can survive for hundreds of millions of years, for long-term energy storage. The carbon comes from CO_2, the gas that is a waste product of cellular respiration.

The **Calvin-Benson cycle** (named for Melvin Calvin [1911–1997] and Andrew Benson [1917–2015]), the biochemical pathway used for fixation of CO_2, is located within the cytoplasm of photosynthetic bacteria and in the stroma of eukaryotic chloroplasts. The **light-independent reactions** of the Calvin cycle can be organized into three basic stages: fixation, reduction, and regeneration (see **Metabolic Pathways** for a detailed illustration of the Calvin cycle).

- **Fixation**: The enzyme **ribulose bisphosphate carboxylase (RuBisCO)** catalyzes the addition of a CO_2 to **ribulose bisphosphate (RuBP)**. This results in the production of **3-phosphoglycerate (3-PGA)**.
- **Reduction**: Six molecules of both ATP and NADPH (from the light-dependent reactions) are used to convert 3-PGA into glyceraldehyde 3-phosphate (G3P). Some G3P is then used to build glucose.
- **Regeneration**: The remaining G3P not used to synthesize glucose is used to regenerate RuBP, enabling the system to continue CO_2 fixation. Three more molecules of ATP are used in these regeneration reactions.

The Calvin cycle is used extensively by plants and photoautotrophic bacteria, and the enzyme RuBisCO is said to be the most plentiful enzyme on earth, composing 30%–50% of the total soluble protein in plant chloroplasts.[1] However, besides its prevalent use in photoautotrophs, the Calvin cycle is also used by many nonphotosynthetic chemoautotrophs to fix CO_2. Additionally, other bacteria and archaea use alternative systems for CO_2 fixation. Although most bacteria using Calvin cycle alternatives are chemoautotrophic, certain green sulfur photoautotrophic bacteria have been also shown to use an alternative CO_2 fixation pathway.

Diversity of Metabolism in Procaryotes

Introduction

A lot of hoopla is made about microbial diversity. The unicellular eucaryotes (protista) exhibit a fair amount of structural diversity, but the procaryotes (bacteria and archaea) lack this distinction. There are but a few basic morphologies, the possibilities of motility and resting cells (spores), and a major differential stain (the Gram stain) that differentiates procaryotes microscopically. So what is all the hoopla about regarding procaryotes? It is about biochemical or **metabolic diversity**, especially as it relates to energy-

generating metabolism and biosynthesis of secondary metabolites. The procaryotes, as a group, conduct all the same types of basic metabolism as eucaryotes, but, in addition, there are several types of energy-generating metabolism among the procaryotes that are non existent in eucaryotic cells or organisms. The diversity of procaryotes is expressed by their great variation in modes of energy generation and metabolism, and this feature allows procaryotes to flourish in all habitats suitable for life on earth.
Even within a procaryotic species, there may be great versatility in metabolism. Consider *Escherichia coli.* The bacterium can produce energy for growth by fermentation or respiration. It can respire aerobically using O_2 as a final electron acceptor, or it can respire under anaerobic conditions, using NO_3 or fumarate as a terminal electron acceptor. *E. coli* can use glucose or lactose as a sole carbon source for growth, with the metabolic ability to transform the sugar into all the necessary amino acids, vitamins and nucleotides that make up cells. A relative of*E. coli*, *Rhodospirillum rubrum,* has all the heterotrophic capabilities as *E. coli*, plus the ability to grow by photoautotrophic, photoheterotrophic or lithotrophic means. It does require one growth factor, however; biotin must be added to its growth media.

Fundamentally, most eucaryotes produce energy (ATP) through alcohol fermentation (e.g. yeast), lactic acid fermentation (e.g. muscle cells, neutrophils), aerobic respiration (e.g. molds, protozoa, animals) or oxygenic photosynthesis (e.g. algae, plants). These modes of energy-generating metabolism exist among procaryotes, in addition to all the following types of energy production which are virtually non existent in eucaryotes.

Unique fermentations proceeding through the Embden-Meyerhof pathway

Other fermentation pathways such as the phosphoketolase (heterolactic) and Entner-Doudoroff pathways

Anaerobic respiration: respiration that uses substances other than O_2 as a final electron acceptor

Lithotrophy: use of inorganic substances as sources of energy

Photoheterotrophy: use of organic compounds as a carbon source during bacterial photosynthesis

Anoxygenic photosynthesis: photophosphorylation in the absence of O_2

Methanogenesis: an ancient type of archaean metabolism that uses H_2 as an energy source and produces methane

Light-driven nonphotosynthetic photophosphorylation: unique archaean metabolism that converts light energy into chemical energy

In addition, among autotrophic procaryotes, there are three ways to fix CO_2, two of which are unknown among eucaryotes, the **CODH (acetyl CoA pathway)** and the **reverse TCA cycle**.

Energy-Generating Metabolism The term **metabolism** refers to the sum of the biochemical reactions required for energy generation AND the use of energy to synthesize cell material from small molecules in the environment. Hence, metabolism has an **energy-generating component**, called **catabolism**, and **an energy-consuming**, **biosynthetic component**, called **anabolism**. Catabolic reactions or sequences produce energy as **ATP**, which can be utilized in anabolic reactions to build cell material from nutrients in the environment. The relationship between catabolism and anabolism is illustrated in Figure 1 below.

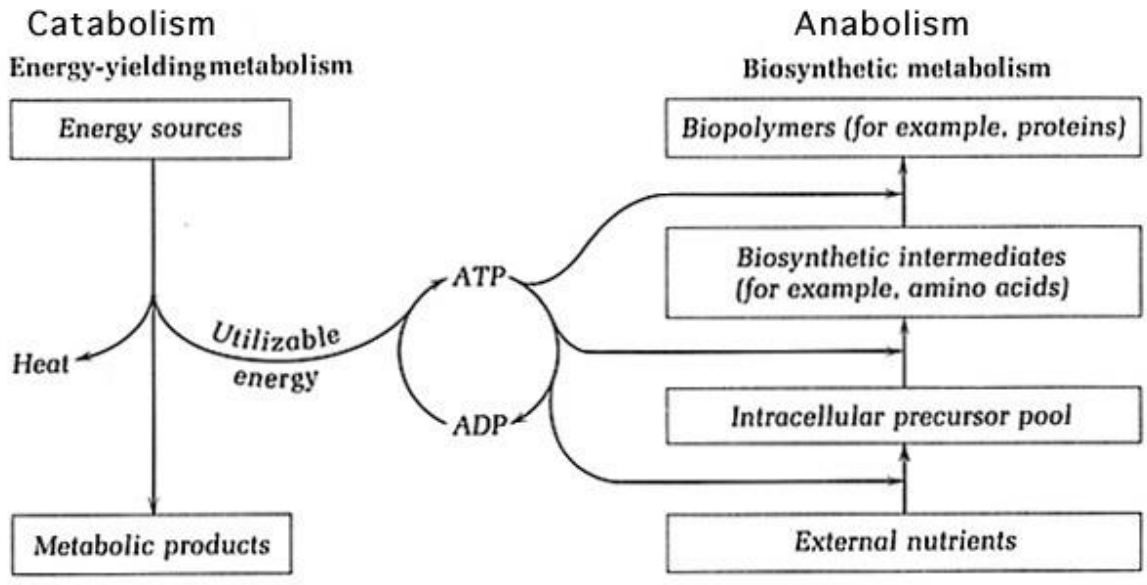


Figure 1. The relationship between catabolism and anabolism in a cell. During catabolism, energy is changed from one form to another, and keeping with the laws of thermodynamics, such energy transformations are never completely efficient, i.e., some energy is lost in the form of heat. The efficiency of a catabolic sequence of reactions is the amount of energy made available to the cell (for anabolism) divided by the total amount of energy released during the reactions.

ATP

During catabolism, useful energy is temporarily conserved in the "high energy bond" of **ATP - adenosine triphosphate**. No matter what form of energy a cell uses as its primary source, the energy is ultimately transformed and conserved as ATP - the universal currency of energy exchange in biological systems. When energy is required during anabolism, it may be spent as the high energy bond of ATP which has a value of about 8 kcal per mole. Hence, the conversion of ADP to ATP requires 8 kcal of energy, and the hydrolysis of ATP to ADP releases 8 kcal.

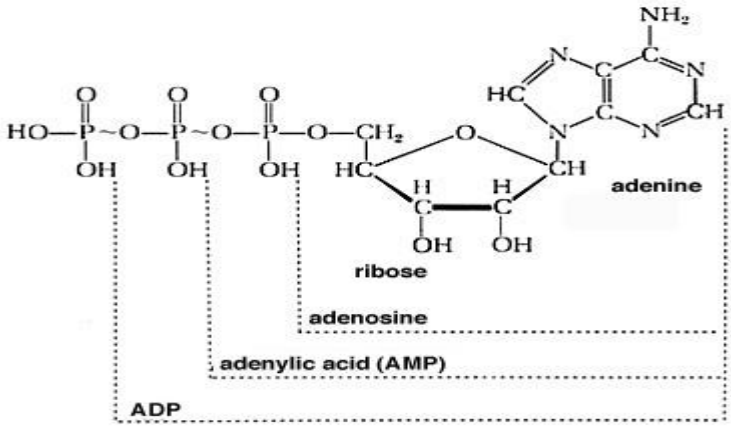


Figure 2. The structure of ATP. ATP is derived from the nucleotide adenosine monophosphate (AMP) or adenylic acid, to which two additional phosphate groups are attached through pyrophosphate bonds (~P). These two bonds are energy rich in the sense that their hydrolysis yields a great deal more energy than a corresponding covalent bond. ATP acts as a coenzyme in energetic coupling reactions wherein one or both of the terminal phosphate groups is removed from the ATP molecule with the bond energy being used to transfer part of the ATP to another molecule to activate its role in metabolism. For example, Glucose + ATP -----> Glucose-P + ADP or Amino Acid + ATP ----->AMP-Amino Acid + PPi.

Because of the central role of ATP in energy-generating metabolism, expect to see its involvement as a coenzyme in most energy-producing processes in cells.

Autotrophic Metabolism

utotrophy generally means the ability of organisms to use inorganic carbon in the form of CO_2 as the sole source of carbon for synthesizing organic compounds necessary to build cell components. This ability is also sometimes called carbon-autotrophy to distinguish the ability of some organisms to use molecular nitrogen as the sole source of nitrogen.
Such organisms are referred to sometimes as nitrogen autotrophs. However, the term autotrophy is commonly used for carbon autotrophy. Primarily, this

property is present in plants, algae and phototrophic bacteria including cyanobacteria.

Besides these organisms, all of which carry out photosynthesis, there are several groups of non-photosynthetic bacteria which can grow using CO_2 as sole source of carbon by virtue of their ability to oxidize inorganic compounds. Such organisms are chemoautotrophic or chemolithotrophic.

CO_2 is the end-product of aerobic respiration, a process which releases the energy of respiratory substrates. Carbon dioxide is, therefore, poor in energy content. In autotrophic metabolism, this energy-poor compound is used to build organic molecules which are much richer in energy content.
It is easily understandable therefore, that conversion of CO_2 to organic compound requires input of energy from an external source. The ultimate source in case of photosynthesis is radiant energy and in case of chemolithotrophy is oxidation energy of inorganic chemical compounds. In either case, the immediate source of energy for driving the endergonic reaction involved in conversion of CO_2 to organic compounds is ATP.
In photosynthesis, ATP is generated with the help of photosynthetic pigments through a process known as photophosphorylation. In chemoautotrophy, the energy of oxidation of inorganic compounds is channelized into the respiratory chain for ATP synthesis by oxidative phosphorylation.

Besides ATP, reduction of CO_2 to organic molecules e.g. glucose, requires an external source of hydrogen and electrons. In green plant photosynthesis, water acts as the terminal hydrogen donor or reductant, whereby water is oxidized to molecular oxygen which is released as by-product. In case of phototrophic anoxygenic bacteria, inorganic or organic molecules, such as H_2, H_2S, $S_2O_3^{--}$ Alcohols etc. act as the external H-donor. Hydrogen and electrons donated by these external donors are transferred to NAD or NADP producing $NADH_2$ or $NADPH_2$ which are the biochemically utilizable reductants used by the cells for reduction of CO_2 to organic compounds. $NADH_2$ and $NADPH_2$ constitute the reducing force of CO_2-reduction.
Thus, autotrophic metabolism can be considered to consist of two sets of reactions. In one set, ATP and reducing force are generated and, in the other set of reactions they are utilized for reduction of CO_2 to organic compounds. The first sets of reactions are different in phototrophic and non- phototrophic autotrophs.

But the second set of reactions is common between the two groups. In majority of autotrophs, the reactions involved in reduction of CO_2 proceed via a cyclic pathway, known as the reductive pentose phosphate pathway or, more commonly, as the Calvin-Benson cycle, although other pathways are also known to operate in some organisms, both in the phototrophic green plants and bacteria. Reduction of CO_2 to yield organic compounds is commonly known as CO_2-fixation.

It is evident, therefore, that there is a close parallelism between the phototrophic and non- phototrophic autotrophic metabolism. While all phototrophic organisms carry out some type of photosynthesis, the non-green autotrophs carry out an allied process which was designated by Winogradsky as chemosynthesis.

The parallelism between these two modes of metabolism is schematically represented in Fig. 8.59:

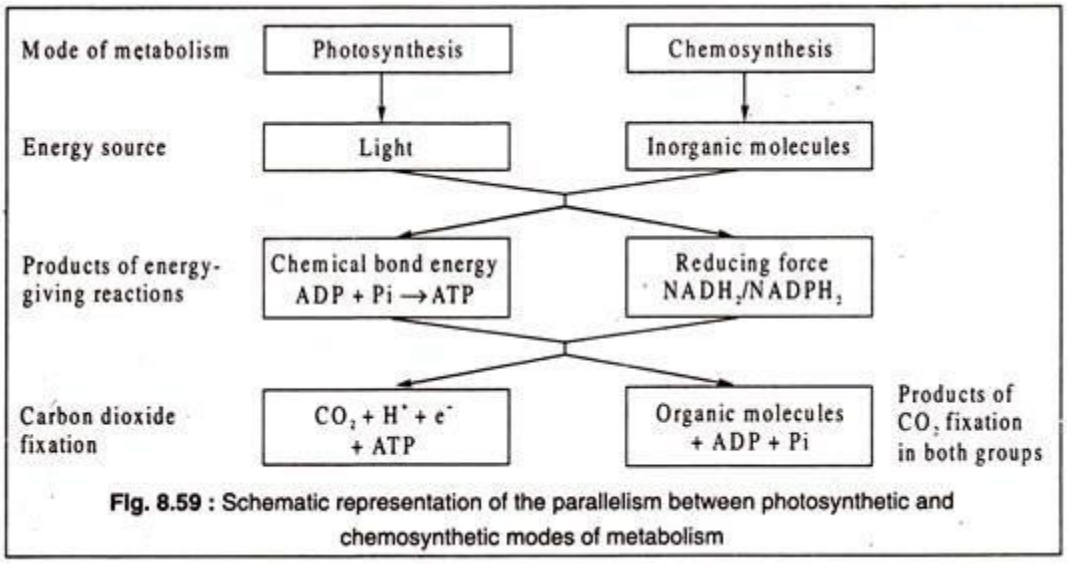


Fig. 8.59 : Schematic representation of the parallelism between photosynthetic and chemosynthetic modes of metabolism

(i) ***Chemoautotrophy****:*

Chemoautotrophy or chemolithotrophy is a mode of metabolism restricted to several specialized groups of non-photosynthetic autotrophic bacteria. Some of these bacteria are capable of growing both chemoorganotrophically and chemo-auto-trophically i.e. they are facultative autotrophs. Examples of this type are Alcaligenes eutrophus and Nitrococcus oceanus.

Other chemoautotrophic bacteria are obligate in nature, because they are unable to utilize organic compounds as carbon source. The best examples of this type are the species of the genera Nitrosomonas and Thiobacillus. Some species of Thiobacillus are, however, facultative.

Depending on the oxidisable inorganic substrate, the chemoautotrophic bacteria can be distinguished into the following groups: the nitrifying bacteria, the sulfur oxidising bacteria, the hydrogen oxidizing bacteria, the iron oxidising bacteria and the carbon monoxide bacteria.

All of them are strictly aerobic, except some which are facultative anaerobes and can grow in absence of free oxygen utilizing nitrate as an alternative electron acceptor (nitrate respiration). Examples of this latter group are Paracoccus denitrificans and Thiobacillus denitrificans.

(a) Nitrifying bacteria and nitrification:
Nitrification is a natural process carried out by the nitrifying bacteria occurring in soil and aquatic bodies. It involves oxidation of ammonia liberated by decomposition of nitrogenous organic matter, like proteins, nucleic acids, urea etc. The oxidation takes place in two steps — ammonia to nitrous acid and nitrous acid to nitric acid.

The acids react with metal ions to produce the corresponding salts, viz. nitrite and nitrate. Nitrate acts as the main nitrogen source of plants, although many plants can also use ammonium ion as nitrogen source. The negatively charged nitrate ions ($N0_3^-$) have greater diffusibility in soil compared to that of positively charged ammonium ion (NH_4^+), because the soil particles (clay), which are also negatively charged, tend to bind positively charged ammonium ions more tightly preventing their diffusion.
The two-step nitrification process is carried out by two different groups of bacteria. The first step involving oxidation of ammonia to nitrous acid is called nitrosification and the organisms are accordingly known as nitrosifying bacteria.

The most important representative of this group is Nitrosomonas. The members of this genus are highly aerobic and strictly autotrophic. They are incapable of utilizing any organic compound and can grow only in purely inorganic salts medium. The energy-yielding oxidation reaction of these bacteria can be represented as $2\ NH4 + 3O_2 \longrightarrow 2\ NO^-_2 + 4H^+ + 2H_2O$. The organisms carrying out this reaction possess the enzyme ammonia dehydrogenase.
The second step of nitrification involves oxidation if nitrous acid to nitric acid and the organisms are known as nitrifying bacteria. The most well-known genus of the group is Nitrococcus. The reaction occurring in these bacteria can be represented as $2NO_2^- + O_2 \longrightarrow 2NO^-_3$. The enzyme catalyzing this reaction is

nitrous acid dehydrogenase. In contrast to the obligately autotrophic Nitrosomonas species, Nitrobacter can grow both auto-trophically as well as using acetate as carbon source. Thus, Nitrobacter species are facultative chemoautotrophs.

The organisms of both groups are capable of generating ATP by oxidative phosphorylation in course of electron transport through the cytochrome system of the respiratory chain and the final electron acceptor is oxygen. ATP generated in this way is utilized for CO_2-fixation by the Calvin- Benson cycle. However, the question of how $NADH_2$ is generated in these bacteria — particularly in the nitrite-oxidizing bacteria — is not easily explainable. This is because the oxidisable substrate, nitrite (NO^-_2) has a more positive (or less negative) redox potential than that of NAD. This does not permit spontaneous flow of electrons from NO_2upstream to NAD, unless energy is expended.

It has been suggested, therefore, that part of the ATP generated by oxidative phosphorylation is spent for driving electrons from nitrite to NAD through a reverse electron transport i.e. from a less negative redox potential to a more negative one. Thus, generation of the reducing force ($NADH_2$) in these bacteria appears to be an energy-consuming process.

(b) Sulfur oxidizing bacteria:

Oxidation of elemental sulfur (S^0) and various reduced sulfur compounds, like sulfide (S^{2-}), thiosulfate ($S_2O^2_3{}^-$) etc. takes place in soil and aquatic bodies mediated by a great variety of bacteria including both eubacteria and archaebacteria. However, all of them are not chemoautotrophic i.e. they cannot utilize the oxidation energy for autotrophic growth. The best-known among the sulfur-oxidising eubacteria are the members of the genus Thiobacillus. Some species, like T. thiooxidans, T. thioparus and T. denitrificans are obligately chemoautotrophic, while other species, like T. novellus or T. intermedius are facultative and can utilize also organic compounds by heterotrophic pathways. Thiobacilli in general are strongly aerobic, but T. denitrificans can also grow anaerobically carrying out nitrate respiration.

Among sulfur-oxidising archaebacteria, Sulfolobus, an aerobic thermophilic as well as acidophilic organism (optimum temperature 75°C and optimum pH 1.5-3.5) is a facultative chemoautotroph. Another one, Thermoproteus is an anaerobic thermophile (70°-90°C) which can grow facultatively as an autotroph oxidizing either sulfur or hydrogen.

Some eubacteria, designated as filamentous sulfur-oxidising bacteria, belonging to the genera Beggiatoa, Thiothrix etc. are able to oxidize sulfide (H_2S) to

elemental sulfur (S^0). However, their ability to use the oxidation energy for CO_2-fixation remains controversial. The anoxygenic sulfur purple and green bacteria, like Chromatium, Chlorobium etc. are also able to oxidize sulfide to sulfur. They use sulfide as hydrogen donor in anoxygenic photosynthesis, just as green plants use water.

A remarkably large spherical bacterium (750 μm in diameter) was discovered in 1999 in the coastal sediments collected from a depth of more than 100 meter near Namibia (South West Africa). The organism has been named as Thiomargarita namibiensis. It is capable of growing autotrophically utilizing oxidation of hydrogen sulfide, and nitrate is used as terminal electron acceptor (nitrate respiration). The organism oxidizes H_2S to S° and the sulfur globules are deposited intracellularly. This happens also in case of Beggiatoa, Thiothrix, Chromatium etc.

Thiobacilli oxidize elemental sulfur or sulfur compounds to sulfuric acid.

The reactions can be represented as:

$$H_2S + 2O_2 \rightarrow H_2SO_4 \; \Delta \; SO_4^{2-} + 2H^+$$

$$2S^0 + 2H_2O + 3O_2 \rightarrow 2H_2SO_4 \; \Delta \; 2SO_4^{2-} + 4H^+$$

$$S_2O_3^{2-} + H_2O + 2O_2 \rightarrow 2SO_4^{2-} + 2H^+$$

Hydrogen and electrons are transferred from the substrates in course of oxidation to specific acceptors which are then end-oxidised via electron transport system to produce ATP. The production of $NADH_2$ is faced with the same problem as with nitrite oxidation, because electrons liberated by oxidation of sulfur, sulfide or thiosulfate cannot be transferred directly to NAD as their redox-potentials are more positive than that of NAD. Therefore, $NADH_2$ is presumably produced by an ATP-consuming reverse electron flow in case of sulfur-oxidising bacteria also.

Thiobacilli fix CO_2 via Calvin-Benson cycle in which ATP and $NADH_2$ provide energy and reducing force, respectively. That this cycle operates in chemoautotrophic bacteria was first demonstrated in Thiobacillus denitrificans.

Thiobacillus thiooxidans is outstanding for its very high acid tolerance. It can grow even at pH 0. This property has been practically utilized for reclamation of alkaline soils. Soils having excess of Ca-carbonate or oxide are treated with elemental sulfur in powder form which stimulates growth of T. thiooxidans in soil. The bacteria produce sulfuric acid and converts calcium salts into

$CaSO_4$ which is more soluble than the carbonate. By leaching, the sulfate can be gradually removed.
Thiobacilli producing plenty of sulfuric acid are also used for extraction of metals by the leaching process. For example, T. ferrooxidans, which is highly resistant to copper, is utilized for extraction of copper from low-grade ores. Such ores are mixed with iron-pyrites (FeS). Thiobacilli grow and produce sulfuric acid from the sulfide which leaches out copper as copper sulfate in the leachate from where copper in recovered.

(c) Hydrogen oxidizing bacteria:
A diverse group of bacteria, both eubacteria and archaebacteria possess the ability to oxidize molecular hydrogen. Many of them can use the energy of hydrogen oxidation for autotrophic growth. However, most of these bacteria are facultative i.e. they can also grow as heterotrophs using a variety of organic compounds as carbon and energy source. Some like Paracoccus denitrificans are mixotrophic a type of metabolism in which part of the carbon requirement is fulfilled by $CO_2$and a part by some organic compound.
Among the well-characterized hydrogen oxidizing bacteria are Alcaligenes (formerly Hydrogenomonas) eutrophus, A. ruhlandii, Pseudomonas facilis, Xanthobacter autotrophicum, Azospirillum lipoferum, Nocardia opaca, Paracoccus denitrificans etc. All of them are facultative chemoautotrophs. They can grow in an inorganic salts medium when the atmosphere contains about 70% H_2, 20% O_2 and 10% CO_2.
Some non-pathogenic mycobacteria can also grow as facultative autotrophs. A number of extremophilic archaebacteria can grow as hydrogen-bacteria e.g. Thermoproteus (facultative), Pyrodictium (anaerobic, obligately chemoautotrophic) and Methanococcus jannaschii (obligate).

Under autotrophic conditions i.e. in absence of any organic carbon source and in presence of an atmosphere containing H_2, O_2 and CO_2, the hydrogen-oxidising enzyme, hydrogenase, is synthesized in the hydrogen bacteria. The energy-yielding hydrogen oxidation catalysed by this enzyme can be represented as $2H_2 + O_2 \text{—>} 2H_2O$ ($-\Delta G$ =112 Kcal).
The enzyme hydrogenase performs two important functions. Firstly, it can transfer hydrogen directly to NAD producing $NADH_2$ which is the reducing force required for CO_2 reduction via Calvin-Benson cycle. Secondly, the enzyme can transfer electrons to the electron transport chain to generate ATP by oxidative phosphorylation. Hydrogenase is a nickel containing enzyme and the

bacteria require Ni^{2+} for autotrophic growth, but not when they grow in organic media carrying out heterotrophic metabolism. Obviously, hydrogenase is synthesized only under autotrophic conditions.

In some hydrogen-bacteria the hydrogenase is of two types — one is in soluble state (cytoplasmic) and the other is particle-bound (membrane associated). In such organisms the two types perform separate functions. While the particulate type is involved in supplying electrons donated by hydrogen to ubiquinone of the respiratory chain, the soluble type transfers hydrogen and electrons for reduction of NAD to $NADH_2$.

