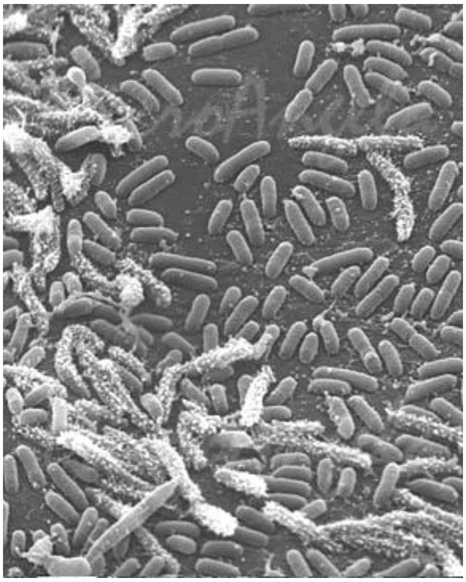
South Valley University
Faculty of Science
Department of Botany and Microbiology

BACTERIOLOGY

For

B Sc. Students

Faculty of Science



By

Prof. Dr. Waiel F. Sayed

كلية العلوم
قسم النبات

مقرر البكتريولوجى

كود المقرر: 307 ن

- **أهداف المقرر القدرة على التعامل مع أنواع البكتريا سواء النافعة أو الممرضة وحل المشكلات المرتبطة بها فى مجالات الصناعة والبيئة والبحث العلمى**
- **المستهدف من تدريس المقرر**

أ- المعلومات والمفاهيم

1- يصف الطالب تركيب الخلية البكتيرية وطرق الصباغة و يحدد مراحل النمو المختلفة للبكتريا (منحنى النمو) والظروف التى تؤثر فيه

2- يذكر الطالب أنواع مثبطات النمو المختلفة والمضادات الحيوية ويصف التخمر والتنفس وسلسلة انتقال الالكترونات

3- يحدد دور البكتريا فى صناعات الألبان والصناعة والبيئة وتفادى أضرارها وأهم أنواع البكتريا الممرضة ويسمى أسس تقسيم البكتريا بناء على الصفات المظهرية والوراثية والخطوات الأساسية لتعريف البكتريا

ب- المهارات الذهنية

1- يميز الطالب بين الكائنات الأولية والحقيقية

2- يقارن الطالب بين أنواع البكتريا على أساس صباغة جرام والصبغات الأخرى والتى تحدد الفرق فى تركيب الخلية

3- يستنتج العناصر الرئيسية للتحكم فى الأيض الخلوى عن طريق مصادر الكربون والطاقة والتفاعلات الحيوية

4- يربط بين الأنواع الشائعة من البكتريا الممرضة والنافعة ودورها فى البيئة والصناعات المختلفة وكيفية مقاومة الأمراض والوقاية منها

جـ- المهارات المهنية الخاصة بالمقرر

1- يستخدم الميكروسكوب الضوئى ووسائل وأدوات التعقيم المختلفة

2- يتناول الأشكال الرئيسية وتركيب البكتريا تحت الميكروسكوب وأشكال المستعمرات فى الأنابيب والأطباق على الأوساط السائلة والصلبة ويستخدم الأوساط والمحاليل المنظمة للنمو

3- يستخدم طرق تقدير نمو البكتريا وعد المستعمرات

4- يتناول تأثير بعض مضادات النمو على البكتريا مثل الصبغات والمركبات الفينولية والمضادات الحيوية بأنواعها و نواتج الأيض المختلفة لأنواع التخمر والتنفس

5- يستخدم طرق عزل البكتريا من الأوساط المختلفة ويجرى خطوات التعريف والتقسيم بالفحص والصباغة والتجارب الأخرى

د- المهارات العامة

1- المناقشة واستحضار المعلومات الأساسية خلال المحاضرات والدروس العملية

2- الاستخدام الأمثل للأدوات والأجهزة الأساسية للتجارب العملية

SUBJECT INDEX

Part (6)- Continued...

INTRODUCTION

The term “microorganism” refers to any organism of microscopic dimensions. According to one of several classifications for living organisms there are three kingdoms namely; plants, animals and microorganisms.

The name "bacteria" is derived from the Greek word “bakterion” that means a small stick. The term “microbiology” means the science of small living things and is derived from three words (Mikros= small, Bios= life and Logos= science).

The **Bacteria** are a group of single- cell microorganisms with **procaryotic** cellular characteristics. The genetic material (DNA) of procaryotic cells exists unbound in the cytoplasm of the cells. There is **no nuclear membrane**, which is the definitive characteristic of eucaryotic cells such as those that make up plants and animals. Until recently, bacteria were the only known type of procaryotic cell, and the discipline of biology related to their study is called **bacteriology**. In the 1980's, with the outbreak of molecular techniques applied to phylogeny of life, another group of procaryotes was defined and informally named "archaebacteria". This group of procaryotes has been renamed **Archaea** and has been allocated in a biological **Domain** on the level with **Bacteria** and **Eucarya (see Figure 1).**

The main differences between eukaryotic and prokaryotic cells are illustrated in Table (1).

Table (1): The main differences between eukaryotic and prokaryotic cells

Eukaryotic cell	**Prokaryotic cell**
The chromosome are enclosed in a double-layered membrane (nucleus)	The chromosome is in the cytoplasm (no membranes)
Chromosome structure is relatively complex	More simple structure
Cell division involve meiosis and mitosis	Cell division does not involve meiosis or mitosis
Two types of ribosomes are present; a larger type in the cytoplasm and a smaller type in the chloroplasts and mitochondria	Only one small type in the cytoplasm
Presence of cell organelles for specific functions such as photosynthesis (chloroplasts) and respiration (mitochondria)	No such organelles