The functions of these two types of hydrogenase are diagrammatically shown in Fig. 8.60:

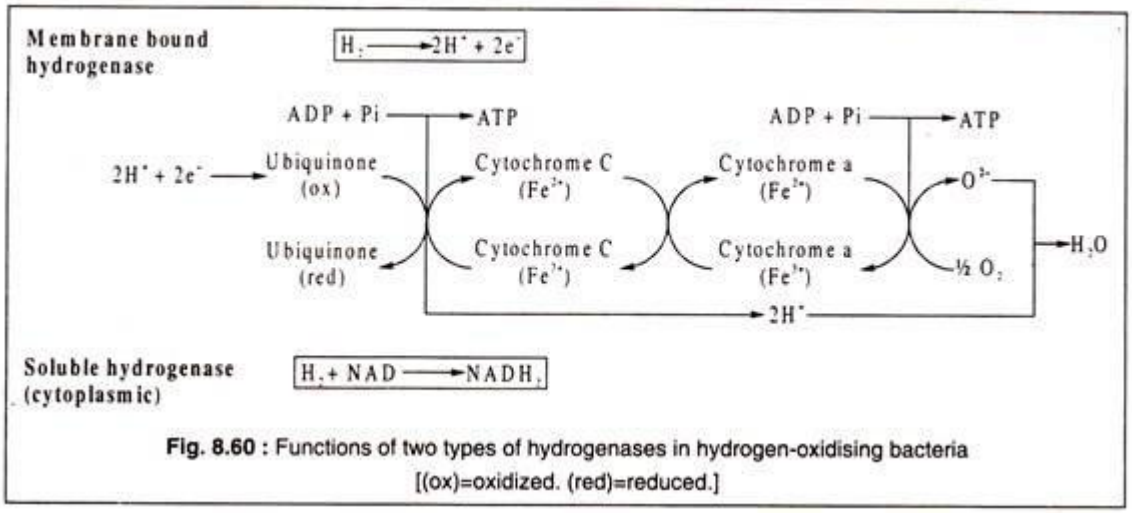


Fig. 8.60 : Functions of two types of hydrogenases in hydrogen-oxidising bacteria [(ox)=oxidized. (red)=reduced.]

Some hydrogen bacteria have both membrane-bound as well as soluble hydrogenases, e.g. Alcaligenes eutrophus, A. ruhlandii, Pseudomonas saccharophila etc. Some others have only one type e.g. Nocardia opaca and N. autotrophica. They contain only the soluble NAD-reducing hydrogenase. On the other hand Paracoccus denitrificans, Aquaspirillum autotrophicum, Xanthobacter autotrophicus, as well as several pseudomonads have only the membrane-bound type.

(d) Carboxido bacteria:

A group of aerobic bacteria are capable of chemoautotrophic growth by virtue of their ability to oxidize carbon monoxide CO to CO_2. These organisms can also grow as hydrogen bacteria. Carboxidobacteria can grow in a mineral-salt medium with an atmosphere containing CO and O_2. The oxidation reaction is catalysed by the enzyme carbon monoxide oxidoreductase. It can be represented as $CO + H_2O \; CO_2 + 2H^+ + 2e^-$.

Carboxidobacteria belong to the genera Alcaligenes, Pseudomonas etc. All of them have a membrane-bound hydrogenase, while the CO-oxidising enzyme is a soluble cytoplasmic protein.

(e) Iron-oxidising bacteria:

The only iron (Fe^{2+}) oxidizing bacterium that is definitely known to be capable of chemoautotrophic growth is Ferrobacillus ferro-oxidans (= Thiobacillus ferrooxidans). It is closely related to Thiobacillus thiooxidans. The organism is able to oxidize Fe^{2+} to Fe^{3+} vigorously in an acidic environment (pH 3.0) according to the following reaction. $4Fe^{2+} + 4H^{+} + O_2 \longrightarrow 4\ Fe^{3+} + 2H_2O$ ($-\Delta G$ = 40 Kcal). The oxidation energy is utilized for fixation of CO_2. For assimilation of one μM of CO_2, 50 μM of Fe^{2+} is oxidized to Fe^{3+}.

Ferro-bacilli can also oxidize elemental sulfur (S^0) to sulphate like the sulfur-oxidising bacteria. This property has been commercially exploited for removal of sulfur from iron ore which improves the quality of the ore and makes it more suitable for extraction of metallic iron.

Several other bacteria, like Gallionella ferruginea, a stalked bacterium and Leptothrix ochracea can oxidize ferrous salts present in the water in which they grow to ferric oxide. The insoluble ferric oxide is deposited on their cell wall as incrustation. However, their ability of chemoautotrophic growth at the expense of the oxidation of ferrous iron to ferric iron has not been decisively proved.

(ii) Photo trophy:

Photo trophy refers to an autotrophic mode of metabolism in which organisms are able to harness light energy with the help of photosynthetic pigments and convert it to chemical bond energy in the form of ATP (photophosphorylation). Phototrophic organisms, like the chemoautotrophs, are able to form organic compounds from carbon dioxide, generally through the Calvin-Benson cycle. The two processes i.e. generation of ATP by photophosphorylation and carbon dioxide fixation, together constitute photosynthesis.

Primarily, photosynthesis is a property of plants and different groups of algae and some chlorophyllous protozoans. All of them, including the cyanobacteria (which are also known as blue- green algae), carry out photosynthesis with oxygen as by-product. Therefore, the type of photosynthesis is known as oxygenic. There are also some bacteria which contain bacteriochlorophylls and can carry out photosynthesis, but without oxygen evolution (anoxygenie).

In a broad sense, photosynthesis can be defined as a process in which light energy is used to promote cellular energy-requiring biochemical events. The capacity of photosynthesis is attributable to the presence of special pigments, called chlorophylls and carotenoids which are associated with lipoproteins to form complexes.

Photosynthesis includes two closely linked, but distinct processes. The first of these comprises the reactions by which light energy is absorbed by the photosynthetic pigments and transformed into chemical bond energy. These reactions are photochemical in nature and are known as light reactions. The second process includes enzyme-catalysed biochemical reactions involving CO_2-fixation in which light has no direct role.
These reactions are called dark-reactions. A notable feature of photochemical reactions which distinguishes them from chemical or biochemical reactions is that the reaction velocity is independent of temperature i.e. the reaction proceeds with the same velocity at, say, zero degree and 30°C. In case of chemical and biochemical reactions, the reaction velocity typically doubles with every 10°C rise of temperature within limits. In enzyme-catalysed reactions, the enzyme protein generally loses catalytic activity above 35° to 40°C.

The usual products of light reactions are ATP and $NADH_2$or $NADPH_2$. These products are used in the dark reactions for synthesis of sugars or other organic compounds from CO_2. The dark reactions are common to both photoautotrophs and chemoautotrophs. The two groups differ mainly on the mode of generation of ATP and reducing force ($NADH_2$/ $NADPH_2$).

(a) Photosynthetic light reactions:

The first step in photosynthesis is the absorption of photons by a series of light harvesting pigments in pigment-protein complexes. These are called antenna-complexes, because they collect light of different wave-lengths. For example, the different chlorophylls generally absorb light of longer wavelengths extending up to infra-red regions by some bacteriochlorophylls.

On the other hand, carotenoids absorb light of shorter wavelengths, generally in the yellow region of the spectrum. The light energy absorbed by the antenna system is transmitted to a special photo-reactive centre, known as the reaction centre which is also a pigment-protein complex located in the photosynthetic lamellae.

The lamellae are part of chloroplasts in case of eukaryotic organisms, and in prokaryotic organisms they are the intracellular membrane system produced by invagination of the cytoplasmic membrane (chromatophores).

The reaction centre pigment complex — on being excited by energy transferred from the antenna pigments — ejects energy-rich electrons which are accepted by the primary electron acceptor, ferredoxin. Electrons from ferredoxin are then transferred to the secondary electron acceptors at a velocity which is faster than that of electron transfer from the reaction centre pigment to ferredoxin.

As a result, the reaction centre remains positively charged (due to loss of electrons). Appropriate positioning of secondary electron acceptors leads to an electron transport in one direction across the membrane and proton transport in an opposite direction with the consequent generation of an electric field (due to charge separation). The charge separation is utilized for ATP generation by the chemiosmotic mechanism.

The overall electron flow resulting in ATP generation in photosynthesis can be of two main types, — cyclic and non-cyclic. In the cyclic type, the high-energy electrons ejected by the reaction centre pigment flow through a series of electron acceptors from a higher energy level to a gradually lower energy level and return to the reaction centre, forming thereby a close circuit. The loss of energy of electrons in this cyclic path is utilized for phosphorylation of ADP to ATP. The only product of cyclic path is ATP. No $NADH_2$ is produced.
In the non-cyclic pathway of photosynthetic electron-transport, electrons ejected by the reaction centre pigment complex and accepted by ferredoxin are used for reduction of NAD/NADP. It becomes necessary, therefore, to draw electrons from an exogenous source, so that the reaction centre can be re-oxidised to its ground state. In case of green plants and cyanobacteria, water acts as the electron donor. Water is photolysed by chlorophyll to yield H^+ and $(OH)^-$. Protons are used for reduction of NADP, while electrons of $(OH)^-$ are passed on to the positively charged reaction centre through cytochromes, and molecular oxygen (O_2) is evolved. In green plant photosynthesis two light reactions occur, Photosystem I and Photosystem II. The pigment complexes of these two systems are called P700 and P680, respectively.
In anoxygenic bacterial photosynthesis the situation is somewhat different. Firstly, in bacteria there is only one photosystem which is Photosystem I. Photosystem II, which is involved in oxygen evolution through photolysis of

water, is absent. In bacterial non-cyclic electron transport, the exogenous electron donor may be H_2, H_2S, S^O, $S_2O_3^{2-}$ or even organic compounds.

The cyclic pathway of electron transport is diagrammatically shown in Fig. 8.61:

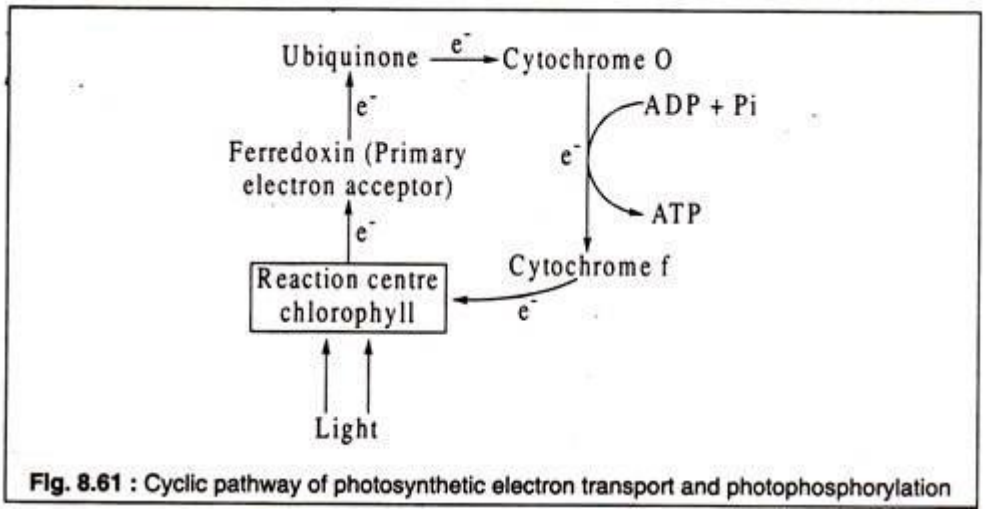


Fig. 8.61 : Cyclic pathway of photosynthetic electron transport and photophosphorylation

The non-cyclic pathway of green plant photosynthesis is shown in Fig. 8.62 and a tentative non- cyclic pathway of anoxygenic photosynthesis is represented in Fig. 8.63 A and 63 B:

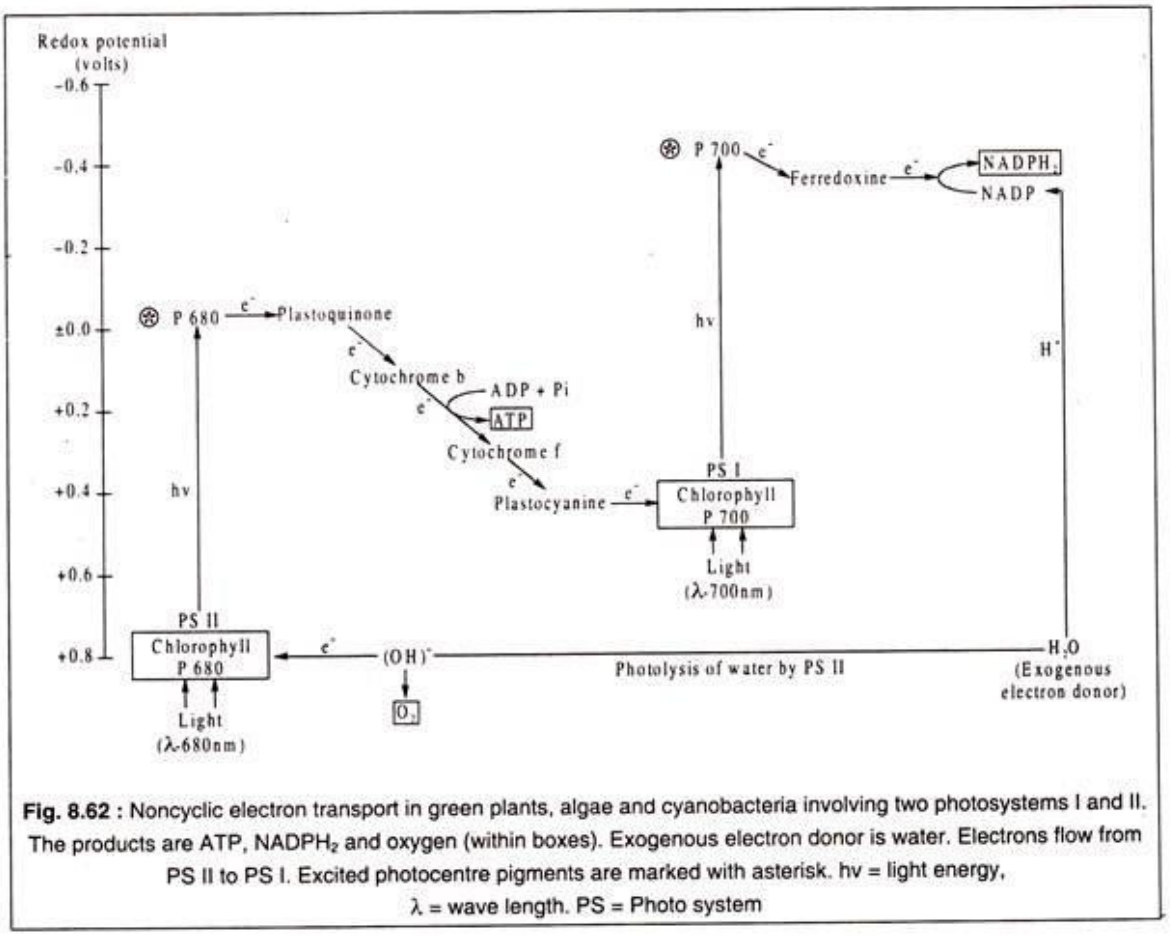


Fig. 8.62 : Noncyclic electron transport in green plants, algae and cyanobacteria involving two photosystems I and II. The products are ATP, $NADPH_2$ and oxygen (within boxes). Exogenous electron donor is water. Electrons flow from PS II to PS I. Excited photocentre pigments are marked with asterisk. hv = light energy, λ = wave length. PS = Photo system

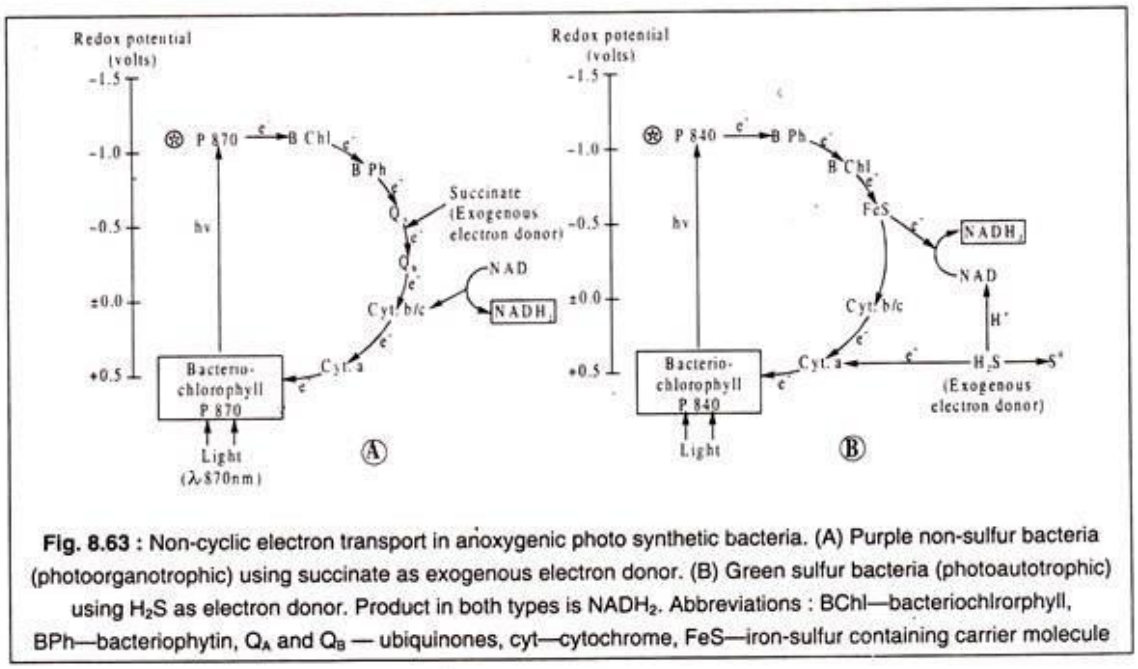


Fig. 8.63 : Non-cyclic electron transport in anoxygenic photo synthetic bacteria. (A) Purple non-sulfur bacteria (photoorganotrophic) using succinate as exogenous electron donor. (B) Green sulfur bacteria (photoautotrophic) using H_2S as electron donor. Product in both types is $NADH_2$. Abbreviations : BChl—bacteriochlrorphyll, BPh—bacteriophytin, Q_A and Q_B — ubiquinones, cyt—cytochrome, FeS—iron-sulfur containing carrier molecule

In the anoxygenic photosynthetic bacteria, ATP is generated by cyclic photo-phorylation. The mode of production of the reducing force, which is $NADH_2$ (not $NADPH_2$ as in green plants and cyanobacteria), is apparently variable in different groups and depends on the exogenous reductant used by the organism.

However, the non-cyclic pathways operating in bacteria are not so well understood. It has been suggested that $NADH_2$ production in photosynthetic bacteria may take place at least in three different ways. Firstly, when hydrogen acts as an exogenous electron donor, NAD can be directly reduced to $NADH_2$:

$$H_2 + NAD^+ \xrightarrow{e^-} NADH_2.$$

Another way may by reverse electron flow as it occurs in the nitrifying bacteria. The reverse electron flow requires input of ATP. It may occur when H2S, SO or organic compounds are used as exogenous reductants by purple sulfur and purple non-sulfur bacteria.

$$H_2S/S^0/\text{organic compounds} + NAD \xrightarrow[ATP \quad ADP]{} S^0/SO_4^{-2} / \text{organic compounds oxidized} + NADH_2$$

A third way of $NADH_2$ production appears to occur in green sulfur bacteria. These bacteria are able to utilize the light energy to transfer electrons from donors like H_2S, $S_2O_3^{2-}$ etc. to NAD via suitable electron transport carriers as shown in Fig. 8.63 (B).

In summarizing the light reactions of oxygenic and anoxygenic photosynthesis, it may be observed that in oxygenic photosynthesis, both ATP and $NADPH_2$ are produced through two light reactions using water as exogenous reductant. In contrast, in the anoxygenic type only one light reaction produces ATP by a cyclic electron transport chain. The mode of reduction of NAD to $NADH_2$may be different in different major groups of bacteria. As exogenous hydrogen donor, H_2, H_2S, elemental sulfur, thiosulfate or even organic compounds may be used for reduction of NAD.

(b) Photo-synthetic dark reactions:

The products of light reactions, ATP and $NADPH_2$ or $NADH_2$ are utilized in the dark reactions for reduction of CO_2 to produce sugars or other organic compounds. Most of the autotrophic organisms, including the chemoautotrophs carry out these reactions via the Calvin-Benson cycle. The green sulfur bacteria are exceptions in this regard.

The dark reactions are purely biochemical in nature catalysed by different enzymes without any direct involvement of light. The overall stoichiometry of CO_2-fixation by the Calvin-Benson cycle is given by the equation

$6CO_2 + 18ATP + 12NAD(P)H_2 + 12H_2O \; C_6H_{12}O_6 + 18ADP + 18Pi + 12NAD(P)$.

In the Calvin-Benson cycle, the acceptor of CO_2 is ribulose 1,5-bis phosphate (RuBP) and the primary product of photosynthesis is 3-phosphoglyceric acid (3-PGA). This product is reduced to triose phosphates — di-hyceraldehyde phosphate (GAP) and dihydroxyacetone phosphate (DHAP). Part of these triose phosphates produce hexose phosphate which is the final product of photosynthesis and part is used to regenerate the acceptor of CO_2 i.e. RuBP.

The cycle has been represented in a very simplified manner showing the stoichiometry of the above equation in Fig. 8.64:

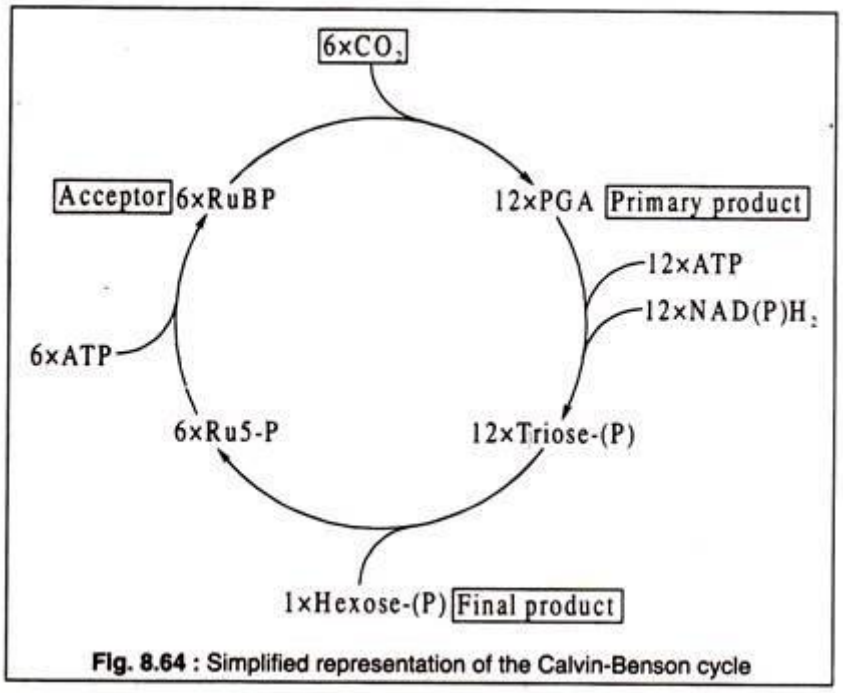
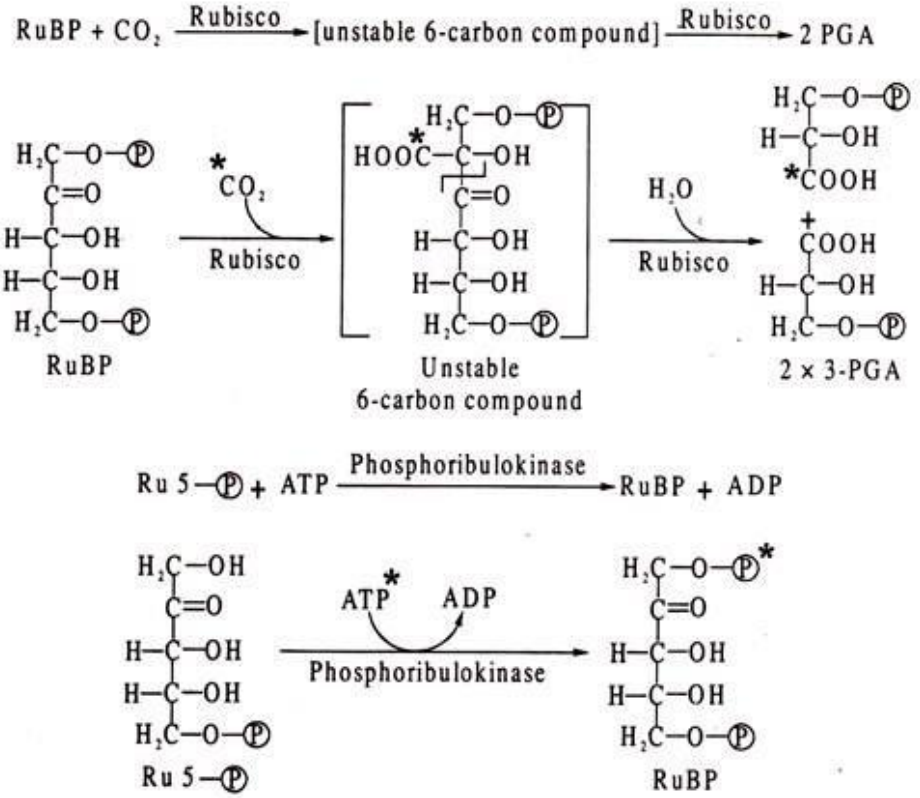


Fig. 8.64 : Simplified representation of the Calvin-Benson cycle

Among the enzymes of the calvinbenson cycle, only two are unique to this pathway, while the rest are present also in other pathways like EMP and PPC. These two enzymes are ribulose bisphosphate carboxylase oxygenase (Rubisco) and ribulose 5-phosphate kinase or phosphoribulokinase.

The reactions catalyzed by these enzymes are:



As each complete turn of the cycle incorporates one CO_2molecule into RuBP, six CO_2 molecules are needed to synthesize one hexose molecules. This means the cycle must operate six times to produce a single hexose molecule.
The primary product 3-PGA is phosphorylated with ATP and 3-PGA kinase to DPGA (1,3-di-phosphoglyceric acid) which is then reduced to GAP by an NAD (P) H_2 linked GAP- dehydrogenase. A portion of GAP is utilized for formation of hexose via condensation of GAP and DHAP by aldolase to produce FBP, fructose 6-phosphate and finally glucose.
All these reactions are common with those of EMP, but the reactions of Calvin-Benson cycle run in reverse direction. The rest of GAP is utilized for regeneration of RuBP for starting another turn of the cycle. Formation of RuBP from Ru5-(P) takes place by the phosphoribulokinase reaction.

Regeneration of Ru5-(P) from GAP takes place mainly by several transfer reactions, catalysed by the transaldolases and transketolases, as well as by some epimerases and isomerases. These enzymes are common between the PPC and the Calvin-Benson cycle.

The reactions of the cycle are shown in Fig. 8.65:

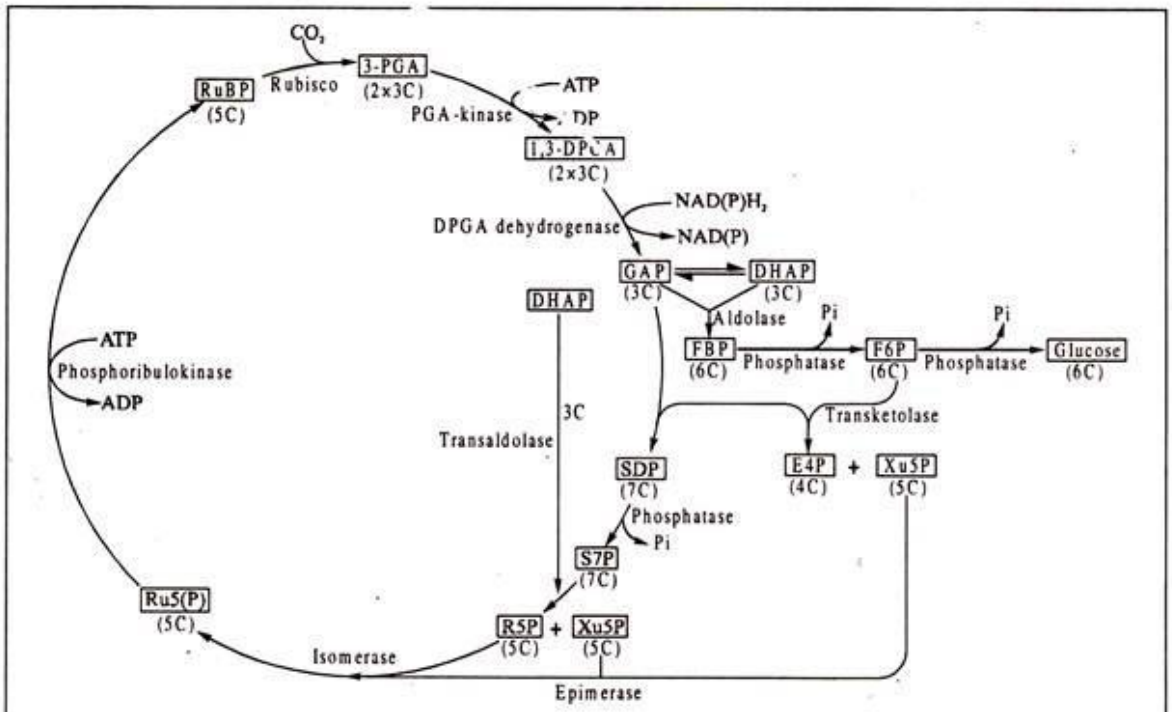


Fig. 8.65 : The Calvin-Benson cycle

Abbreviations : RuBP = Ribulose 1,5 bisphosphate, 3-PGA = 3-Phosphoglyceric acid, DPGA = 1,3-diphosphoglyceric acid, GAP = Glyceraldehyde 3-phosphate, DHAP = Dihydroxyacetone phosphate, SDP = Sedoheptulose 1,7-diphosphate, S7P = Seduheptulose 7-phosphate, FBP = Fructose 1,6-bisphosphate, F6P = Fructose 6-phosphate, E4P = Erythrose 4-phosphate, R5P = Ribose 5-phosphate, Ru5P = Ribulose 5-phosphate, Xu5P = Xylulose 5-phosphate

Formation of ribulose 5-phosphate in the cycle from glyceraldehyde 3-phosphate takes place through several transfer reactions:

1. Fructose 6-phosphate (F6P) + glyceraldehyde 3-phosphate —> xylulose 5-phosphate (Xu5P) + Erythrose 4-phosphate (E4P).

The reaction is catalysed by the TPP-linked transketolase which transfers a two-carbon group from fructose 6-phosphate to glyceraldehyde 3-phosphate producing a four-carbon sugar (erythrose 4-phosphate) and a five-carbon sugar (xylulose 5-phosphate).

2. Dihydroxyacetone phosphate (DHAP) + Erythrose 4-phosphate (E4P) —> Sedoheptulose 1,7-diphosphate (SDP)

The reaction is catalysed by transaldelose.

3. Sedoheptulose 1,7-diphosphate (SDP) —> Sedoheptulose 7-phosphate (S7P). A phosphate group is removed through the action of a phosphatase.

4. Sedoheptulose 7-phosphate (S7P) + Glyceraldehyde 3-phosphate (GAP) —> Ribose-5 phosphate (R5P) + Xylulose 5-phosphate (Xu5P)

This reaction is also catalysed by transketolose.

5. Ribose 5-phosphate (R5P) —> Ribulose 5-phosphate (Ru5P)

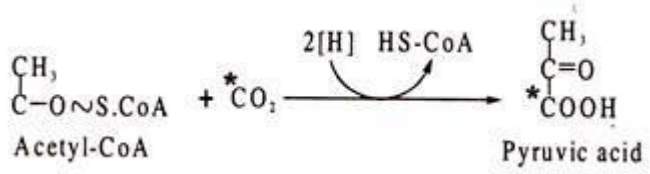
The aldosugar R5P is changed into a ketosugar Ru5P through the action of an isomerase.

6. Xylulose 5-phosphate (Xu5P) —> Ribulose 5-phosphate (Ru5P)

Intra-molecular rearrangements of H and OH groups of the two keto-sugar is catalysed by an epimerase.

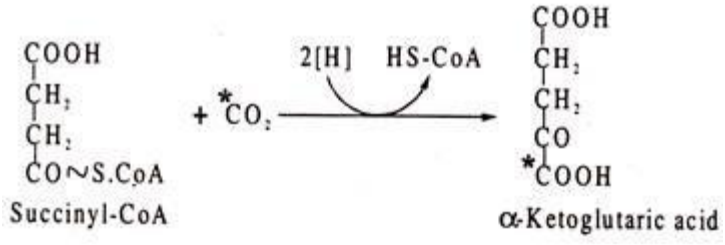
Finally, ribulose 5-phosphate produced in reactions (5) and (6) are phosphorylated by ATP and phosphoribulokinase to ribulose bisphosphate.

The obligately anaerobic green sulfur bacteria do not use the Calvin-Benson cycle for photosynthetic CO_2-fixation. So far as it is known, these bacteria employ some reactions which are normally involved in oxidative decarboxylation of substrates.
These reactions are normally irreversible, but the green sulfur bacteria can generate a strongly electronegative reductant by its Photosystem I which is able to force the reversal of the decarboxylation reactions leading to CO_2-fixation. This reductant may be ferredoxine.
So, in these photosynthetic bacteria, CO_2probably enters through reversal of reactions like oxidative decarboxylation of pyruvic acid and of α-ketoglutaric acid, as shown:



Normally, in aerobic and also in many fermentative organisms, pyruvic acid produced by glucose dissimilation is decarboxylated to acetyl-CoA.

The green photosynthetic bacteria possess special capacity to run the reaction in opposite direction leading to CO_2 fixation:



This reaction occurs in the TCA cycle in aerobic organisms, where it runs in opposite direction for decarboxylation of α-ketoglutaric acid to succinyl CoA. But in green sulfur bacteria, it is employed for CO_2incorporation and not elimination. Thus, these bacteria probably use a reductive TCA cycle for CO_2-fixation.

Photosynthesis is the biological process by which all green plants, photosynthetic bacteria and other autotrophs convert light energy into chemical energy. In this process, glucose is synthesised from carbon dioxide and water in the presence of sunlight. Furthermore, oxygen gas is released out into the atmosphere as the byproduct of photosynthesis.

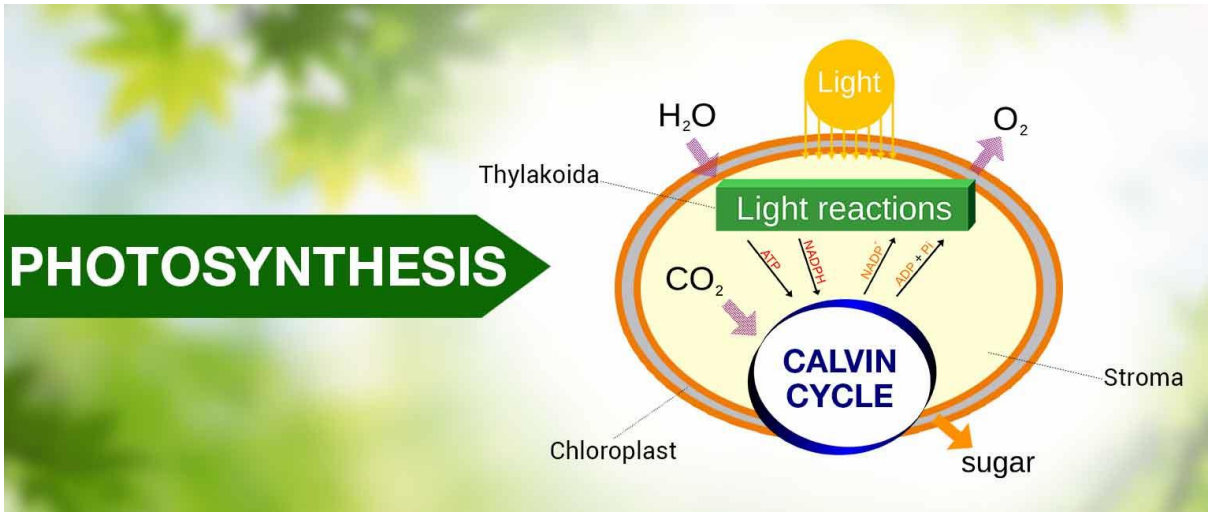
The balanced chemical equation for the photosynthesis process is as follows:

$$\mathbf{6CO_2 + 6H_2O \longrightarrow C_6H_{12}O_6 + 6O_2}$$

Sunlight is the ultimate source of energy. Plants use this light energy to prepare chemical energy during the process of photosynthesis. The whole process of photosynthesis takes place in two phases- photochemical phase and biosynthetic phase.

In-depth Reading: Photosynthesis

The photochemical phase is the initial stage where ATP and NADPH for the biosynthetic phase are prepared. In the biosynthetic phase, the end product glucose is produced. Let us focus more on pathways in biosynthetic phase.



Photosynthetic Pathways

During the biosynthetic phase, carbon dioxide and water combine to give carbohydrates i.e. sugar molecules. This reaction of carbon dioxide is termed as carbon fixation. Different plants follow different pathways for carbon fixation.

Based on the first product formed during carbon fixation there are two pathways: the C3 pathway and C4 pathway.

The Pathway of Photosynthesis

C3 Pathway (Calvin Cycle)

The majority of plants produce 3-carbon acid called 3-phosphoglyceric acid (PGA) as a first product during carbon dioxide fixation. Such a pathway is known as the C3 pathway which is also called the Calvin cycle.

Calvin Cycle occurs in three steps:

- carboxylation
- reduction
- regeneration

In the first step, the two molecules of 3-phosphoglyceric acid (PGA) are produced with the help of the enzyme called RuBP carboxylase. Later in the second and third steps, the ATP and NADPH phosphorylate the 3-PGA and ultimately produces glucose. Then the cycle restarts again by regeneration of RuBP.

Beans, Rice, Wheat, and Potatoes are an example of plants that follow the C3 pathway

C4 Pathway (Hatch and Slack Pathway)

Every photosynthetic plant follows Calvin cycle but in some plants, there is a primary stage to the Calvin Cycle known as C4 pathway. Plants in tropical desert regions commonly follow the C4 pathway. Here, a 4-carbon compound called oxaloacetic acid (OAA) is the first product by carbon fixation. Such plants are special and have certain adaptations as well.

The C4 pathway initiates with a molecule called phosphoenolpyruvate (PEP) which is a 3-carbon molecule. This is the primary CO2 acceptor and the carboxylation takes place with the help of an enzyme called PEP carboxylase. They yield a 4-C molecule called oxaloacetic acid (OAA).

Eventually, it is converted into another 4-carbon compound known as malic acid. Later, they are transferred from mesophyll cells to bundle sheath cells. Here, OAA is broken down to yield carbon dioxide and a 3-C molecule.

The CO2 thus formed is utilized in the Calvin cycle whereas 3-C molecule is transferred back to mesophyll cells for regeneration of PEP.

Corn, sugarcane and some shrubs are examples of plants that follow the C4 pathway. Calvin pathway is a common pathway in both C3 plants and C4 plants but it takes place only in the mesophyll cells of the C3 Plants but not in the C4 Plants.

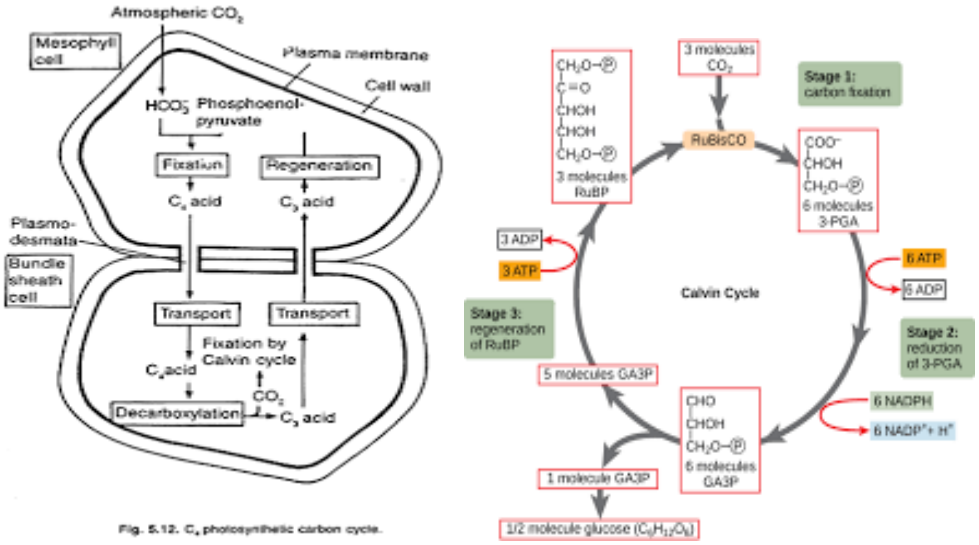
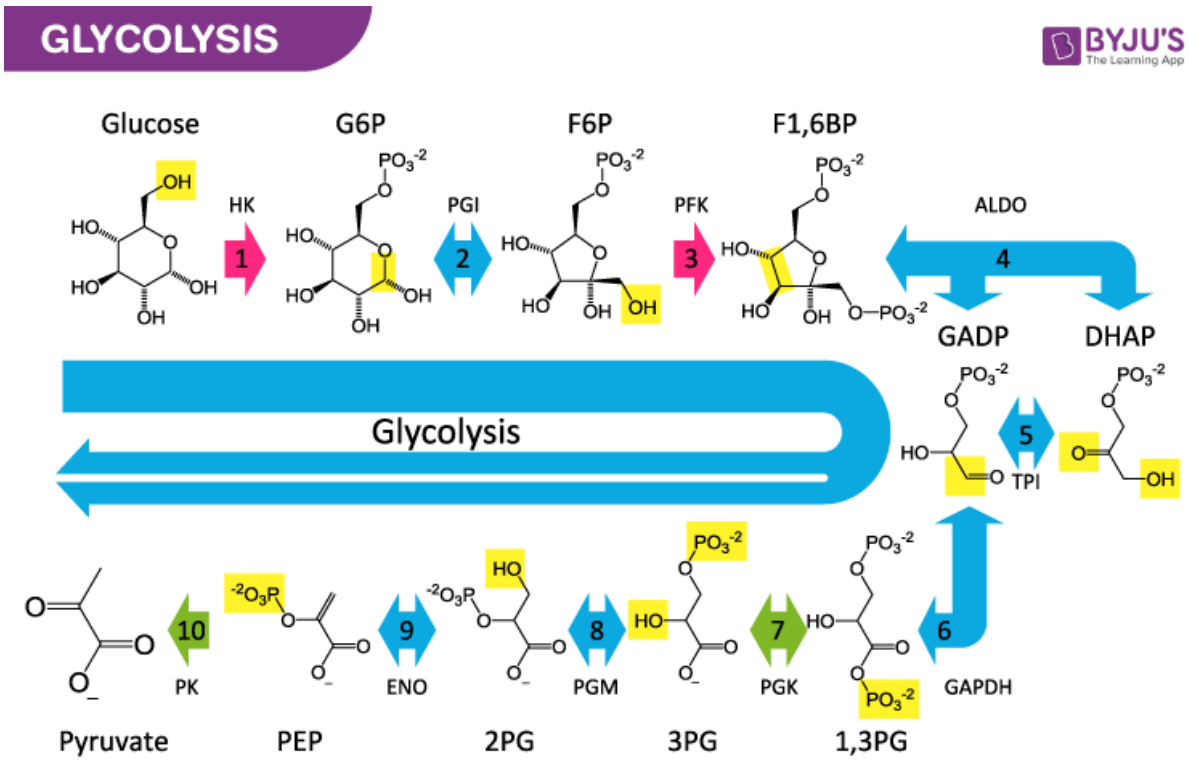


Fig. 5.12. C_4 photosynthetic carbon cycle.

What is Glycolysis?

Glycolysis is the process in which glucose is broken down to produce energy. It produces two molecules of pyruvate, ATP, NADH and water. The process takes place in the cytosol of the cell cytoplasm, in the presence or absence of oxygen.



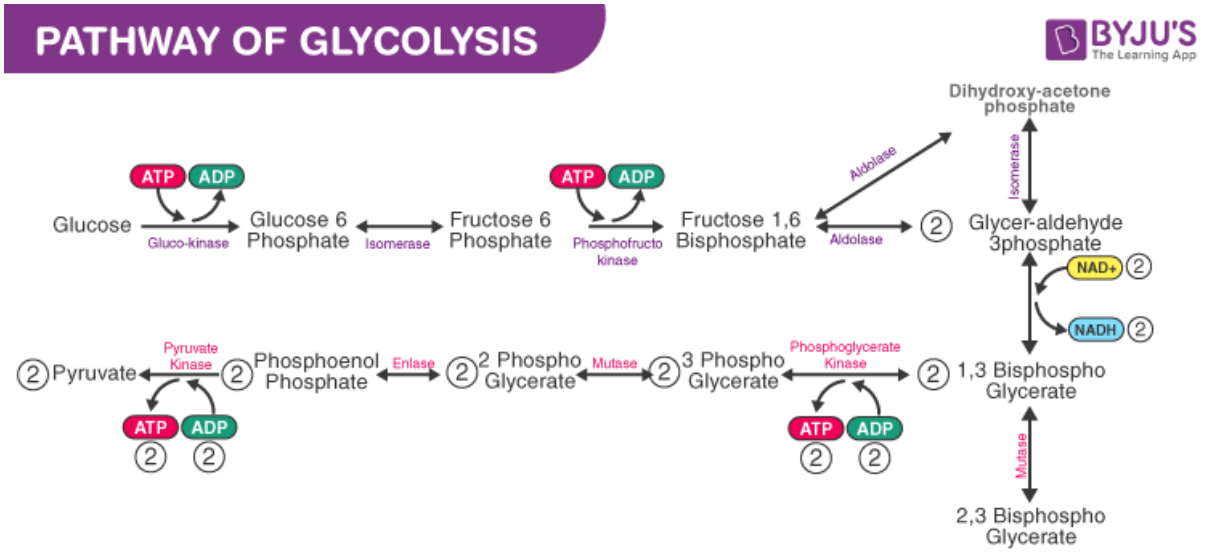
Glycolysis is the primary step of cellular respiration. In the absence of oxygen, the cells take small amounts of ATP through the process of fermentation.

This metabolic pathway was discovered by three German biochemists- Gustav Embden, Otto Meyerhof, and Jakub Karol Parnas in the early 19th century and is known as EMP pathway (Embden–Meyerhof–Parnas).

Also Read: TCA cycle

Glycolysis Pathway(EMP PATHWAY)

The glycolysis pathway occurs in the following stages:



Stage 1

- A phosphate group is added to glucose in the cell cytoplasm, by the action of enzyme hexokinase.
- In this, a phosphate group is transferred from ATP to glucose forming glucose,6-phosphate.

Stage 2

Glucose-6-phosphate is isomerized into fructose,6-phosphate by the enzyme phosphoglucomutase.

Stage 3

The other ATP molecule transfers a phosphate group to fructose 6-phosphate and converts it into fructose 1,6-bisphosphate by the action of enzyme phosphofructokinase.

Stage 4

The enzyme aldolase breaks down fructose 1,6-bisphosphate into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, which are isomers of each other.

Step 5

Triose-phosphate isomerase converts dihydroxyacetone phosphate into glyceraldehyde 3-phosphate which is the substrate in the successive step of glycolysis.

Step 6

This step undergoes two reactions:

- The enzyme glyceraldehyde 3-phosphate dehydrogenase transfers 1 hydrogen molecule from glyceraldehyde phosphate to nicotinamide adenine dinucleotide to form NADH + H^+.
- Glyceraldehyde 3-phosphate dehydrogenase adds a phosphate to the oxidized glyceraldehyde phosphate to form 1,3-bisphosphoglycerate.

Step 7

Phosphate is transferred from 1,3-bisphosphoglycerate to ADP to form ATP with the help of phosphoglycerokinase. Thus two molecules of phosphoglycerate and ATP are obtained at the end of this reaction.

Step 8

The phosphate of both the phosphoglycerate molecules is relocated from the third to the second carbon to yield two molecules of 2-phosphoglycerate by the enzyme phosphoglyceromutase.

Step 9

The enzyme enolase removes a water molecule from 2-phosphoglycerate to form phosphoenolpyruvate.

Step 10

A phosphate from phosphoenolpyruvate is transferred to ADP to form pyruvate and ATP by the action of pyruvate kinase. Two molecules of pyruvate and ATP are obtained as the end products.

Entner-Doudoroff (ED) pathway

- This pathway occurs in both aerobic and anaerobic condition
- Occur in prokaryotes only
- It occurs in cytoplasm
- Pyruvate and glyceraldehyde-3-phosphate produced from glucose by ED pathway

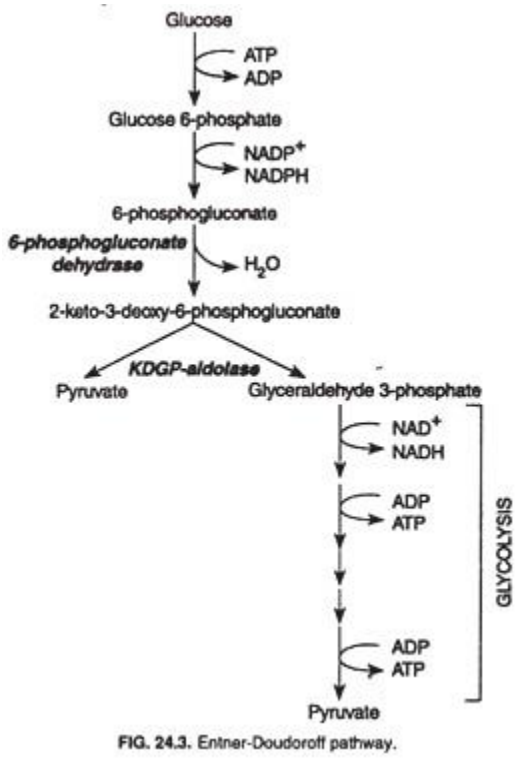


FIG. 24.3. Entner-Doudoroff pathway.

- At first glucose is phosphorylated to glucose -6-phosphate by the enzyme hexokinase.
- Glucose-6-phosphate is then oxidized to 6-phosphogluconolactone releasing a molecule of NADPH. This reaction is catalyzed by the enzyme glucose-6-phosphate dehydrogenase.
- Hydrolase enzyme converts 6-phopshogluconolactone to 6-phosphogluconate.
- 6-phosphogluconate undergoes dehydration reaction catalyzed by 6-phosphogluconate dehydratase to form 2-keto 3-deoxy 6-Phosphogluconate (KDPG).
- KDPG splits to form pyruvate and glceraldehyde-3-phosphate. It is catalyzed by KDPG aldolase enzyme
- Glyceraldehyde-3-phosphate is then metabolized by glycolysis to form pyruvate.

Significance of ED pathway

- This pathway used two specific enzymes ie. 6-phosphogluconate dehydratase and KDPG aldolase.
- This pathway generates 1 ATP, 1 NADH and 1 NADPH from one glucose molecule.

Glyoxylate cycle

- Glyoxylate cycle occurs in some microorganisms when acetate is sole source of carbon
- This cycle has two unique enzyme- **isocitrate lyase** and **malate synthase** which bypass some of the reaction of TCA cycle.

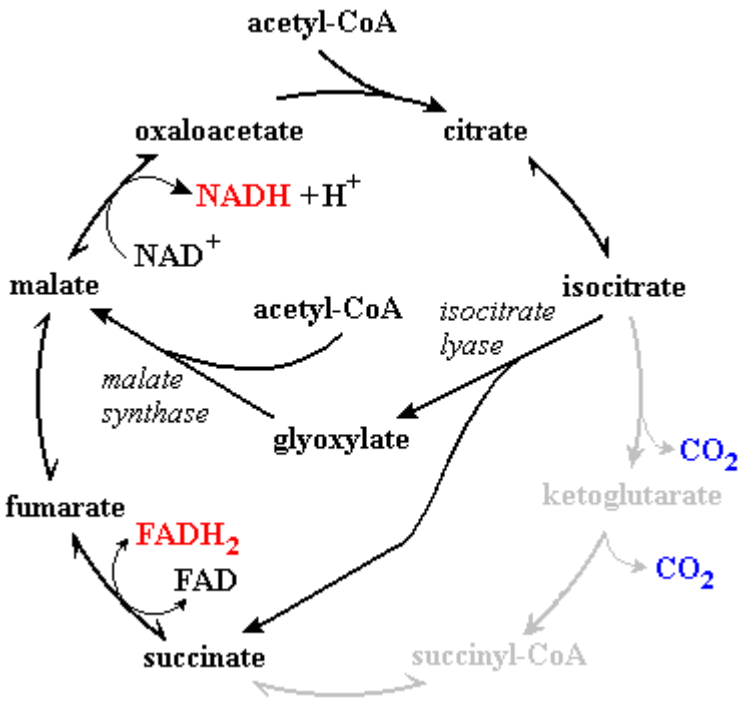


figure: Glyoxylate cycle

- Glyoxylate cycle is absent in higher organism.
- At first acetylcoA is produced from acetate or by oxidation of higher fattyacids.
- AcetylcoA then enter into TCA cycle and condensed with oxaloacetate to form citrate.
- Citrate then isomerized to isocitrate.
- Isocitrate lyase bypass the TCA cycle by splitting isocitrate into **succinate** and **glycoxylate**.
- Succinate metabolized by TCA whereas Glycoxylate condenses with another molecule of acetylcoA to form malate in the presence of malate synthase.
- Malate is converted into oxaloacetate by the enzyme malate dehydrogenase.

Significance of Glyoxylate cycle

- It is bypass reaction of TCA cycle
- It occurs in bacteria when they are cultured in acetate rich carbon source.
- When Higher fattyacids are oxidized into acetylcoA without forming puruvate acids, then acetylcoA enters into glyoxylate cycle.

Citric acid cycle or Krebs cycle or Tri-carboxylic acid (TCA) cycle

- Citric acid cycle is a central metabolic pathway for metabolism of carbohydrates, fats and proteins. Citric acid cycle occurs in aerobic condition in mitochondria.
- At first carbohydrates, fats and proteins are catabolized by separate pathway to form acetyl-coA then Acetyl-coA enters into Citric acid cycle.
- It is also known as Krebs cycle or Tri carboxylic acid (TCA) cycle.

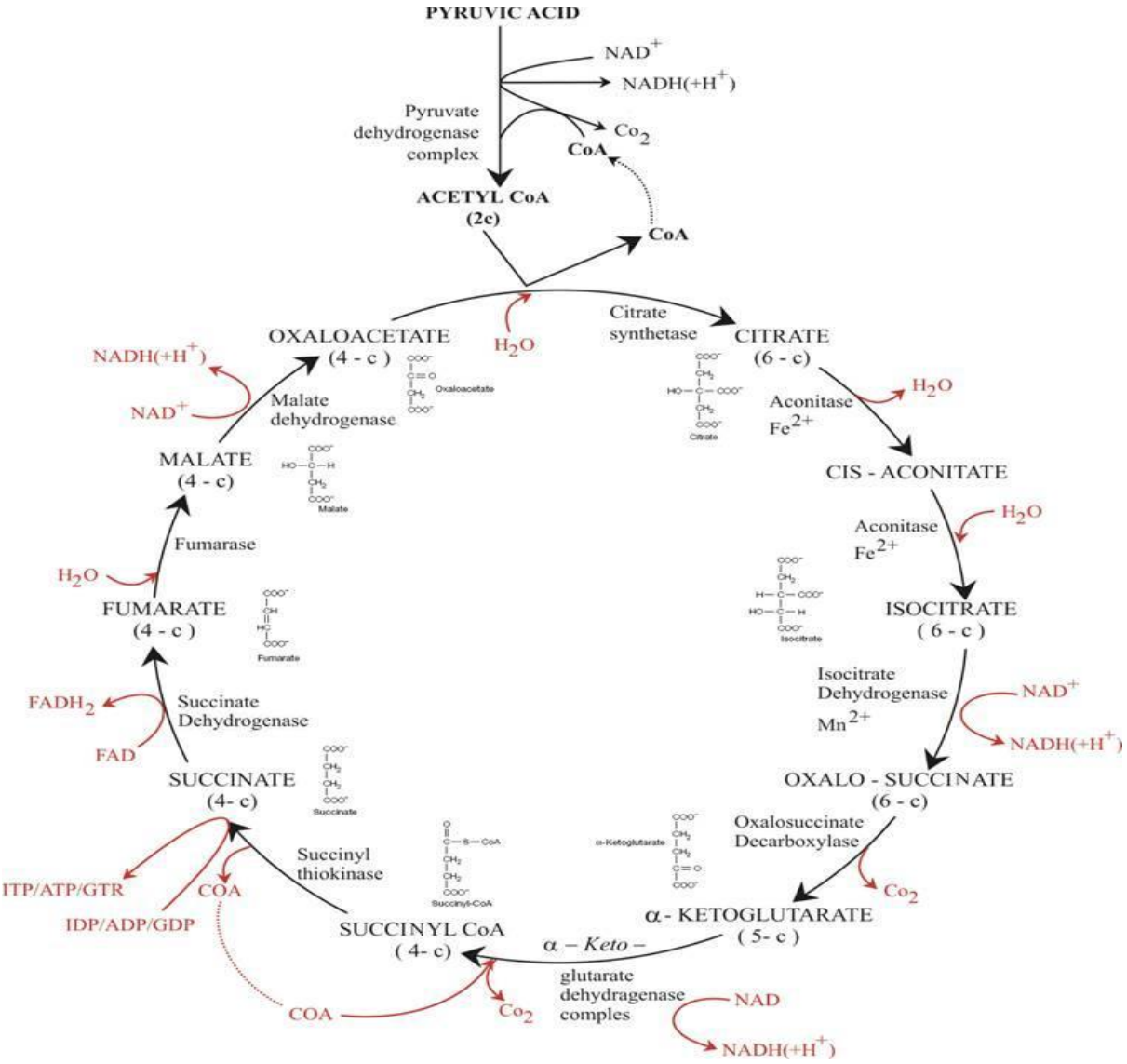


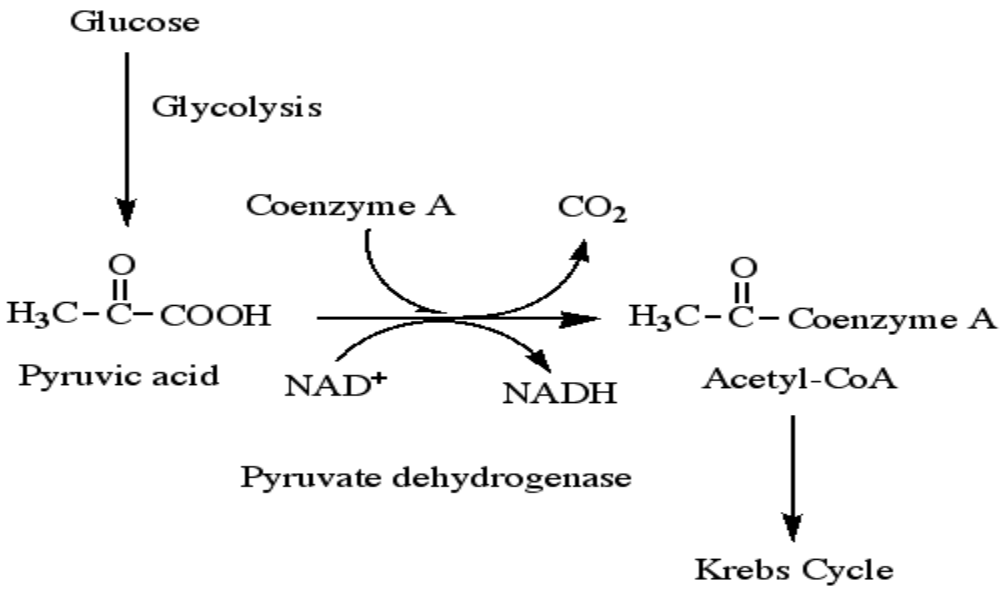
Figure: Citric acid cycle

Two major reactions involved in citric acid cycle.

1. Formation of acetyl-coA
2. Reactions of citric acid cycle

1. Formation of acetyl-coA:

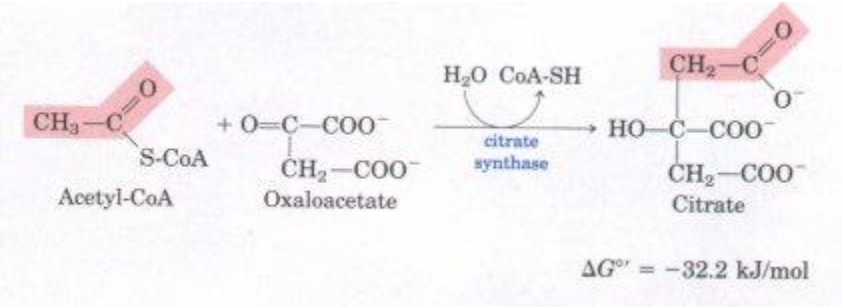
- Before entering into Krebs cycle, carbohydrates, fats and proteins are catabolized by separate pathway to form Acetyl-coA.
- For example: pyruvate formed by aerobic glycolysis is oxidized into acetyl-coA and CO2 by the enzyme Pyruvate dehydrogenase complex.



- It is an irreversible oxidative decarboxylation reaction in which a molecule of carbon in the form of CO2 is removed from pyruvate.
- In this reaction a molecule of pyruvate generate 1 NADH.
- This step is link between glycolysis and Krebs cycle.

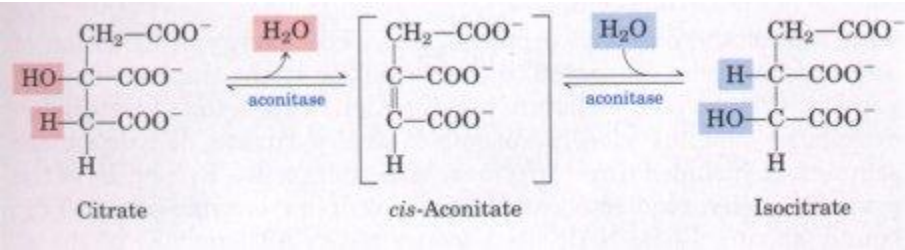
2. Reactions of citric acid cycle

i. Formation of citrate (citric acid):



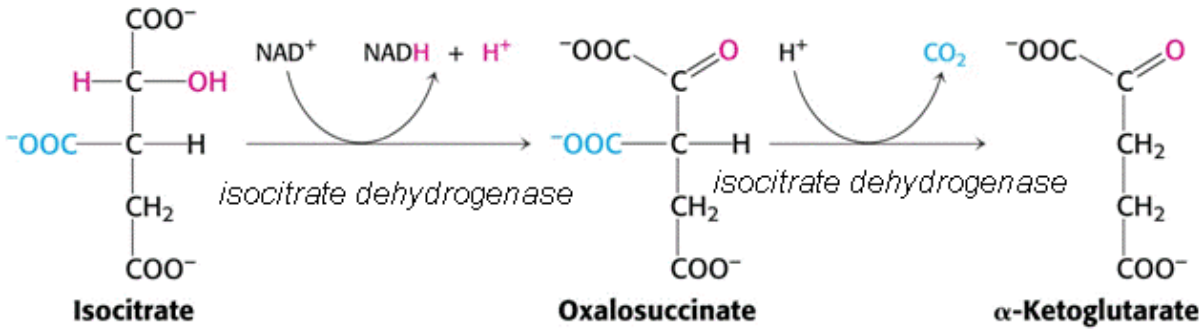
- It is a condensation reaction. Acetyl-coA condensed with oxaloacetate to form citrate and the reaction is catalyzed by the enzyme Citrate synthase.
- Oxaloacetate play catalytic role in citric acid cycle and at the end of process oxaloacetate is regenerated.

ii. Isomerization of citrate to Isocitrate:



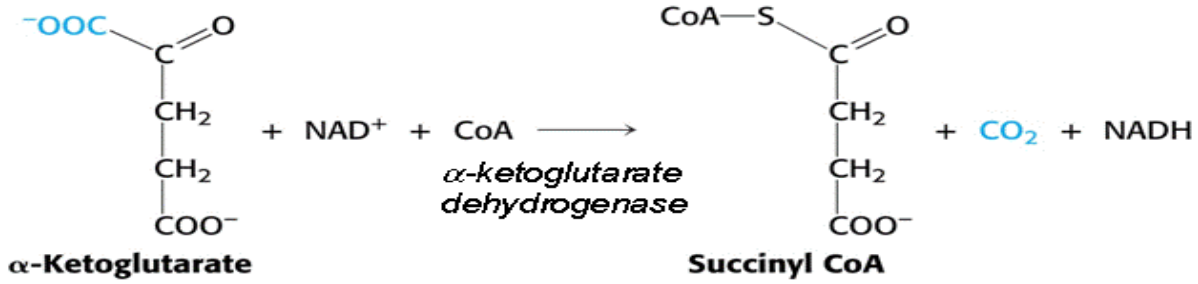
- The enzyme aconitase catalyzes the isomerization of citrate to isocitrate with intermediate cis-aconitate.
- This is a reversible reaction.
- This reaction occurs in two steps- first dehydration and second hydration.

iii. Formation of α-ketoglutarate:



- This is an oxidative decarboxylation reaction.
- The enzyme isocitrate dehydrogenase catalyzes the oxidation of isocitrate to form α-ketoglutarate and CO2.
- In this step 1 molecule of NADH is generated.
- It is an irreversible reaction.

iv. Formation of succinyl-coA:

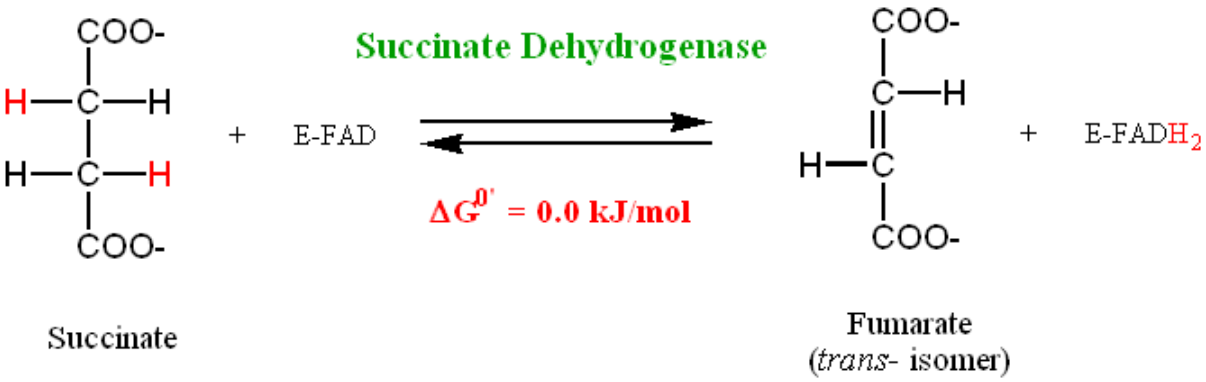


- This is also an oxidative decarboxylation reaction catalyzed by α-ketoglutarate dehydrogenase enzyme in which α-ketoglutarate is oxidized into succinylcoA and CO2.
- In this reaction, coA serves as carriers of succinyl group and NAD+ serves as electron acceptor.
- One molecule of NADH is generated in this step.

v. Formation of Succinate:

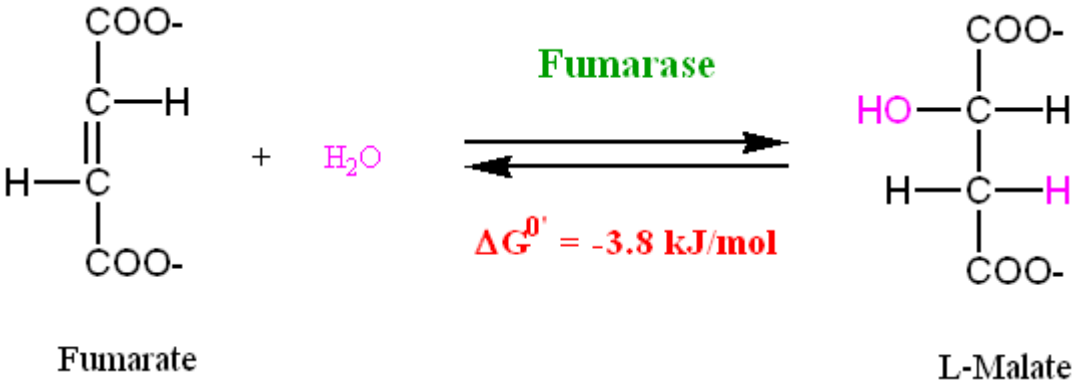
- Conversion of succinyl-coA to succinate is catalyzed by succinyl-coA synthetase or succinic thiokinase enzyme.
- This is a substrate level phosphorylation reaction in which CoA group ultimately donates its phosphate group to GDP forming energy rich GTP.
- A molecule of CO2 is released in this step.

vi. Formation of fumarate:



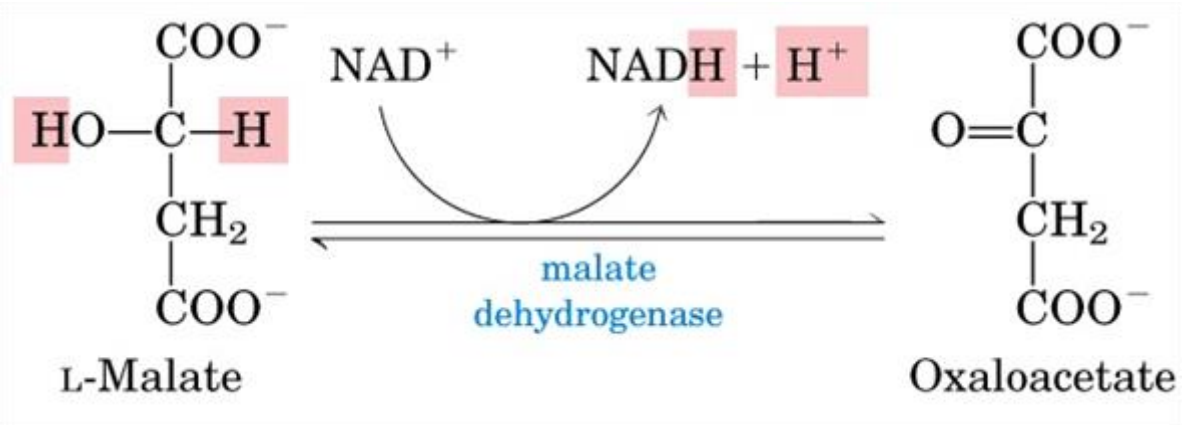
- Succinate dehydrogenase catalyzed the oxidation of succinate to from fumarate.
- It is reversible reaction.
- In this step a molecule of FADH2 is generated.

vii. Formation of malate:



- This reaction is catalyzed by fumarase (fumarate hydratase) in which fumarate is converted into malate.
- This is a hydration and reversible reaction.

viii. Formation of oxaloacetate: regeneration of oxaloacetate:



- Malate is oxidized into oxaloacetate generating a molecule of NADH.
- This reaction is catalyzed by malate dehydrogenase enzyme.
- Oxaloacetate is regenerated in this step and combines with acetylcoA and continues the cycle.

Significance of TCA cycle: Role of TCA cycle

i. Role in Central metabolic pathway:

- TCA cycle is a final common metabolic pathway of carbohydrates, fattyacids and aminoacids.
- At first all these biomolecules are catabolized by their separate metabolic pathways to generate acetyl-coA then acetyl-coA enters TCA cycle for further metabolism in aerobic condition.
- TCA is more efficient in energy conservation than other pathways of metabolism.

ii. TCA is an amphibolic pathway:

- It plays role in both catabolism and anabolism.

Catabolic role:

- TCA is a catabolic pathway because it oxidizes acetyl-coA completely into CO2 and H2O and releases large amount of energy.

Anabolic role:

- TCA is an anabolic pathway because it provides precursors for biosynthesis of other molecules in cells. Such as citrate, α-ketoglutarate, succinylcoA and oxaloacetate act as precursors for biosynthesis of various molecules.
- Glucose, purine and pyrimidine are synthesized from oxaloacetate.
- Fattyacids and steroids are synthesized from succinylcoA.
- Some aminoacids, purine and pyrimidine are synthesized from α-ketoglutarate.

iii. Citric acid cycle is an aerobic process:

- NAD+ and FAD are electron acceptors in the TCA cycle. These are regenerated by Electron transport chain which requires oxygen as final electron acceptor. Hence overall TCA and ETC are aerobic process.

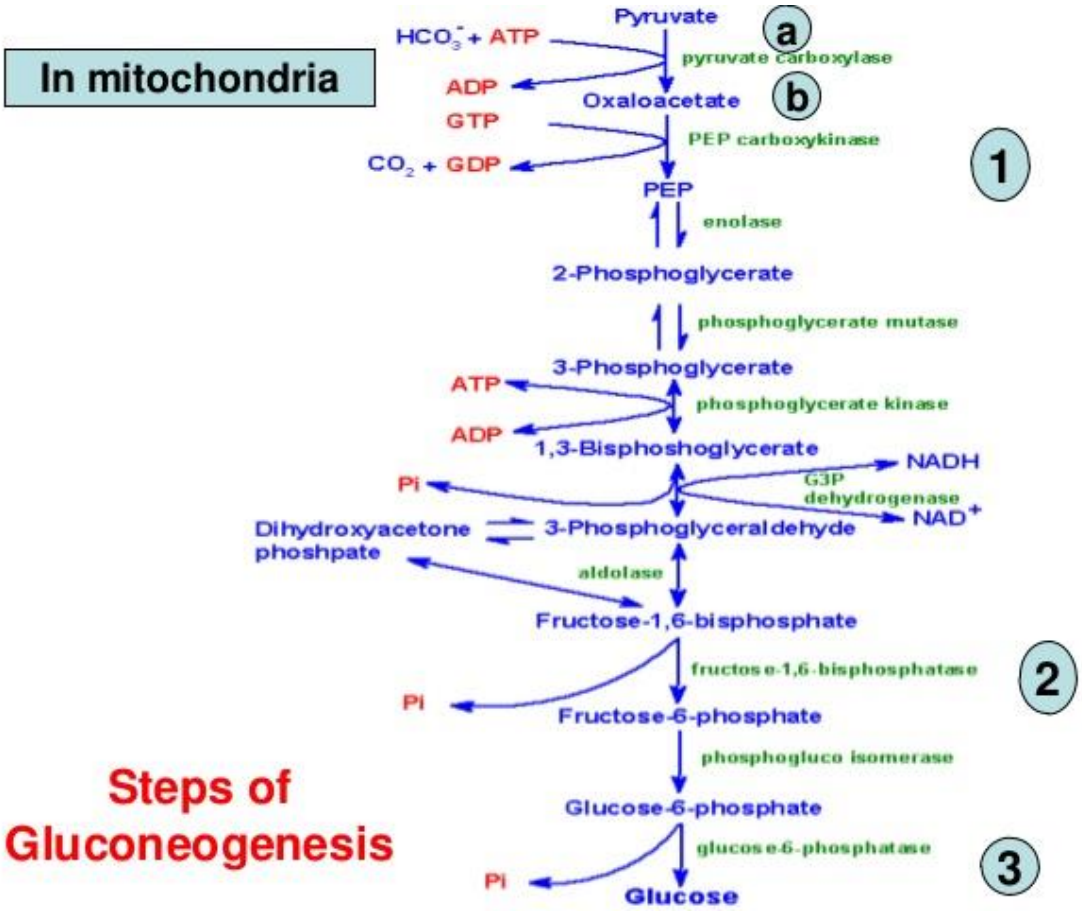
Gluconeogenesis

- It is the process of formation of new glucose molecule from carbohydrate or non-carbohydrate precursor. The important precursor are lactate, pyruvate and glycerol as well as certain aminoacids.

Gluconeogenesis from Pyruvate

- The end product aerobic glycolysis is pyruvate. But glucose cannot be produced by reversing the glycolysis process because there are three irreversible steps in glycolysis.

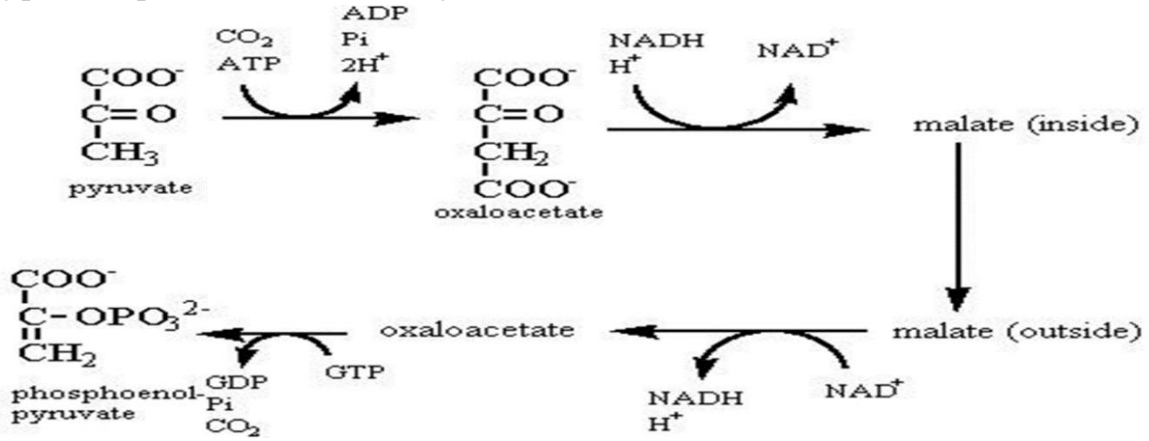
- Gluconeogenesis from pyruvate share 7 reversible steps of glycolysis and the 3 irreversible steps are bypassed by the separate sets of enzymes.



The bypass reaction that occur during conversion of pyruvate to glucose are

1. **Conversion of pyruvate to Phosphoenolpyruvate (PEP)**
2. **Conversion of Fructose-1,6-bisphosphate to Fructose-6-phosphate**
3. **Conversion of Glucose-6-phosphate to glucose**

Bypass step I: Conversion of Pyruvate to PEP:



- It is the first bypass reaction in gluconeogenesis

- The conversion of pyruvate to PEP occurs in both cytosol and mitochondria.
- First pyruvate is transported from cytosol into mitochondira or it is generated in mitochondria from alanine by transamination (urea cycle)
- Then pyruvate carboxylase) coenzyme-biotin) converts pyruvate to Oxaloacetate within mitochondria.

Pyruvate + HCO3- +ATP——————— Oxaloacetate + ADP + pi

- The mitochondrial membrane do not have transporter for Oxaloacetate. So, oxaloacetate is reduced to malate by mitochondrial enzyme malate dehydrogenase

Oxaloacetate + NADH + H+——————— malate + NAD+

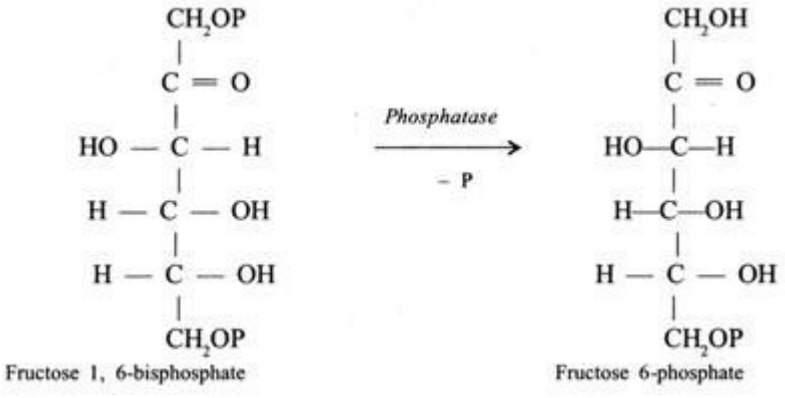
- Malate leaves the mitochondria through special transporter and in cytosol it is reoxidized into oxaloacetate by the cytosolic enzyme malate dehydrogenase.

Malate + NAD+———————- Oxaloacetate + NADH + H+

- The oxaloacetate is then converted to Phosphoenol pyruvate (PEP) by an enzyme phosphoenolpyruvate carboxykinase. This reaction is Mg++ dependent and require GTP.

Oxaloacetate + GTP——————- PEP + GDP + CO2

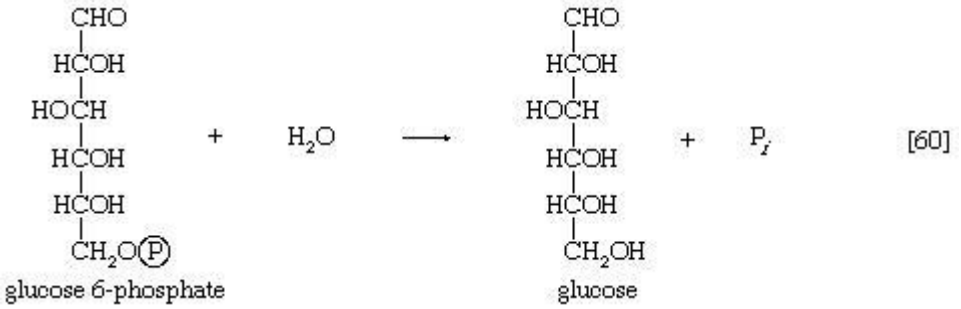
Bypass Step II: conversion of Fructose-1,6-bisphosphate to Fructose-6-phosphate



- This reaction is catalyzed by an enzyme fructose-1,6-bisphospatase (FBPase-1)which causes the irreversible hydrolysis of phosphate at C1.

Fructose-1,6-bisphosphate +H2O————————fructose-6-phosphate + Pi

Bypass step III: conversion of glucose-6-phosphate to glucose

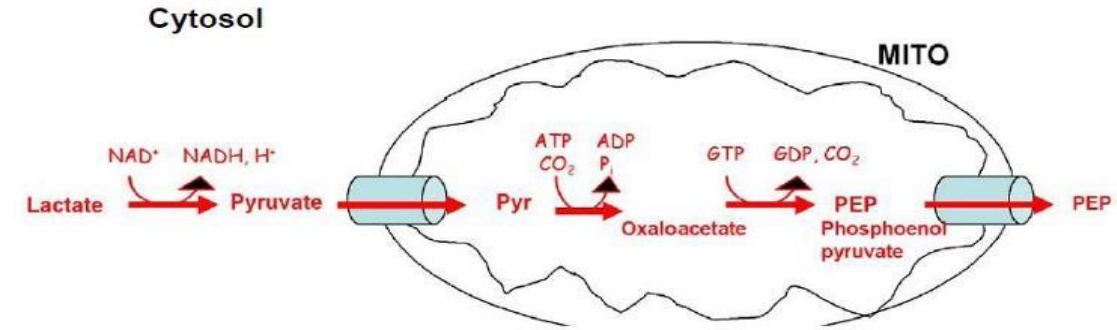


- This reaction is catalyzed by glucose-6-phosthatase which hydrolyses the phosphate at C6 yielding glucose.

Glucose-6-phosphate +H2o ———————— Glucose + Pi

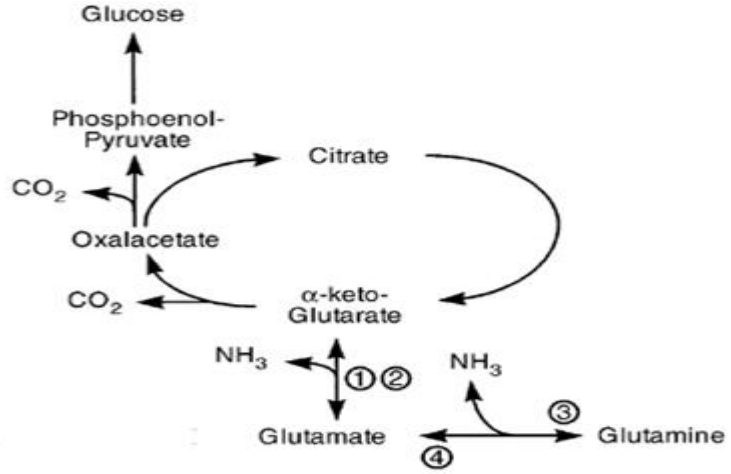
Other precursors such as lactate, intermediates of TCA cycle and some glucogenic aminoacids can also convert into glucose.

1. Conversion of lactate:(cori's cycle)



- Lactate generates during anaerobic respiration in Erythrocytes and in muscle during heavy exercise is converted into pyruvate by an enzyme lactate dehydrogenase.
- Pyruvate then enter into mitochondria from cytosol and convert into Oxaloacetate by the enzyme pyruvate carboxylase
- Oxaloacetate directly convert into PEP (phospoenolpyruvate) by an isoenzyme PEP carboxykinase within Mitochondria.
- PEP is then transported outside of mitochondria to continue gluconeogenesis.

2. conversio of TCA intermediate and aminoacids into glucose:



- Citrate, isocitrate, alfa-ketoglutarate, succinyl coA, succinate, fumarate, malate etc all intermediates of TCA cycle are oxidized to oxaloacetate which then converts in glucose.
- The glucogenic aminoacids such as alanine, glutamine etc. are converted to pyruvate which in turn converts to glucose.

Significance of gluconeogenesis:

1. Glucose is universal building molecule and provides energy to all cells. Mainly brain cell and nervous tissue as well as erythrocytes, testes, renal medulla require glucose as sole source of energy.
2. Glycogen stored in adipose tissue and in skeletal muscle are converted to glucose by glycogenolysis. However the stored glycogen may not be sufficient during heavy exercise, diabetic conditions, etc. so during shortage glucose is synthesized from carbohydrate or non-carbohydrate precursor by the process called gluconeogenesis.

Fermentation

Fermentation, another example of heterotrophic metabolism, requires an organic compound as a terminal electron (or hydrogen) acceptor. In fermentations, simple organic end products are formed from the anaerobic dissimilation of glucose (or some other compound). Energy (ATP) is generated through the dehydrogenation reactions that occur as glucose is broken down enzymatically. The simple organic end products formed from this incomplete biologic oxidation process also serve as final electron and hydrogen acceptors. On reduction, these organic end products are secreted into the medium as waste

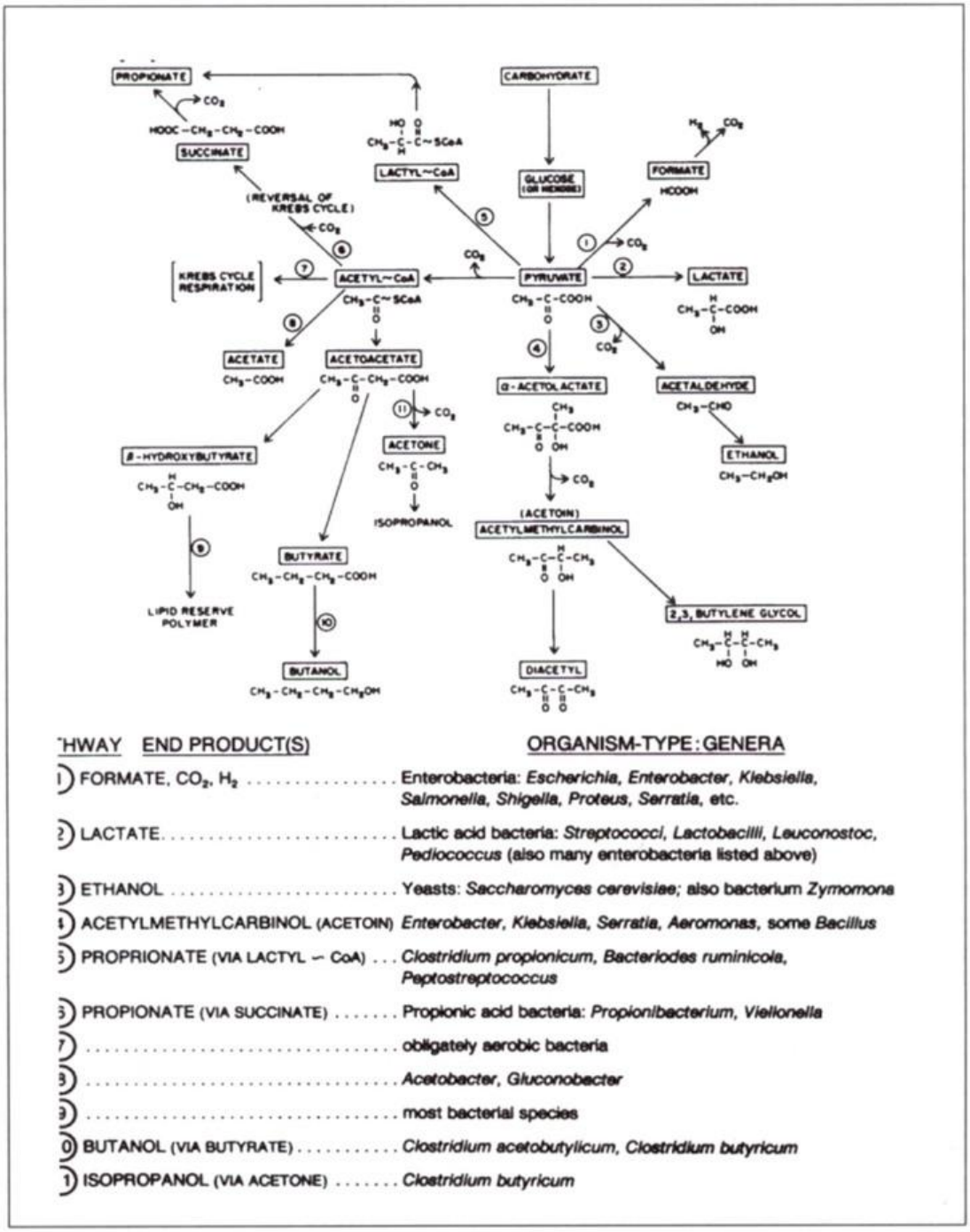
metabolites (usually alcohol or acid). The organic substrate compounds are incompletely oxidized by bacteria, yet yield sufficient energy for microbial growth. Glucose is the most common hexose used to study fermentation reactions.

In the late 1850s, Pasteur demonstrated that fermentation is a vital process associated with the growth of specific microorganisms, and that each type of fermentation can be defined by the principal organic end product formed (lactic acid, ethanol, acetic acid, or butyric acid). His studies on butyric acid fermentation led directly to the discovery of anaerobic microorganisms. Pasteur concluded that oxygen inhibited the microorganisms responsible for butyric acid fermentation because both bacterial mobility and butyric acid formation ceased when air was bubbled into the fermentation mixture. Pasteur also introduced the terms aerobic and anaerobic. His views on fermentation are made clear from his microbiologic studies on the production of beer (from *Etudes sur la Biere*, 1876):

In the experiments which we have described, fermentation by yeast is seen to be the direct consequence of the processes of nutrition, assimilation and life, when these are carried on without the agency of free oxygen. The heat required in the accomplishment of that work must necessarily have been borrowed from the decomposition of the fermentation matter…. Fermentation by yeast appears, therefore, to be essentially connected with the property possessed by this minute cellular plant of performing its respiratory functions, somehow or other, with the oxygen existing combined in sugar.

For most microbial fermentations, glucose dissimilation occurs through the glycolytic pathway (Fig. 4-1). The simple organic compound most commonly generated is pyruvate, or a compound derived enzymatically from pyruvate, such as acetaldehyde, α-acetolactate, acetyl ~ SCoA, or lactyl ~ SCoA (Fig. 4-5). Acetaldehyde can then be reduced by NADH + H^+ to ethanol, which is excreted by the cell. The end product of lactic acid fermentation, which occurs in streptococci (e.g., *Streptococcus lactis*) and many lactobacilli (e.g., *Lactobacillus casei*, *L. pentosus*), is a single organic acid, lactic acid. Organisms that produce only lactic acid from glucose fermentation are homofermenters. Homofermentative lactic acid bacteria dissimilate glucose exclusively through the glycolytic pathway. Organisms that ferment glucose to multiple end products, such as acetic acid, ethanol, formic acid, and CO_2, are referred to as heterofermenters. Examples of heterofermentative bacteria

include *Lactobacillus*, *Leuconostoc*, and *Microbacterium* species. Heterofermentative fermentations are more common among bacteria, as in the mixed-acid fermentations carried out by bacteria of the family Enterobacteriaceae (e.g., *Escherichia coli*, *Salmonella*, *Shigella*, and *Proteus* species). Many of these glucose fermenters usually produce CO_2 and H_2 with different combinations of acid end products (formate, acetate, lactate, and succinate). Other bacteria such as *Enterobacter aerogenes*, *Aeromonas*, *Serratia*, *Erwinia*, and *Bacillus* species also form CO_2 and H_2 as well as other neutral end products (ethanol, acetylmethylcarbinol [acetoin], and 2,3-butylene glycol). Many obligately anaerobic clostridia (e.g., *Clostridium saccharobutyricum*, *C. thermosaccharolyticum*) and *Butyribacterium* species ferment glucose with the production of butyrate, acetate, CO_2, and H_2, whereas other *Clostridum* species (*C. acetobutylicum* and *C. butyricum*) also form these fermentation end products plus others (butanol, acetone, isopropanol, formate, and ethanol). Similarly, the anaerobic propionic acid bacteria (*Propionibacterium* species) and the related *Veillonella*species ferment glucose to form CO_2, propionate, acetate, and succinate. In these bacteria, propionate is formed by the partial reversal of the Krebs cycle reactions and involves a CO_2fixation by pyruvate (the Wood-Werkman reaction) that forms oxaloacetate (a four-carbon intermediate). Oxaloacetate is then reduced to malate, fumarate, and succinate, which is decarboxylated to propionate. Propionate is also formed by another three-carbon pathway in *C. propionicum*, *Bacteroides ruminicola*, and *Peptostreptococcus* species, involving a lactyl ~ SCoA intermediate. The obligately aerobic acetic acid bacteria (*Acetobacter* and the related *Gluconobacter* species) can also ferment glucose, producing acetate and gluconate. Figure 4-5 summarizes the pathways by which the various major fermentation end products form from the dissimilation of glucose through the common intermediate pyruvate.



Fermentative pathways of bacteria and the major end products formed with the organism type carrying out the fermentation.

For thermodynamic reasons, bacteria that rely on fermentative process for growth cannot generate as much energy as respiring cells. In respiration, 38 ATP molecules (or approximately 380,000 cal/mole) can be generated as biologically useful energy from the complete oxidation of 1 molecule of glucose (assuming 1 NAD(P)H = 3 ATP and 1 ATP → ADP + Pi = 10,000

cal/mole). Table 4-3 shows comparable bioenergetic parameters for the lactate and ethanolic fermentations by the glycolytic pathway. Although only 2 ATP molecules are generated by this glycolytic pathway, this is apparently enough energy to permit anaerobic growth of lactic acid bacteria and the ethanolic fermenting yeast, *Saccharomyces cerevisiae*. The ATP-synthesizing reactions in the glycolytic pathway) specifically involve the substrate phosphorylation reactions catalyzed by phosphoglycerokinase and pyruvic kinase. Although all the ATP molecules available for fermentative growth are believed to be generated by these substrate phosphorylation reactions, some energy equivalents are also generated by proton extrusion reactions (acid liberation), which occur with intact membrane systems and involve the proton extrusion reactions of energy conservation) as it applies to fermentative metabolism.

TABLE 4-3 Energy Obtained from Bacterial Fermentations by Substrate Phosphorylations

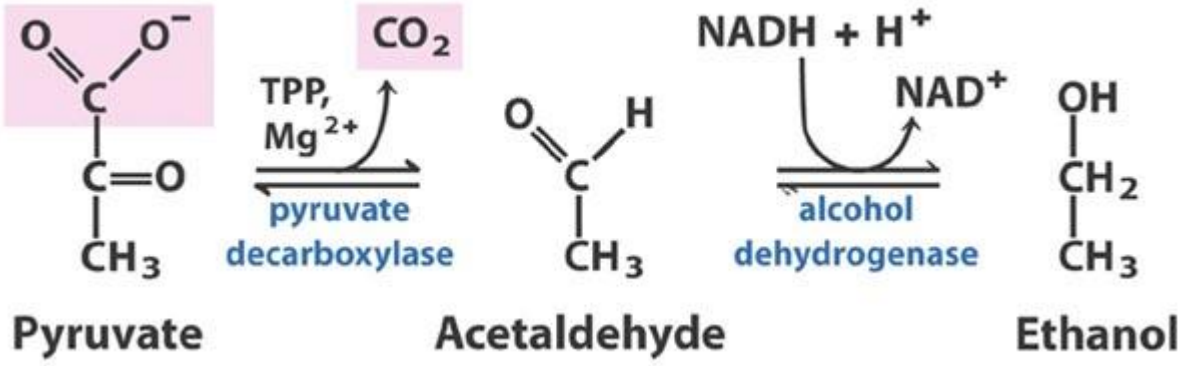
	Fermentation	Actual Energy (cal/mole)	Theoretical Energy (cal/mole)	Efficiency (%)
Homolactic	$C_6H_{12}O_6$ (Glucose) $\xrightarrow{\text{Glycolysis}}$ $2CH_3-\underset{\underset{OH}{|}}{C}H-COOH$ (Lactic acid) +	−20,000	57,000	35
Alcoholic	$C_6H_{12}O_6$ (Glucose) $\xrightarrow{\text{Glycolysis}}$ $2CH_3-\underset{\underset{H}{|}}{C}H-OH + 2CO_2$ (Ethanol) +	−20,000	58,000	34

Different Fermentation pathway of bacteria

1. Alcoholic fermentation

- In this pathway first glucose is converted into Pyruvate by glycolysis. And then alcohol dehydrogenase reduces the pyruvate into ethanol and CO2.
- Metabolism of pyruvate to produce ethanol occurs in two steps.
- 1st step: pyruvate is first decarboxylated into Acetaldehyde and CO2. This reaction is catalyzed by the enzyme Pyruvate decarboxylase with Thymine pyrophosphate (TPP) as co-enzyme.

- 2nd step: Acetaldehyde is then reduced to ethanol by NADH2. This reaction is catalyzed by enzyme Alcohol dehydrogenase. NAD+ is regenerated in this step.



Net equation for alcoholic fermentation is

Glucose+ 2ADP +2Pi……. 2Ethanol +2CO2+2ATP

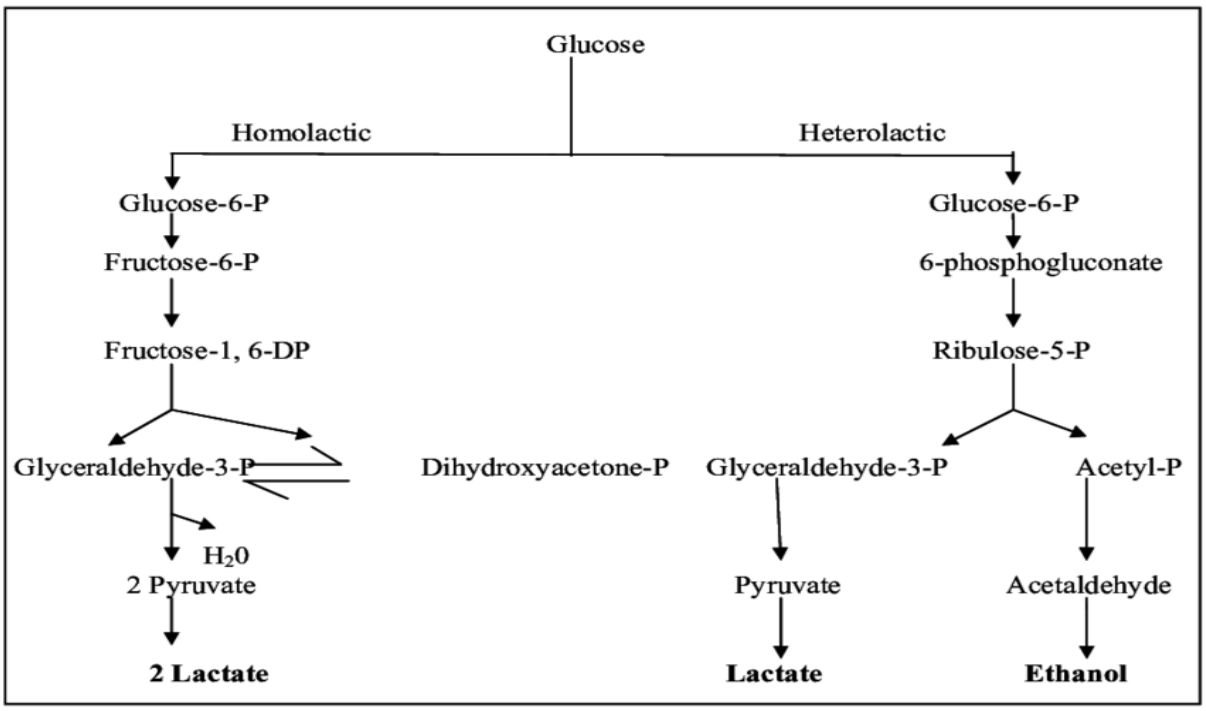
- Examples: *Sacharomyces cereviseae*, *Pseudomonas*

Application:

- Alcoholic fermentation is important in food and industrial microbiology and is used to produce beer, wine, distilled sprits etc.
- It is also used in production of fermented food products.

2. Lactic acid fermentation:

- In this pathway pyruvate is reduced to lactic acid. This is a single step reaction carried out by Lactic acid bacteria (LAB)
- There are two types of lactic acid fermentation.i. Homo lactic fermentationii. Hetero lactic fermentation



Homolactic fermentation:

- In homolactic fermentation, end product is lactic acid.
- Pyruvate is reduced to lactate or lactic acid by the enzyme lactate dehydrogenase (Pyruvate reductase).
- **Homolactic bacteria:** *Streptococcus thermophiles, Streptococcus lactis, lactobacillus lactis, Lactobacillus bulgarius, Pediococcus, Enterococcus*

Application:

- Homolactic fermentation is important in dairy industry for souring of milk to produce various fermented products
- Streptococcus mutant, a bacteria responsible for dental caries is a homolactic fermenting bacteria.
- Lactobacillus spp in the digestive tract of human helps in digestion of lactose present in milk.
- Lactobacillus spp are used as probiotic.

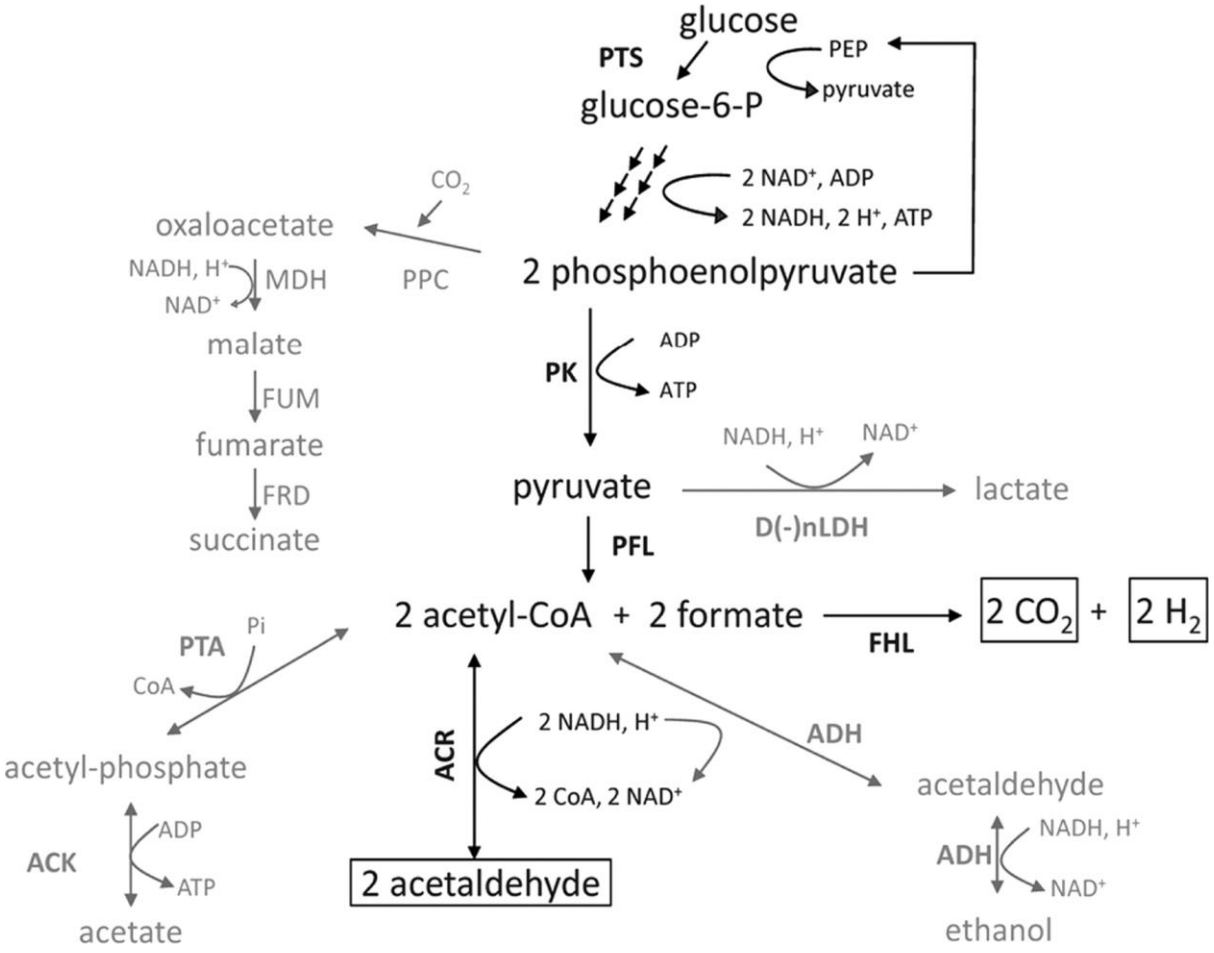
Heterolactic fermentation:

- In hetero lactic fermentation, end product is ethanol and CO2 in addition to lactic acid.
- In this reaction glucose is first metabolized to pyruvate, acetic acid and CO2 by Pentose phosphate pathway.

- Pyruvate is then reduced to lactic acid whereas acetic acid is reduced to ethanol and CO2.
- **Heterolactic bacteria*:*** *Leuconostoc mesenteroides, Lactobacillus bifermentous, Leconostoc lactis*

3. Mixed acid fermentation:

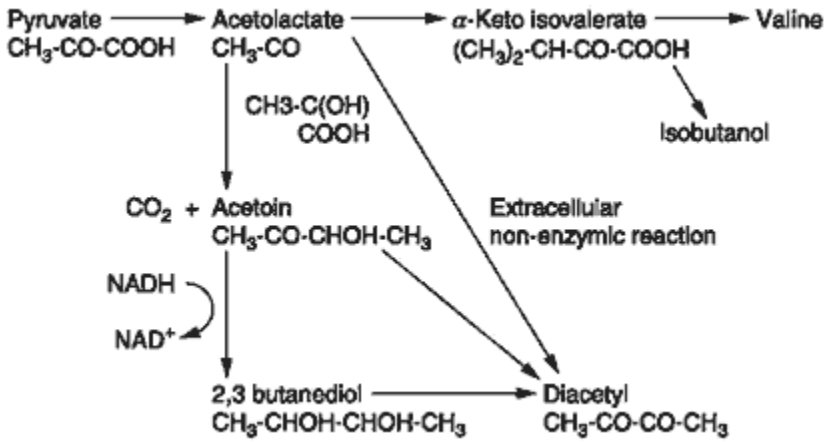
- In mixed acid fermentation, mixture of acids such as lactic acid, acetic acid, ethanol, formic acid etc are produced as the end product.
- At first pyruvate is cleaved by the enzyme Pyruvate formate lyase to yield formic acid and Acetyl coA.
- From formic acid various other end products such as acetic acid, lactic acid, succinic acid, ethanol or CO2 and water are formed according to types of pathway and types of bacteria. However formic acid is always the intermediate product in this pathway.



- This pathway is followed by member of Enterobacteriaceae family such as *E. coli, Salmonella, Klebsiella* etc.
- This fermentative pathway is the basis of Methyl red test.

4. 2,3-Butanediol fermentation:

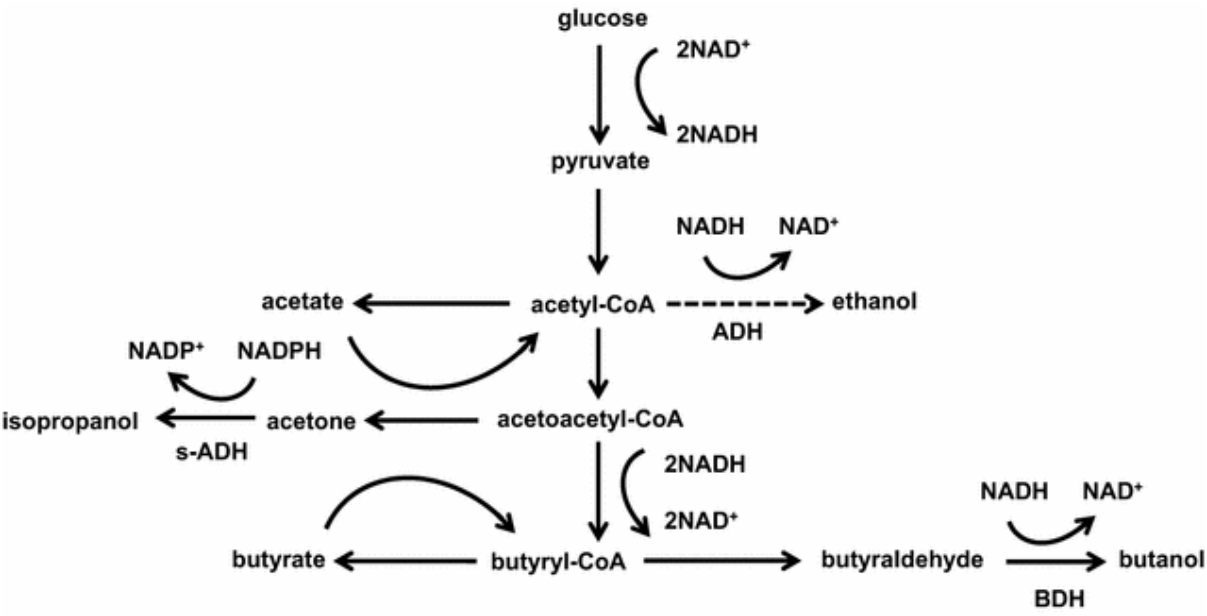
- In this pathway 2,3-butanediol is the end product.
- Some Pyruvate produced during glycolysis is metabolized as in mixed acid fermentation but most of the pyruvate is condensed to form α-acetolactate.
- α-acetolactate undergoes decarboxylation in the presence of enzyme pyruvate decarboxylase to produce Acetoin (acetyl methylcarbainol) which is reduced by NADH2 to form 2,3-butanediol.



- This pathway is followed by some member of Enterobacteriaceae famil. Eg Klebsiella
- This fermentative pathway is the basis of VP test.

5. Butanol fermentation:

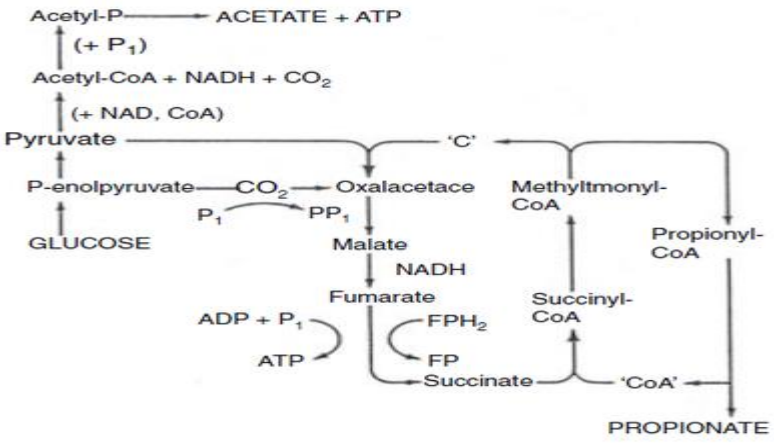
- In this pathway pyruvate is converted into butanol or butyrate. Other end product such as Acetone and CO2 or Isopropyl alcohol and CO2 may formed by this pathway.
- This pathway is present in *Clostridium* spp
- At first *Clostridium* spp convert pyruvate into AcetylcoA aerobically.



- Two molecule of acetylcoA condenses in the presence of enzyme acetyl-transferase to from AcetoacetylcoA.
- AcetoacetylCoA is reduced to β-hydroxybutyrylcoA by NADH2 in the presence of enzyme hydroxybutyrate dehydrogenase.
- β-hydroxybutyrylcoA is reduced by enoylcoA hydratase to form CrotonylcoA and water.
- CrotonylcoA is further reduced to butyrylcoA by an enzyme NAD-linked dehydrohenase.
- ButyrylcoA and acetate act together with fatty acid coA transferase to form acetylcoA and butyrate. Acetyl coA then recycle in the reaction.

6. Propionic acid fermentation:

- In this pathway propionic acid and CO2 is the end product.
- This pathway is carried out by Propionic acid bacteria (PAB).
- These bacteria ferment glucose or lactate to propionic acid under anaerobic condition.



- Pyruvate reacts with methyl malonyl coA to form Propionyl coA and Oxaloacetate.
- Oxaloacetate give rise to malate, fumarate and succinate by reverse TCA cycle.
- Propionyl coA transfer its coA to succinate to form succinylcoA and propionate
- Example: *Propioonibacterium*

UNIT 5

Endospore

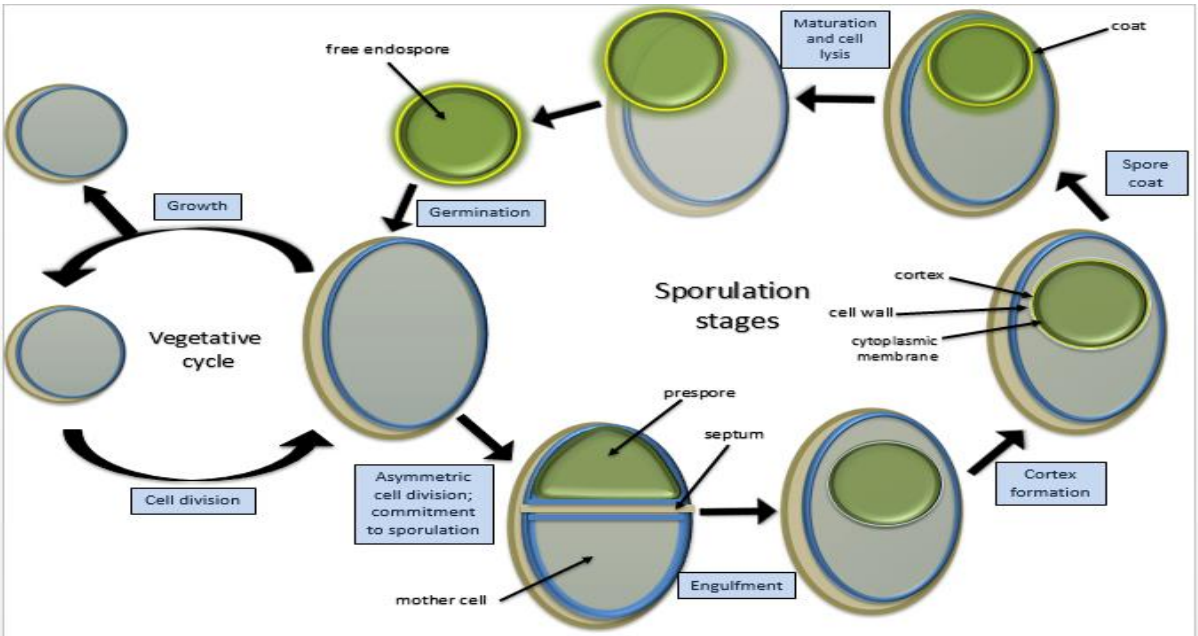
An **endospore** is a dormant, tough, and non-reproductive structure produced by some bacteria in the phylum Firmicutes. The name "endospore" is suggestive of a spore or seed-like form (*endo* means within), but it is not a true spore (i.e., not an offspring). It is a stripped-down, dormant form to which the bacterium can reduce itself. Endospore formation is usually triggered by a lack of nutrients, and usually occurs in gram-positive bacteria. In endospore formation, the bacterium divides within its cell wall, and one side then engulfs the other. Endospores enable bacteria to lie dormant for extended periods, even centuries. There are many reports of spores remaining viable over 10,000 years, and revival of spores millions of years old has been claimed. There is one report of viable spores of *Bacillus marismortui* in salt crystals approximately 250 million years old. When the environment becomes more favorable, the endospore can reactivate itself to the vegetative state. Most types of bacteria cannot change to the endospore form. Examples of bacterial genera that can form endospores include *Bacillus cereus*, *Bacillus anthracis*, *Bacillus thuringiensis*, *Clostridium botulinum*, and *Clostridium tetani*.

The endospore consists of the bacterium's DNA, ribosomes and large amounts of dipicolinic acid. Dipicolinic acid is a spore-specific chemical that appears to help in the ability for endospores to maintain dormancy. This chemical accounts for up to 10% of the spore's dry weight.

Endospores can survive without nutrients. They are resistant to ultraviolet radiation, desiccation, high temperature, extreme freezing and chemical disinfectants. Thermo-resistant endospores were first hypothesized by Ferdinand Cohn after studying *Bacillus subtilis* (pictured to the right) growth on cheese after boiling the cheese. His notion of spores being the reproductive mechanism for the growth was a large blow to the previous suggestions of spontaneous generation. Astrophysicist Steinn Sigurdsson said "There are viable bacterial spores that have been found that are 40 million years old on Earth – and we know they're very hardened to radiation." Common antibacterial agents that work by destroying vegetative cell walls do not affect endospores. Endospores are commonly found in soil and water, where they may survive for long periods of time. A variety of different microorganisms form "spores" or

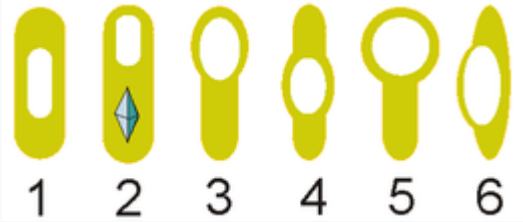
"cysts," but the endospores of low G+C gram-positive bacteria are by far the most resistant to harsh conditions.

Some classes of bacteria can turn into exospores, also known as microbial cysts, instead of endospores. Exospores and endospores are two kinds of "hibernating" or dormant stages seen in some classes of microorganisms.



Formation of an endospore through the process of sporulation.

Structure



Variations in endospore morphology: (1, 4) central endospore; (2, 3, 5) terminal endospore; (6) lateral endospore

Bacteria produce a single endospore internally. The spore is sometimes surrounded by a thin covering known as the exosporium, which overlies the *spore coat*. The spore coat, which acts like a sieve that excludes large toxic molecules like lysozyme, is resistant to many toxic molecules and may also contain enzymesthat are involved in germination. In *Bacillus subtilus* endospores, the spore coat is estimated to contain more than 70 coat proteins, which are organized into an inner and an outer coat layer. The X-ray

diffraction pattern of purified *B. subtilis*endospores indicates the presence of a component with a regular periodic structure, which Kadota and Iijima speculated might be formed from a keratin-like protein. However, after further studies this group concluded that the structure of the spore coat protein was different from keratin. When the *B. subtilis* genome was sequenced, no ortholog of human keratin was detected. The *cortex* lies beneath the spore coat and consists of peptidoglycan. The *core wall* lies beneath the cortex and surrounds the protoplast or *core* of the endospore. The core contains the spore chromosomal DNA which is encased in chromatin-like proteins known as SASPs (small acid-soluble spore proteins), that protect the spore DNA from UV radiation and heat. The core also contains normal cell structures, such as ribosomes and other enzymes, but is not metabolically active.

Up to 20% of the dry weight of the endospore consists of calcium dipicolinate within the core, which is thought to stabilize the DNA. Dipicolinic acid could be responsible for the heat resistance of the spore, and calcium may aid in resistance to heat and oxidizing agents. However, mutants resistant to heat but lacking dipicolinic acid have been isolated, suggesting other mechanisms contributing to heat resistance are also at work. Small acid-soluble proteins (SASPs) are found in endospores. These proteins tightly bind and condense the DNA, and are in part responsible for resistance to UV light and DNA-damaging chemicals.

Visualising endospores under light microscopy can be difficult due to the impermeability of the endospore wall to dyes and stains. While the rest of a bacterial cell may stain, the endospore is left colourless. To combat this, a special stain technique called a Moeller stain is used. That allows the endospore to show up as red, while the rest of the cell stains blue. Another staining technique for endospores is the Schaeffer-Fulton stain, which stains endospores green and bacterial bodies red. The arrangement of spore layers is as follows:

- Exosporium
- Spore coat
- Spore cortex
- Core wall

Location

The position of the endospore differs among bacterial species and is useful in identification. The main types within the cell are terminal, subterminal, and

centrally placed endospores. Terminal endospores are seen at the poles of cells, whereas central endospores are more or less in the middle. Subterminal endospores are those between these two extremes, usually seen far enough towards the poles but close enough to the center so as not to be considered either terminal or central. Lateral endospores are seen occasionally.

Examples of bacteria having terminal endospores include *Clostridium tetani*, the pathogen that causes the disease tetanus. Bacteria having a centrally placed endospore include *Bacillus cereus*. Sometimes the endospore can be so large the cell can be distended around the endospore. This is typical of *Clostridium tetani*.

Under conditions of starvation, especially the lack of carbon and nitrogen sources, a single endospore forms within some of the bacteria through a process called sporulation.[13]

When a bacterium detects environmental conditions are becoming unfavourable it may start the process of endosporulation, which takes about eight hours. The DNA is replicated and a membrane wall known as a *spore septum* begins to form between it and the rest of the cell. The plasma membrane of the cell surrounds this wall and pinches off to leave a double membrane around the DNA, and the developing structure is now known as a forespore. Calcium dipicolinate, the calcium salt of dipicolinic acid, is incorporated into the forespore during this time. The dipicolinic acid helps stabilize the proteins and DNA in the endospore.[14]:141Next the peptidoglycan cortex forms between the two layers and the bacterium adds a spore coat to the outside of the forespore. In the final stages of endospore formation the newly forming endospore is dehydrated and allowed to mature before being released from the mother cell.[3] The cortex is what makes the endospore so resistant to temperature. The cortex contains an inner membrane known as the core. The inner membrane that surrounds this core leads to the endospore's resistance against UV light and harsh chemicals that would normally destroy microbes.[3] Sporulation is now complete, and the mature endospore will be released when the surrounding vegetative cell is degraded.

Endospores are resistant to most agents that would normally kill the vegetative cells they formed from. Unlike persister cells, endospores are the result of a morphological differentiation process triggered by nutrient limitation (starvation) in the environment; endosporulation is initiated by quorum sensing within the "starving" population.[14]:141 Most disinfectants such as

household cleaning products, alcohols, quaternary ammonium compounds and detergents have little effect on endospores. However, sterilant alkylating agents such as ethylene oxide (ETO), and 10% bleach are effective against endospores. To kill most anthrax spores, standard household bleach (with 10% sodium hypochlorite) must be in contact with the spores for at least several minutes; a very small proportion of spores can survive longer than 10 minutes in such a solution.[15] Higher concentrations of bleach are not more effective, and can cause some types of bacteria to aggregate and thus survive.

While significantly resistant to heat and radiation, endospores can be destroyed by burning or by autoclaving at a temperature exceeding the boiling point of water, 100 °C. Endospores are able to survive at 100 °C for hours, although the larger the number of hours the fewer that will survive. An indirect way to destroy them is to place them in an environment that reactivates them to their vegetative state. They will germinate within a day or two with the right environmental conditions, and then the vegetative cells, not as hardy as endospores, can be straightforwardly destroyed. This indirect method is called tyndallization. It was the usual method for a while in the late 19th century before the introduction of inexpensive autoclaves. Prolonged exposure to ionising radiation, such as x-rays and gamma rays, will also kill most endospores.

The endospores of certain types of (typically non-pathogenic) bacteria, such as *Geobacillus stearothermophilus*, are used as probes to verify that an autoclaved item has been rendered truly sterile: a small capsule containing the spores is put into the autoclave with the items; after the cycle the content of the capsule is cultured to check if anything will grow from it. If nothing will grow, then the spores were destroyed and the sterilization was successful.[16]

In hospitals, endospores on delicate invasive instruments such as endoscopes are killed by low-temperature, and non-corrosive, non-toxic, ethylene oxide sterilizers. ETO is the only low-temperature sterilant to stop outbreaks on these instruments.[17] In contrast, "high level disinfection" does not kill endospores but is used for instruments such as a colonoscope that do not enter sterile bodily cavities. This latter method uses only warm water, enzymes, and detergents.

Bacterial endospores are resistant to antibiotics, most disinfectants, and physical agents such as radiation, boiling, and drying. The impermeability of the spore

coat is thought to be responsible for the endospore's resistance to chemicals. The heat resistance of endospores is due to a variety of factors:

- Calcium dipicolinate, abundant within the endospore, may stabilize and protect the endospore's DNA.
- Small acid-soluble proteins (SASPs) saturate the endospore's DNA and protect it from heat, drying, chemicals, and radiation. They also function as a carbon and energy source for the development of a vegetative bacterium during germination.
- The cortex may osmotically remove water from the interior of the endospore and the dehydration that results is thought to be very important in the endospore's resistance to heat and radiation.
- Finally, DNA repair enzymes contained within the endospore are able to repair damaged DNA during germination.

Reactivation

Reactivation of the endospore occurs when conditions are more favourable and involves *activation*, *germination*, and *outgrowth*. Even if an endospore is located in plentiful nutrients, it may fail to germinate unless activation has taken place. This may be triggered by heating the endospore. Germination involves the dormant endospore starting metabolic activity and thus breaking hibernation. It is commonly characterised by rupture or absorption of the spore coat, swelling of the endospore, an increase in metabolic activity, and loss of resistance to environmental stress.

Outgrowth follows germination and involves the core of the endospore manufacturing new chemical components and exiting the old spore coat to develop into a fully functional vegetative bacterial cell, which can divide to produce more cells.

Endospores possess five times more sulfur than vegetative cells. This excess sulfur is concentrated in spore coats as an amino acid, cysteine. It is believed that the macromolecule accountable for maintaining the dormant state has a protein coat rich in cystine, stabilized by S-S linkages. A reduction in these linkages has the potential to change the tertiary structure, causing the protein to unfold. This conformational change in the protein is thought to be responsible for exposing active enzymatic sites necessary for endospore germination.[18]

Endospores can stay dormant for a very long time. For instance, endospores were found in the tombs of the Egyptian pharaohs. When placed in appropriate medium, under appropriate conditions, they were able to be reactivated. In 1995, Raul Cano of California Polytechnic State University found bacterial spores in the gut of a fossilized bee trapped in amber from a tree in the Dominican Republic. The bee fossilized in amber was dated to being about 25 million years old. The spores germinated when the amber was cracked open and the material from the gut of the bee was extracted and placed in nutrient medium. After the spores were analyzed by microscopy, it was determined that the cells were very similar to *Bacillus sphaericus* which is found in bees in the Dominican Republic today.

Importance

As a simplified model for cellular differentiation, the molecular details of endospore formation have been extensively studied, specifically in the model organism *Bacillus subtilis*. These studies have contributed much to our understanding of the regulation of gene expression, transcription factors, and the sigma factor subunits of RNA polymerase.

Endospores of the bacterium *Bacillus anthracis* were used in the 2001 anthrax attacks. The powder found in contaminated postal letters was composed of extracellular anthrax endospores. This intentional distribution lead to 22 known cases of anthrax (11 inhalation and 11 cutaneous) making the case fatality rate among patients with inhalation anthrax 45% (5/11). The six other individuals with inhalation anthrax and all the individuals with cutaneous anthrax recovered. Had it not been for antibiotic therapy many more might have sbeen stricken.

According to WHO veterinary documents, *B. anthracis* sporulates when it sees oxygen instead of the carbon dioxide present in mammal blood; this signals to the bacteria that it has reached the end of the animal, and an inactive dispersable morphology is useful.

Sporulation requires the presence of free oxygen. In the natural situation, this means the vegetative cycles occur within the low oxygen environment of the infected host and, within the host, the organism is exclusively in the vegetative form. once outside the host, sporulation commences upon exposure to the air and the spore forms are essentially the exclusive phase in the environment.

Biotechnology

Bacillus subtilis spores are useful for the expression of recombinant proteins and in particular for the surface display of peptides and proteins as a tool for fundamental and applied research in the fields of microbiology, biotechnology and vaccination.

Endospore-forming bacteria

Examples of endospore-forming bacteria include the genera:

- *Acetonema*
- *Actinomyces*
- *Alkalibacillus*
- *Ammoniphilus*
- *Amphibacillus*
- *Anaerobacter*
- *Anaerospora*
- *Aneurinibacillus*
- *Anoxybacillus*
- *Bacillus*
- *Brevibacillus*
- *Caldanaerobacter*
- *Caloramator*
- *Caminicella*
- *Cerasibacillus*
- *Clostridium*
- *Clostridiisalibacter*
- *Cohnella*
- *Coxiella* (i.e. *Coxiella burnetii*)
- *Dendrosporobacter*
- *Desulfotomaculum*
- *Desulfosporomusa*
- *Desulfosporosinus*
- *Desulfovirgula*
- *Desulfunispora*
- *Desulfurispora*
- *Filifactor*
- *Filobacillus*
- *Gelria*
- *Geobacillus*
- *Geosporobacter*
- *Gracilibacillus*
- *Halobacillus*
- *Halonatronum*
- *Heliobacterium*
- *Heliophilum*
- *Laceyella*
- *Lentibacillus*
- *Lysinibacillus*
- *Mahella*
- *Metabacterium*
- *Moorella*
- *Natroniella*
- *Oceanobacillus*
- *Orenia*
- *Ornithinibacillus*
- *Oxalophagus*
- *Oxobacter*
- *Paenibacillus*
- *Paraliobacillus*

Definition: What is Sporulation?

Essentially, sporulation refers to the formation of spores from vegetative cells during unfavorable environmental conditions. As such, it may be described as an adaptive response that allows the organism to survive given adverse conditions (radiation, extreme heat or cold, lack of nutrition etc).

Compared to vegetative cells, spores (formed during sporulation) are multilayered structures that tend to be dormant (or relatively dormant). These characteristics make it possible for some of the spores to preserve the genetic content of the organism during harsh environmental conditions.

During certain unfavorable conditions (depending on the organism), some of the vegetative cells go through a series of morphological changes (and some level of programmed gene expression) that ultimately produce spores. Apart from genetic material, spores also contain some cytoplasm, specific acids, ribosome, and the appropriate enzymes among others that allow the spore to germinate during favorable environmental conditions.

The term "spore" comes from the Greek word for Seed.

Some of the organisms that produce spores include:

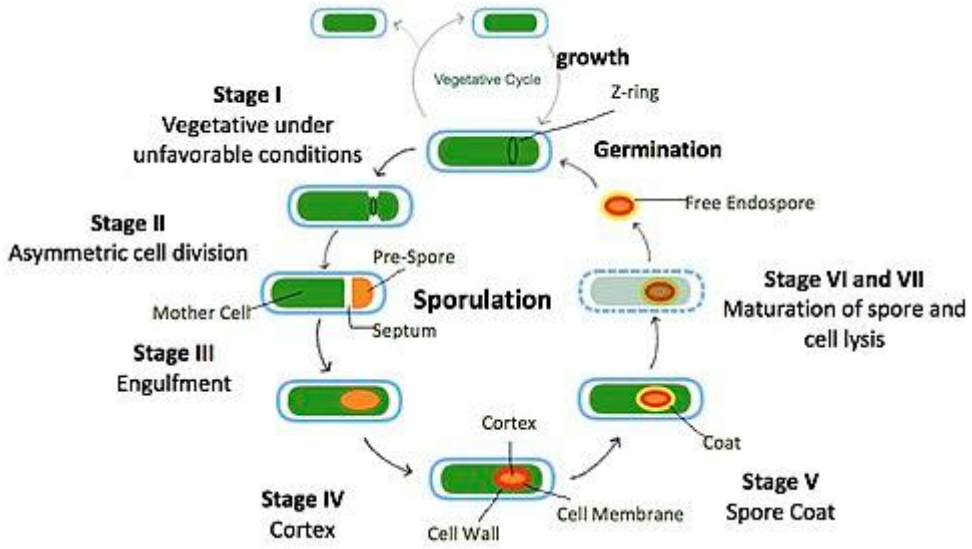
- Plants
- Algae
- Protozoa
- Bacteria
- Fungi

Depending on the organism, spores vary significantly.

There are also different types of spores including:

- Asexual spores (e.g. exogenous spores produced by Conidia oidia)
- Sexual spores such as Oospores and Zygote
- Vegetative spores (e.g. Chlamydospores)
- Megaspores of plants (female gametophyte)
- Microspores of plants (develop to formmale gametophyte)
- Endospores - Type of spores that are produced within the organism (e.g. bacteria)

- Exospores - Exospores are a type of spores that are produced outside the cell (e.g. in the genus Methylosinus)



Sporulation in Bacillus subtilis in stages:]

Sporulation in Bacteria

A majority of spore-forming bacteria are Gram-negative bacilli (rod-shaped). These include aerobic Bacillus and anaerobic Clostridium species. Although some Gram-negative bacteria have been shown to be capable of producing spores, it is only a few species found in a few genera.

***** Some filamentous cocci have also been shown to be capable of sporulation (producing endospores)

Depending on the type of bacteria, four types of spores are produced.

These include:

Endospores: Endospores are the most common types of spores. They are typically produced by Clostridium (e.g. Clostridium botulinum), Bacillus (e.g. Bacillus anthracis) and Sporosarcina bacteria (e.g. Sporosarcina ureae).

Cysts: Cysts are a group of cells that join together in order to survive harsh environmental conditions. When conditions in the environment become unfavorable, the cells come together in a manner that allows them to survive. They are common in Azotobacter species

***** Unlike endospores, cysts are non-reproductive cells. They are also less resistant to tough environmental conditions and have a tough cell wall that is waterproof in place of the peptidoglycan layer found on the cell wall of bacterial endospores

Myxospores: A myxospore is a type of spore that is produced by myxobacteria/ Myxobacterates (slimy molds bacteria). Referred to as resting cells in some books, myxospores are capable of surviving such harsh environmental conditions as UV light, high temperatures, and desiccation. They are formed within the fruiting body of the bacteria and in contrast to other resting cells; they are formed from specialized spore-bearing structures.

Exospores: Exospores are a type of spore that are produced by members of the phylum Actinobacteria. These spores are produced through the budding of mycelium and can survive in harsh environmental conditions better than the vegetative cells.

***** Although spores play an important role in the life cycle of bacteria, they are not an obligatory stage. As such, they are typically common during unfavorable environmental conditions.

***** Sporulating bacteria are ubiquitous and can be found in soil, water, and air among other environments in nature.

***** Compared to the other types of spores, endospores are the most defensive structures capable of surviving harsher environmental conditions. This is largely due to the fact that beneath the thin and delicate exosporium, they have a spore coat that is made up of layers of proteins.

Some of the other characteristics associated with the high resistance capacity of endospores include:

High dipicolinic acid content

- High calcium concentration
- High amounts of SASP (small acid soluble DNA binding proteins)

Although unfavorable conditions such as diminishing sources of nutrition play an important role in sporulation, in that they trigger the process, sporulation does not necessarily start taking place right away. Rather, the bacteria will start by trying to move to an area with favorable conditions.

For instance, in the event that food sources diminish, the bacteria, using such structures will attempt to move to a new location through such processes as chemotaxis. Other mechanisms to survive may involve an attempt to adapt to the new conditions or changing their mode of reproduction among others. Therefore, sporulation occurs as the last survival strategy. Before the cell commits to sporulation, several conditions must be met.

These include:

- Chromosome integrity
- Functional Krebs cycle

The following is a basic mode of sporulation of Bacillus subtilis:

The end of vegetative growth

Phosphorelay system (consisting of a number of kinases (histidine kinases)) transmit information regarding the environmental conditions and conditions of the cell to the Spo0A (master transcriptional regulator) thus activating it - This phase also plays an important role in determining the state of phosphorylation of the intercellular pool of the master transcriptional regulator

* Here, Spo0A with a phosphyl group play an important role in regulating expression of about 121 genes some of which are involved in sporulation - High levels of Spo0A~P promote the process while low levels result in the formation of biofilm.

Stage 1: Axial filamentation and chromosome division - Activation of the master transcriptional regulator is followed by chromosome replication with each chromosome moving to the opposite poles of the cell – One of the chromosomes remains in the mother cell while the other is contained in the forespore

Stage II: Asymmetric septation - Origin-proximal regions of the cell are tethered to the opposite poles of the cell

Chromosomal DNA stretch forming axial filaments

Stage III: Engulfment - As the cell divides, 30 percent of the origin-proximal portion containing chromosome is covered. This is known as the forespore – A

section of the cell containing chromosome is engulfed by a membrane as the peptidogycan is synthesized.

Stage IV-V: Cortex and Coat assembly - Synthesis of the peptidoglycan cortex followed by formation of proteinaceous spore coat: In this stage, the spore (mature) is enclosed in two different layers that include the outer coat consisting of different types of proteins and the cortex that consists of a special type of peptidoglycan.

Lysis of the mother cell - The mother cell undergoes lysis/disintegration. Some material from the mother cell may be used to build on the spore coat.

Spore release - Mature spores are released into the environment and are capable of surviving high temperatures, certain chemicals that can destroy the vegetative cell and radiation among other extreme environments. Once environmental conditions improve, the spore can germinate as the organism returns to the original vegetative growth.

***** Mutation has been shown to result in the production of twin endospores (two viable spores). Some mutations may also produce more than two endospores.

Sporulation of Fungi

Fungi are some of the most studied spore-producing organisms in the world. They produce a wide variety of spores that significantly vary in size, shape and other surface features that suit their environment (for dispersal etc).

Whereas the spores produced sexually (through meiosis) remain dormant for survival (e.g. ascospores), those that are produced asexually (mitospores) are for dispersal.

Produced through mitosis, asexual spores are released in high numbers and are genetically identical. This allows them to play an important role in reproduction when they land on the appropriate substrate in the environment following dispersal.

* **Chlamydospore** - This is a type of fungal spore that develops from the hyphal structures during unfavorable conditions. Chlamydospores are characterized by a thick, melanized wall that protects the contents of the spore.

Different types of fungi may produce different types of spores.

The following are some of the main groups of fungi and the type of spores they produce:

· Zygomycota - Members of Zygomycota are known as zygomeycetes. They produce both sexual (zygospores) and asexual (sporangiospores) spores.

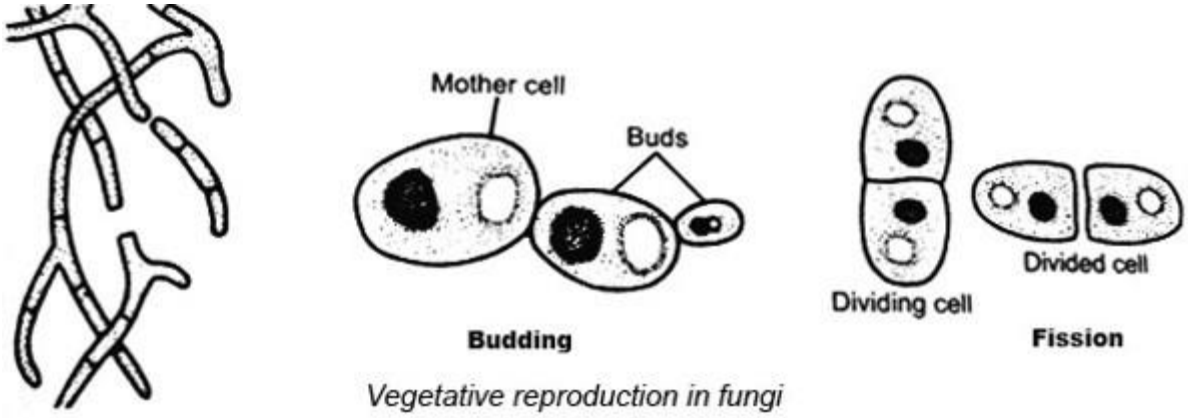
· Ascomycota - Ascomycetes also produce both sexual (ascospores) and asexual (conidia) spores.

· Basidiomycota - Compared to the other groups of fungi, basidiomyecetes are largely known to produce sexual spores that are known as basidiospores.

Reproduction in fungi: asexual and sexual methods

Asexual reproduction in fungi:

1. fission of somatic cell
2. Budding of somatic cell
3. Fragmentation or disjoining of hyphae
4. Asexual spore formation



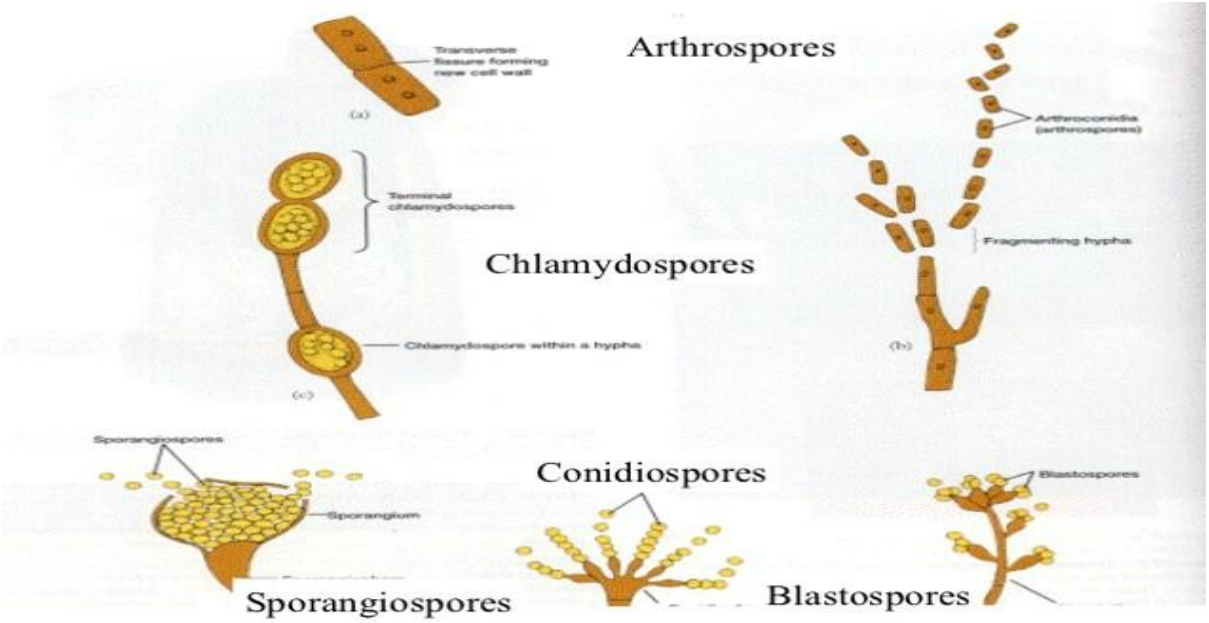
Vegetative reproduction in fungi

1. Fission:

- In binary fission a mature cell elongates and its nucleus divides into two daughter nuclei.
- The daughter nuclei separates, cleaves cytoplasm centripetally in the middle till it divides parent protoplasm into two daughter protoplasm.
- A double cross wall is deposited in the middle to form two daughter cell.

- Ultimately the middle layer of double cross wall degenerates and daughter cells are separated.
- Examples: Saccharomyces pobbe, Psygosaccharomyces

2. Budding:
   - The cell wall bulge out and softens in the area probably by certain enzymes brought by vesicles.
   - The protoplasm also bulge out in this region as small protuberance.
   - The parent nucleus also divides into two, one of the daughter nucleus migrates into bud, the cytoplasm of bud and mother remain continuous for some time
   - As the bud enlarges, a septum is laid down at the joining of bud with mother cell. Then bud separates and leads independent life.
   - Some time, bud starts reproducing while still attached with mother cell. This gives branching appearance.
   - Budding is the typical reproductive characteristics of Ascomycetes.
   - Examples: yeast
3. Fragmentation:
   - In some fungi, fragmentation or disjoining of hyphae occurs and each hyphae become a new organism
4. Asexual spore of fungi:
   - Spore formation is the characteristic feature of fungi.
   - Different fungi forms different types of spore,



Types of asexual spore:

i. Sporangiospore:

- These asexual spore are produced in a sac like structure called sporangia (singular;saprangium).
- Sporangium are produced at the end of special aerial hyphae called sporangiophore
- Sporangium contains large numbers of haploid spores, which are released by rapture of sporangial wall
- Examples: *Rhizopus*

ii. Conidiospore:

- Conidiospore or conidia are single celled, bicelled or multicelled structure born on the tip or side of aerial hyphal structure called conidiophore
- Conidia are different from sporangiospore as these are not produced inside sporangium or any sac like structure.
- Conidia are born singly or in chain
- Examples: *Penicillium, Apergillus*

iii. Arthrospore:

- Arthrospore are very primitive type of spore formed by the breaking up of fungal mycelium
- A spore is formed by separation followed by fragmentation of hyphae
- Examples: *Trichosporium, Geotrichum, Coccididious imitis*

iv. Chlamydospore:

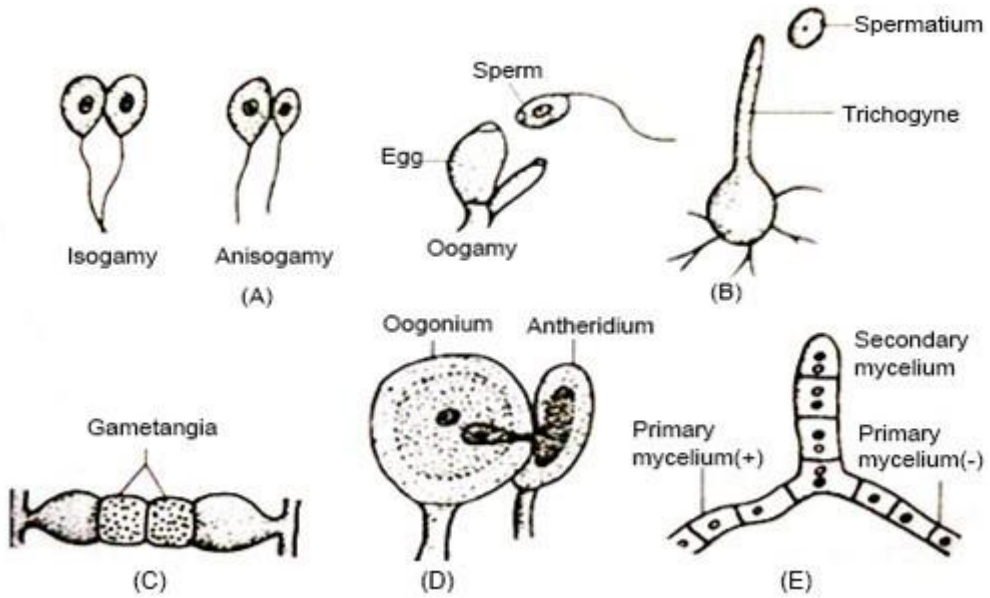
- These are usually formed during unfavorable condition and are thick walled single celled spore, which are highly resistant to adverse condition.
- Hyphal cell or portion of hyphae contracts, loose water, round up and develops into thick walled chalmydospore.
- When favorable condition returns, each chlamydospore give rise to a new individual fungi.
- Examples: ascomycetes, basidiomycetes, zygomycetes,
- *Histoplasma capsulatum, Candida albicans*

v. Blastospore:

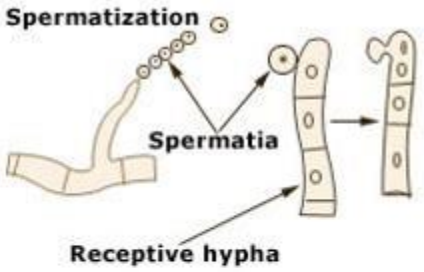
- It is a budding spores usually formed at the terminal end of hyphae.
- These spore may remains attached to hyphae and bud further to gibe branching chain of blastospores
- Examples: ascomycetes, basidiomycetes, zygomycetes

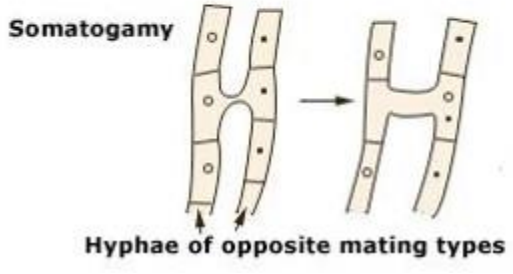
Sexual reproduction in fungi:

- Sexual reproduction is carried out by diffusion of compatable nuclei from two parent at a definite state in the life cycle of fungi.
- The process of sexual reproduction involves three phases:
    - Plasmogamy: fusion of protoplasm
    - Karyogamy: fusion of nucleus
    - Meiosis: reductional nuclear division
- Various methods by which compatible nuclei are brought together in plasmogamy. Some are:
    - Gametic copulation
    - Gamete-gametangial copulation
    - Gametangial copulation
    - Somatic copulation
    - Spermatization



1. Gametic copulation:
   - Fusion of two naked gametes, one or both of them are motile
     - Isogamous
     - Anisogamous
     - Oogamous
2. Gamete-gametangial copulation:
   - Male and female gametangia comes into contact but do not fuse.
   - A fertilization tube formed from where male gametangium enters the female gametangium and male gamate passes through this tube
3. Gametangial copulation;
   - Two gametangia or their protoplast fuse and give rise to zygospore
4. Somatic copulation:
   - Also known as somatogamy.
   - In this process fusion of somatic cell occurs
   - This sexual fusion of undifferentiated vegetative cell results in dikaryotic hyphae, so the process is also called dikarotization
5. Spermetization:
   - It is an union of special male structure called spertatium with a female receptive structure.
   - Spermatium empties its content into receptive hyphae during plasmogamy





Sexual spores of fungi

- As a result of sexual reproduction sexual sores are produced.
- Sexual spores are fewer in number than asexual spores.

Types of sexual spores

i. Ascospore:

- It is usually single celled produced in a sac called ascus (plural;asci) and usually there are 4-8 ascospore in an ascus but the number may vary from species to species
- The ascospore are usually arranged in a linear order. In some case ascospores are long, narrow and are arranged in parallel order.

ii. Basidiospore:

- It is a reproductive spore produced by basidiomycetes.
- This single celled spores are born in a club shaped structure called basidium
- These basidiospore aerves as main air dispersal unit for the fungi.

iii. Zygospore:

- Zygospores are thick walled spores formed when two sexually compatible hyphae or gametangia of certain fungi fuse together.
- In suitable condition, zygospore germinates to produce a single vertical hyphae which forms a aporangium and releases its spores

iv. Oospore:

- These are formed within a special female structure called Oogonium.

- Fertilization of egg by male gamete in female sex organ give rise to oospoes.
- There are one or more oospores in each oogonium.

Reproduction in Algae:

ode # 1. Vegetative Reproduction:
In this type, any vegetative part of the thallus develops into new individual. It does not involve any spore formation and there is no alternation of generations. It is the most common method of reproduction in algae.

The vegetative reproduction in algae is of the following types:
a. Cell division or fission:
It is the simplest method of reproduction. The unicellular forms of algae commonly reproduce by this simple process, often called binary fission as found in Chlamydomonas, Synechococcus (Fig. 3.16A), diatoms etc. In this method the vegetative cell divides mitotically into two daughter cells, those finally behave as new individual.

b. Fragmentation:
In this method, the multicellular filamentous thallus breaks into many-celled fragments, each of which gives rise to a new individual. The fragmentation may be accidental or by the formation of separation discs or by some other mechanical force or injury. It is found in Spirogyra, Ulothrix, Oedo- gonium, Zygnema, Cylindospermum (Fig. 3.16B) etc.

c. Hormogonia:
This method of vegetative reproduction is found in blue-green algae. The trichomes of blue-green algae break up within the sheath into many-celled segments called hormogonia or hormogones. They remain delimited by the formation of heterocysts, separation discs or necridia or by the death and decay of intercalary cells of the trichome. Hormogonia are commonly found in Nostoc, Oscillatoria, Cylindosporium etc.

d. Formation of Adventitious Branches:
Adventitious branches are formed in different large thalloid algae, which, when detached from the plant body, develop into new individuals (e.g., Fucus,

Dictyota). Protonema-like adventitious branches are formed from the internodes of Chara, stolons of Cladophora glomareta etc.

e. Bulbils:

Tuber-like outgrowths are developed due to storage of food at the tip of rhizoids and on the lower nodes of Chara, called bulbils (Fig. 3.16C). After detachment from the plant body, bulbils grow into new plants.

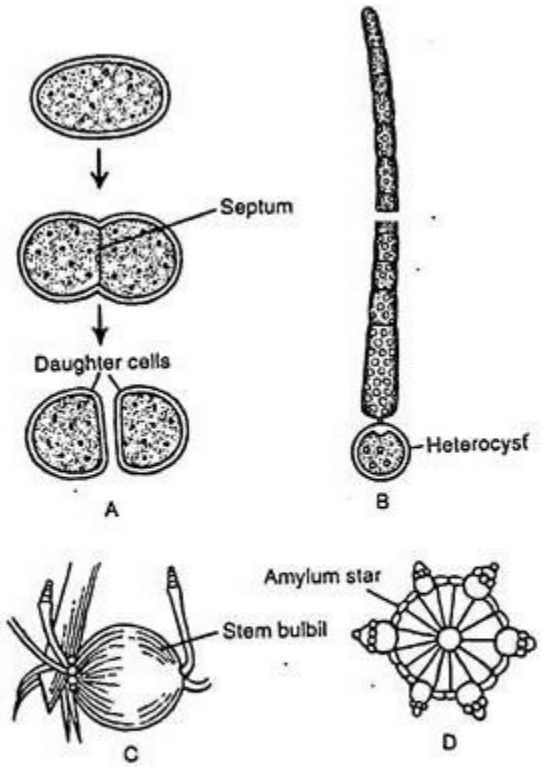


Fig. 3.16 : Vegetative reproduction in algae : A. Cell division (*Synechococcus* sp.) B. Fragmentation of filament (*Cylindrospermum* sp.) C. Stem bulbil (*Chara* sp.) and D. Amylum star (*Chara* sp.)

f. Amylum stars:

Star-shaped aggregation of starch containing cells develops on the lower nodes of Chara. These structures are called amylum stars (Fig. 3.16D). When detached from the plant body, they grow into new plants.

g. Budding:

In Protosiphon bud-like structures are formed due to proliferation of vesicles delimited from the parental body by a septum, which, after detachment, grow into a new plant.

Mode # 2. Asexual Reproduction:

Asexual reproduction involves the formation of certain type of spores — either naked or newly walled. It is a process of rejuvenation of the protoplast without any sexual fusion. Each and every spore germinates into a new plant. In this method, there is no alternation of generations.

The asexual spores may be of various types:

a. Zoospores:

These are motile naked spores provided with two, four or many flagella and called as bi-, quadri- or multiflagellate zoospores, respectively. Biflagellate zoospores are found in Chlamydomonas, Ulothrix (Fig. 3.17A) Ectocarpus etc., quadriflagellate zoospores are found in Ulothrix (Fig. 3.17B) and multiflagellate zoospores are found in Oedogonium (Fig. 3.17C).

But the multinucleate and multiflagellate zoospores as found in Vaucheria (Fig. 3.17D) are called synzoospores. Each zoospore has a chloroplast and an eye spot. The zoospores may be either haploid or diploid.

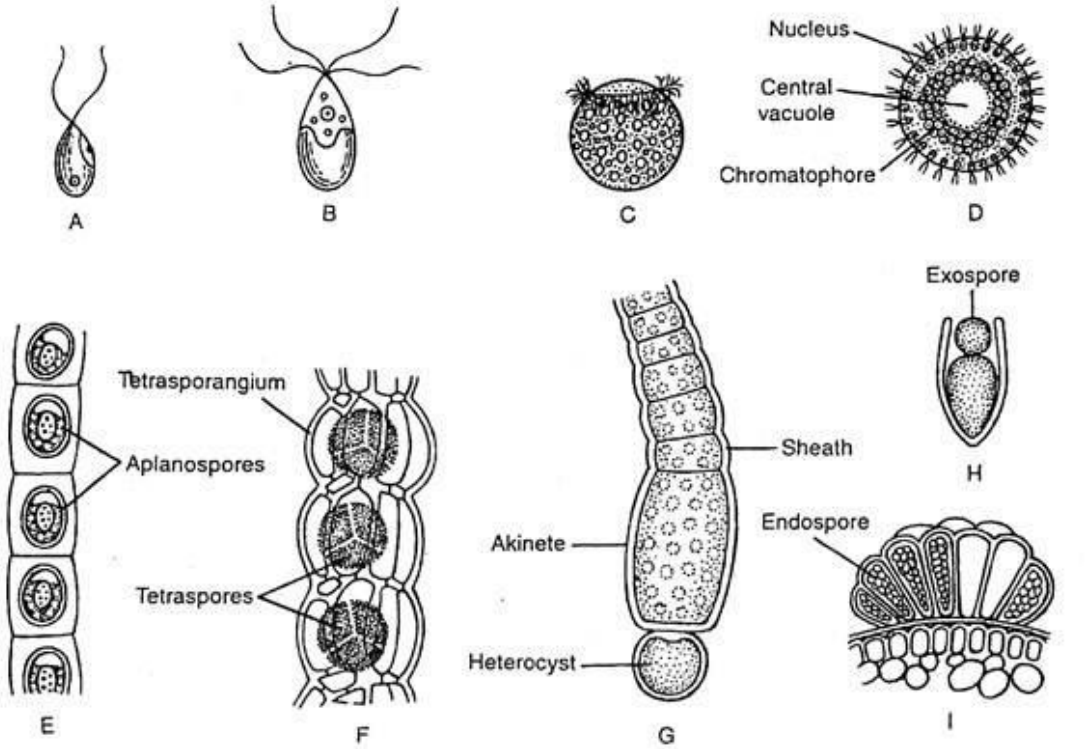


Fig. 3.17 : Asexual spores in algae : A. Biflagellate microzoospore, and B. Quadriflagellate microzoospore of *Ulothrix* sp., C. Multiflagellate zoospore of *Oedogonium* sp., D. Synzoospore of *Vaucheria* sp., E. Aplanospores of *Ulothrix* sp., F. Tetraspores of *Polysiphonia* sp., G. Akinete of *Gloeotrichia* sp., H. Exospore of *Chamaesiphon incrustans*, and I. Endospores of *Dermocarpa prasina*

They are formed within the zoosporangium. There may be single zoospore (e.g., Oedogonium) or many zoospores (e.g., Cladophora) per zoosporangium.

Zoospores are either haploid or diploid depending on the nature of plant body, gametophytic or sporophytic on which it develops.

The zoospores are liberated either by the disintegration of the zoosporangial wall or by the formation of an apical pore on the zoosporangium. After liberation the zoospores swim for a while, then withdraw their flagella, encyst and ultimately germinate into new plants.

b. Aplanospores:
Aplanospores are non- motile spores. These spores are formed either singly or its protoplast may divide to form many aplanospores inside sporangium during unfavourable conditions, especially in drought (e.g., Ulothrix (f ig. 3.17E), Microspora). The aplanospores may also be formed in certain algae of semiaquatic habitat.

When they appear identical to the parent cell, they are referred to as autospores (e.g., Scenedesmus, Chlorella etc.). Aplanospores with thickened wall and abundant food reserve are known as hypnospores (e.g., Pediastram, Sphaerella etc.).

They are formed to overcome prolonged period of desiccation. With the onset of favourable condition the hypnospores either directly germinate into a new individual or their protoplasts may form zoospores. Due to deposition of haematochrome pigment in their walls, the hypnospores of Chlamydomonas nivalis are red in colour.

c. Tetraspores:
Diploid plants of some algae (e.g., Polysiphonia, Fig. 3.17F) produce a special type of haploid aplanospores, called tetraspores, formed within tetrasporangium. The diploid nucleus of a tetrasporangium divides meiotically to form four haploid nuclei which — with little amount of protoplasm — are developed into four tetraspores. After liberation the tetraspores germinate to form male and female gametophytes.

d. Akinetes:
The vegetative cells of certain filamentous algae develop into elongated thick-walled spore-like structures with abundant food reserves, called akinetes (e.g., Gloeotrichia, Fig. 3.17G). They can tide over the unfavourable conditions. With the onset of favourable condition they germinate into new individuals.

e. Exospores:
In some algae, spores are regularly cut off at the exposed distal end of the protoplast in basipetal succession, called exospores. These spores aggregate in groups and develop new colonies, e.g., Chamaesiphon (Fig. 3.17H).

f. Endospores:
These are small spores formed by the divisions of the mother protoplast. They are also called conidia or gonidia. They are set free after the dissolution of mother wail. Without taking rest, the spores germinate directly and develop into a new plant, e.g., Dermocarpa (Fig. 3.17 I).

Mode # 3. Sexual Reproduction:
All algae except the members of the class Cyanophyceae reproduce sexually. During sexual reproduction gametes fuse to form zygote (Fig. 3.18). The new genetic set up can develop by the fusion of gametes coming from the different parents.

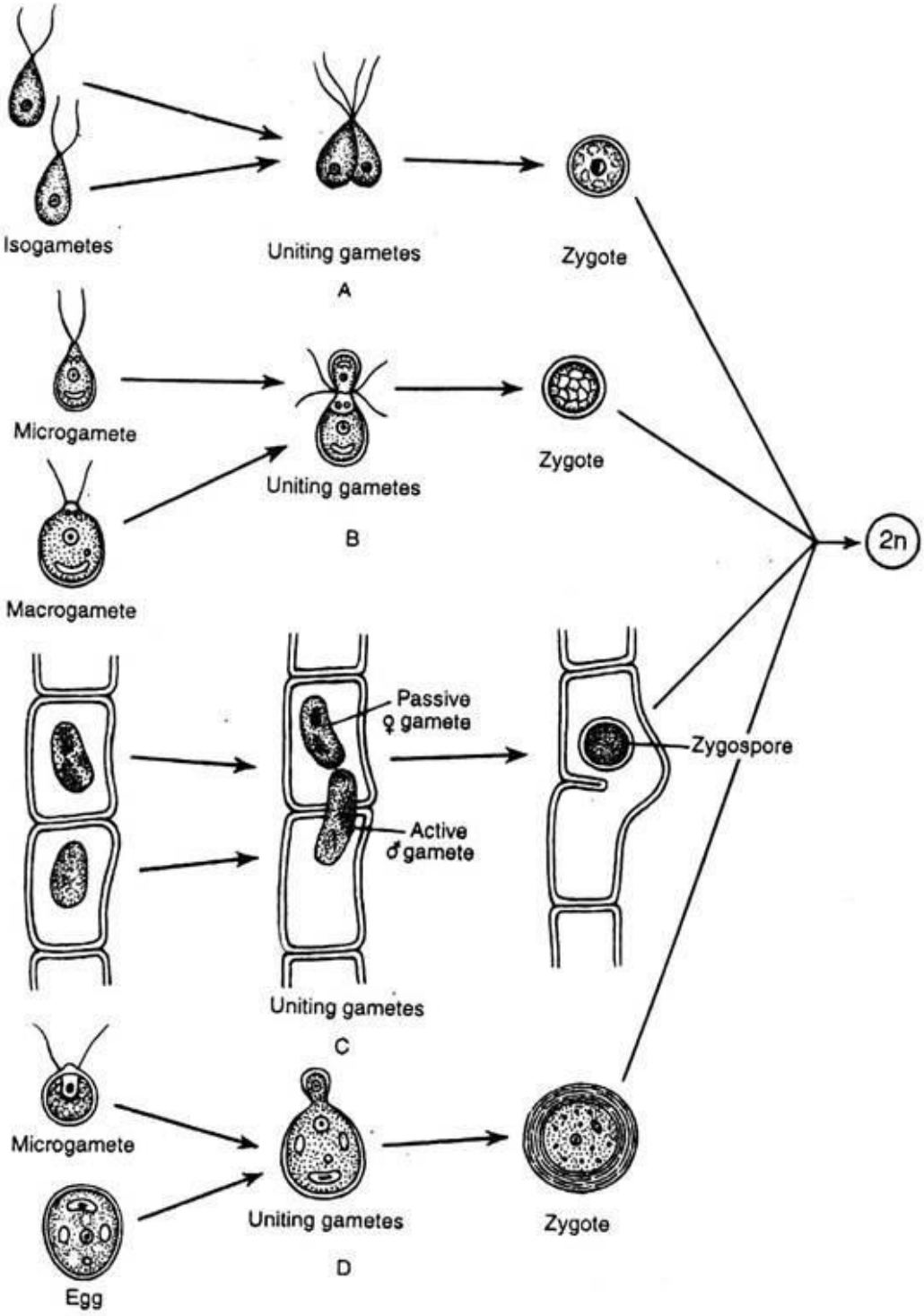


Fig. 3.18 : Types of sexual reproduction in algae : A. Isogamy in *Chlamydomonas* sp., B. Anisogamy in *Ectocarpus* sp. C. Physiological anisogamy in *Spirogyra* sp., and D. Oogamy in *Chlamydomonas* sp.

Depending on the structure, physiological behaviour and complexity of sex organs, sexual reproductions are of the following five types:

a. Autogamy:

In this process the fusing gametes are developed from the same mother cell and after fusion they form zygote. For the above, plant developed through autogamy

does not show the introduction of any new characteristic, e.g., Diatom (Amphora normani).

b. Hologamy:
In some unicellular member the vegetative cells of different strains (+ and -) behave as gametes and after fusion they form zygote. It is an inefficient process considering the point of multiplication, but new genetic combinations are developed by this process, e.g., Chlamydomonas.

c. Isogamy:
It is the process of union, between two gametes which are morphologically and physiologically similar — after fusion they form zygote. The gametes are called isogametes. Usually they are flagellate, e.g., Chlamydomonas eugametos, Ulothrix etc.

d. Anisogamy:
In this process the uniting gametes are morphologically and physiologically different. The smaller and more active one is the microgamete (male), whereas the larger and less active one is the macrogamete (female), e.g., Chlamydomonas braunii.

Deviating from the typical anisogamy, when the uniting gametes show morphological similarity with physiological difference, it is called physiological anisogamy. e.g., Zygnema, Spirogyra etc.

e. Oogamy:
It is an advanced process where fertilisation takes place between a small motile (non-motile in Rhodo- phyceae) male gamete (sperm or antherozoides) with a large non-motile female gamete (egg or ovum). Male gametes develop within antheridium, whereas the female gamete within the oogonium, e.g., Oedogonium, Vaucheria, Chara, Laminaria, Sargassum, Polysiphonia, Batrachospermum etc

Characters of Cyanobacteria:
The cyanobacteria (the earlier blue-green algae), or the blue-green bacteria, represent a group of photosynthetic, mostly photolysis-mediated oxygen-evolving monerans (prokaryotes).

These are the only organisms able to perform oxygenic photosynthesis that can also fix nitrogen. These organisms are amongst the oldest organisms known dating back to the early Precambrian period 3.6 x 10^9years ago and probably played a crucial role in the evolution of higher plants.
Cyanobacterial thallus ranges from unicellular, colonial to filamentous; multi-seriate branched filamentous condition is the highest level of organization attained by them.

Because of their close architectural, physiological and biochemical similarities with bacteria, especially the gram-negative ones, the cyanobacteria have been placed under a separate division, namely, 'The Cyanobacteria' in Bergey's Manual of Determinative Bacteriology (8th edition, 1974), which has been accepted and used widely as the standard reference for bacterial taxonomy.

This manual, however, has recognized the Kingdom-Monera of Whittaker, but has called it Kingdom-Prokaryotae because of the prokaryotic nature of all the monerans.

The cyanobacteria possess various distinguishing characters, which can be summarized as:

(i) Cyanobacteria can grow in diverse habitats, but one striking feature in their occurrence and predominance in habitats alternating between photo-aerobic and photo-anaerobic conditions can be correlated with their preference for low oxygen tension and low redox-potential. These properties stem from their recently discovered dual-capacity of oxygenic photosynthesis and facultative an-oxygenic photosynthesis,

(ii) The cyanobacteria possess various morphologically distinctive structures, e.g., akinetes and heterocysts.

(iii) The main cell wall constituent of cyanobacteria is peptidoglycan.

(iv) The cyanobacterial cytoplasm is traversed extensively by flattened vesicular structures called thylakoids or lamellae, the photosynthetic sites,

(v) The principal photosynthetic pigment of all cyanobacteria is chlorophyll a. Besides, they possess β-carotene and other accessory pigments, namely, phycobiliproteins. The phycobiliproteins are phycocyanin (PC), allophycocyanin (AP), allophycocyanin B (APB), and phycoerythrin, and

(vi) Most filamentous cyanobacteria show a gliding motility at some stage of development; they lack flagella.

Classification of Cyanobacteria:
Rippka (1979) have proposed a modern scheme of cyanobacteria classification taking mainly their physiology, cell constituents and DNA characteristics into consideration. They have created five sub-groups called 'sections'. (Table 6.5)

TABLE 6.5. Rippka *et al.* (1979) system of classification of cyanobacteria

Section	*Cell type*	*Mode of reproduction*	*Range of G + C%*	*Recognized Genera*	*Other properties*
I	Unicells or aggregates	Binary fission or budding	35-71	*Synechococcus, Synechocystis, Gloeobacter, Gloeothece, Gloeocapsa, Chamesiphon*	Almost always nonmotile
II	Unicells or aggregates	Multiple fission to form baeocytes	38-47	*Dermocarpa, Xenococcus, Myxosarchina, Chroococcidiopsis*	Usually some baeocytes are motile
III	Filaments; unbranched trichomes with only vegetative cells	Binary fission in a single plane	40-67	*Spirulina, Lyngbya, Oscillatoria, Pseudoanabaena, Phormidium, Plectonema*	Usually motile
IV	Filaments can form heterocysts; no true branching	Hormogonia formed; binary fission in a single plane	38-47	*Anabaena, Nodularia, Nostoc, Cylindrospermum Scytonema, Calothrix, Tolypothrix, Rivularia*	Often motile; may form akinetes
V	Filaments can form heterocysts and true branches	Hormogonia, Akinetes, Hormocysts; binary fission in more than one plane	42-46	*Chlogloeopsis, Fischerella, Mastigocladus*	Greatest morphological complexity and differentiation in cyanobacteria

Reproduction in Cyanobacteria:
Like bacteria, the cyanobacteria also reproduce asexually and the commonest mode of reproduction in them is transverse binary fission. In addition, there are certain specialized structures such as akinetes, hormogonia, hormocysts and spores, which are partly involved in the process of reproduction.

So far as the sexual reproduction in its true sense is concerned, it is absent in them and the requirements of sexuality are considered to be met by some alternative pathways referred to as parasexual-pathways.

1. Akinetes:

Most filamentous cyanobacteria develop perennating structures (dormant structures) in adverse condition. These structures are larger than the vegetative cells, are equipped with thick walls, and are called akinetes (Fig. 6.12). When favourable conditions return, they germinate and produce new filaments.

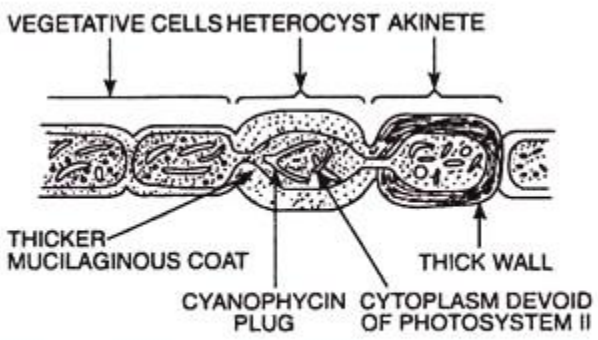


FIG. 6.12. Trichome of *Anabaena* possessing heterocyst and akinete.

2. Hormogonia:

All filamentous cyanobacteria reproduce by fragmentation of their filaments (trichomes) at more or less regular intervals to form short pieces each consisting of 5-15 cells. These short pieces of filaments are called hormogonia. The latter show gliding motility and develop into new full- fledged filaments.

3. Hormocysts:

Some cyanobacteria produce hormocysts, which are multicellular structures having a thick and massive sheath. They may be intercalary or terminal in position and may germinate from either end or both the ends to give rise to the new filaments.

4. Spores:

Non-filamentous cyanobacteria generally produce spores such as endospores, exospores and nanocysts which contribute by germinating and giving rise to new vegetative cells when the unfavourable condition is over. Endospores are produced endogenously like those in bacteria; exospores are the result to exogenous budding of cells, and the nanocysts are produced endogenously like endospores.

The difference between an endospore and a nanocyst is that in endospore formation the parent cell concomittantly enlarges in size, whereas in nanocyst formation there is no such enlargement of the cell.

Parasexuality in Cyanobacteria:

The knowledge of cyanobacterial genetics is relatively new and was pioneered by Kumar in 1962 who obtained penicillin and streptomycin resistant strains of Anacystis nidulans, crossed them, and successfully demonstrated the appearance of a third type of recombinant strain resistant to both the antibiotics.

However, the mechanisms of genetic recombination in cyanobacteria are thought to be the same as those in bacteria.

The existence of the process of transformation in cyanobacteria was established experimentally in 1979 by Doolittle. Stevens and Porter in 1980 has successfully demonstrated this process in Agmenellum quadruplicatum. The transforming principle was shown to be DNA. Some cyanobacteriologists, however, have found that the transformation is mediated in some cases by complexes of DNA and RNA.

The first report on conjugation in a cyanobacterium, namely, Anacysts nidulans was by Kumar and Ueda in 1984. The frequency of conjugation is very low (about 1 in 10^6 cells) and cells conjugate by means of a conjungation tube. The knowledge of transduction in these microorganisms at present is restricted to some preliminary reports. However, the occurrence of different cyanophages, e.g., LIP 1-7, SM-1, N-1 and infection of several cyanobacteria by them prompts one to imagine that the virus-mediated method of genetic transfer (transduction) in cyanobacteria may be conclusively established in the near future.

Heterocyst in Cyanobacteria:

As stated earlier, the cyanobacteria are the only organisms able to perform oxygenic photosynthesis that can also fix nitrogen; many, but not all, are vigorous nitrogen fixers.

The coexistence of the processes of oxygenic photosynthesis and intrinsically anaerobic nitrogen fixation process in a single organism presents an obvious paradox because nitrogenase, the key enzymes, is rapidly and irreversibly inactivated by an exposure even to low partial pressure of oxygen.

However, the nitrogen fixing cyanobacteria produce a specialized type of cell, the heterocyst, within which nitrogen is fixed.

Filamentous forms of cyanobacteria such as Anabaena form large distinctive cyst-like cells, the heterocysts, at intervals along the trichome (filament), (Fig. 6.12). The latter develop from normal vegetative cells particularly in conditions deficient in NH^{+}_{3} or NO^{-}_{3}, and are considered to be the site of nitrogen fixation. The conversion of atmospheric nitrogen to ammonia takes place under highly anaerobic conditions, that only the heterocysts are able to provide. For instance, the oxygen-evolving part of the photosynthetic mechanism (photosystem II) is blocked in heterocysts, and the remaining photosynthetic machinery becomes geared to provide energy for the reduction of nitrogen to NH_3^+.

Economic Importance of Cyanobacteria:

1. Cyanobacteria are one of the early colonizers of bare and barren areas and generate such conditions that favour the growth of other organisms even in the most hostile environment.

2. They are good food source for several aquatic animals. Moreover, the cyanobacteria are now-the-days exploited as food for animals including humans. Spirulina, a filamentous cyanobacterium, is now incorporated in food supplement as well as animal feed through ‘single cell protein’ manufacture because of its high protein content (upto 70%).

Some Indian dishes, for instance, like ‘puri’ ‘idli’ and ‘sandwich’ prepared by supplementing 5-10% S. fusiformis have been found to be palatable. In parts of Rajasthan Anabaena and Spirulina are collected from Sambar lake and used as fodder and manure.

3. N_2-fixation is the characteristic feature of many cyanobacteria and this function is performed by heterocysts present in them. Aulosira, Nostoc, Anabaena, etc. are some such cyanobacteria that are now regularly inoculated in the rice fields for nitrogen supply. This saves consumption of nitrogen fertilizers.

4. N_2-fixing cyanobacteria (e.g., Nostoc, Anabaena) are often used for reclamation of ‘user’ soils. They produce acidic chemicals for counteracting alkalinity of the soil and they supply nitrogen compounds which are generally deficient in these soils.

5. Species of Anabaena and Aulosira do not allow mosquito larvae to grow nearby. Such cyanobacteria can be inoculated in village ponds to prevent the growth of mosquitoes.

6. Extracts of Lyngbia are used to manufacture antibiotic-like compounds.

7. Certain cyanobacteria such as Microcystis aeruginosa (= Anacystis cyanea), Anabaena flos-aquae and Aphanizomenon flos-aquae produce toxins harmful to most aquatic animals. These toxins may prove equally harmful to humans drinking or bathing in such water.

8. Cyanobacteria generally grow on walls and roofs of buildings during the rainy seasons and cause discolouration, corrosion, and leakage.

Actinomycetes: Economic Importance and Reproduction

Characteristics of Actinomycetes:

The Actinomycetes or Streptomycetes or Actinomycetales as they are called are a group or Gram-positive bacteria which form branched filamentous hyphae having resemblance with fungal hyphae. But their hyphal diameter is approximately 1µm, whereas in fungi it is 5 to 10 µm.

These organisms reproduce by asexual spores which are termed conidia when they are naked or sporangiospores when enclosed in a sporangium. Although these spores are not heat-resistant, they are resistant to desiccation and aid survival of the species during periods of drought.

These filamentous bacteria are mainly harmless soil organisms, although a few are pathogenic for humans (Streptomyces somaliensis causes actinomycetoma of human), other animals (Actinomyces bovis causes lumpy-jaw disease of cattle), or plants (Streptomyces scabies causes common scab in potatoes and sugar beets).

In soil they are saprophytic and chemoorganotrophic, and they have the important function of degrading plant or animal resides.

Again some are best known for their ability to produce a wide range of antibiotics useful in treating human diseases. These organisms excrete extracellular enzymes which are decomposers of dead organic material. These enzymes lyse bacteria and thereby keep the bacterial population in check and thus help to maintain the microbial equilibrium of the soil.

The Actinomycetes superficially resemble fungi for having subterranean and aerial hyphae and chains of spores. But their hyphal diameter, cytology and chemical composition of cell walls are quite decidedly bacterial in pattern

2. Historical Review of Actinomycetes*:*

The early exploratory studies by McCormack (1935) and Alexopoulos and Herrick (1938-1942) were followed by the intensive studies by Professor S. A. Waksman and his students (1943-1951) which culminated in the discovery of streptomycin and other new and potentially useful chemotherapeutic agents.

Nearly 100 antibiotic substances have been reported in the literature as metabolites of the Actinomycetes. A few of these have been isolated in pure form and their chemistry studied in detail, while others have been described only as concentrates or in a preliminary way.

3. Economic Importance of Actinomycetes:

The Actinomycetes, forming soil micro-flora have gained the greatest importance in recent years as producers of therapeutic substances.

Many of the Actinomycetes have the ability to synthesize metabolites which hinder the growth of bacteria; these are called antibiotics, and, although harmful to bacteria are more or less harmless when introduced into the human or animal body. Antibiotics have in modern times great therapeutical and industrial value.

The past decade has seen considerable interest in the Actinomycetes as producers of antibiotic substances. The successful use in chemotherapy of streptomycin, chloromphenicol (Chloromycetin is the trade name of this substance), aureomycin and terramycin all metabolites of the Actinomycetes, has stimulated the search for new Actinomycetes and new antibiotics among the Actinomycetes.

The genus Streptomyces is the largest and the most important one, antibiotically speaking.

4. Distribution and Mode of Nutrition of Actinomycetes:

The Actinomycetes are essentially mesophilic and aerobic in their requirements for growth and thus resemble both bacteiia and fungi. They along with other microorganisms, form the soil microflora and produce powerful enzymes by means of which they are able to decompose organic matter.

The majority of these are soil organisms and are associated with rotting material. The characteristic odour of soil after it is ploughed or wetted by rain is largely due to the presence of the Actinomycetes.

Some are pathogens. The Actinomycetes grow slowly and on artificial media produce hard and chalky colonies which smell decaying leaves 01 musty earth. They are particularly abundant in forest soil because of the abundance of organic matter. They occur mainly in soils of neutral pH, although some prefer acidic or alkaline soil. The Actinomycetes can grow in soils having less water content than that needed for most others bacteria.

The Actinomycetes are capable of utilizing a large number of carbohydrates as energy sources when the carbohydrates are present in the media as sole sources of meta- bolizable carbon.

Most of the Actinomycetes are quire proteolytic and attack proteins and poly-peptides, and are also able to utilize nitrates and ammonia as sources of nitrogen. Nearly all synthesize vitamin B_{12} when grown on media containing cobalt salts, and many are able to synthesize rather complex organic molecules which have antibiotic properties. The mechanism of synthesis of these substances is not understood.

5. Somatic Structures of Actinomycetes:

Most of the Actinomycetes are mycelioid. They begin their development as unicellular organisms but grow into branched filaments or hyphae which grow profusely by producing further branches constituting the mycelium. The width of the hyphae is usually 1 µm. The delicate mycelia often grow in all directions from a central point and produce an appearance that has been compared with the rays of sun or of a star.

Therefore, the Actinomycetes are also called **'ray fungi'.**They often produce complicated designs and resemble some of the drawings in modern art exhibitions. They are Gram-positive. The protoplasm of the young hyphae appears to be undifferentiated, but the older parts of the mycelium show definite granules, vacuoles and nuclei.

Many Actinomycetes at first produce a very delicate, widely branched, mycelium that may embed itself into the soil, or, if grown in culture, into the solid medium. This kind of mycelium is therefore called the **'substratum or primary mycelium'**(Fig. 338).

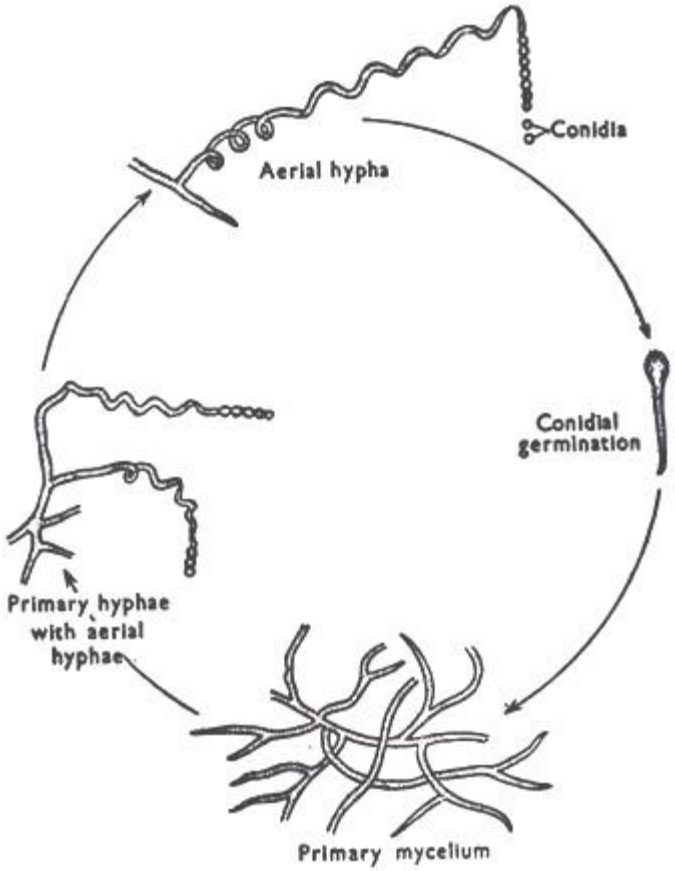


Fig. 338. Life cycle of an actinomycete.

After a period of growth, hyphae of a different kind develop, which raise themselves up from the substratum mycelium and grow into the air. These ate called aerial hyphae, and the corresponding mycelium is the aerial or secondary mycelium. The aerial mycelium may be white yellow, violet, red, blue, green, or grey and many form pigments that are excreted into the medium.

The aerial mycellium is usually slightly wider than the substratum mycelium. The aerial hyphae possess an extra ceil wall layer (sheath). The hyphal tip undergoes septation within this sheath to form a chain of conidia. Conidial cell contains a plump, deeply staining, oval or rod-shaped nuclear body.

6. ***Reproduction in Actinomycetes****:*

Most species reproduce by conidia which are developed in chains from the aerial hyphae. The chains may be straight, flexuous (wavy) or coiled to various degrees. The conidia bearing filaments are often spirally twisted. Sometimes the whole length of the aerial hypha, sometimes only its upper part is transformed into conidia.

Each conidium has a roundish nucleus and is surrounded by a firm outer wall. The conidial wall may be smooth, warty, spiny, or hairy.

The conidia can persist in the dry state for many years. Even the vegetative forms of the Actinomycetes are quite hardy and are able to adapt themselves to the changing soil conditions.

The conidia appear as a fine powdery coat on the surface of cultures. When the conidia have been scattered on the ground and conditions are favourable they germinate producing one to three or even occasionally four little germ tubes which give rise to mycelioid condition (Fig. 338).

The primary mycelium in some species commonly breaks up into small fragments called arthrospores, which often look like bacterial cells and which might easily be mistaken for the latter.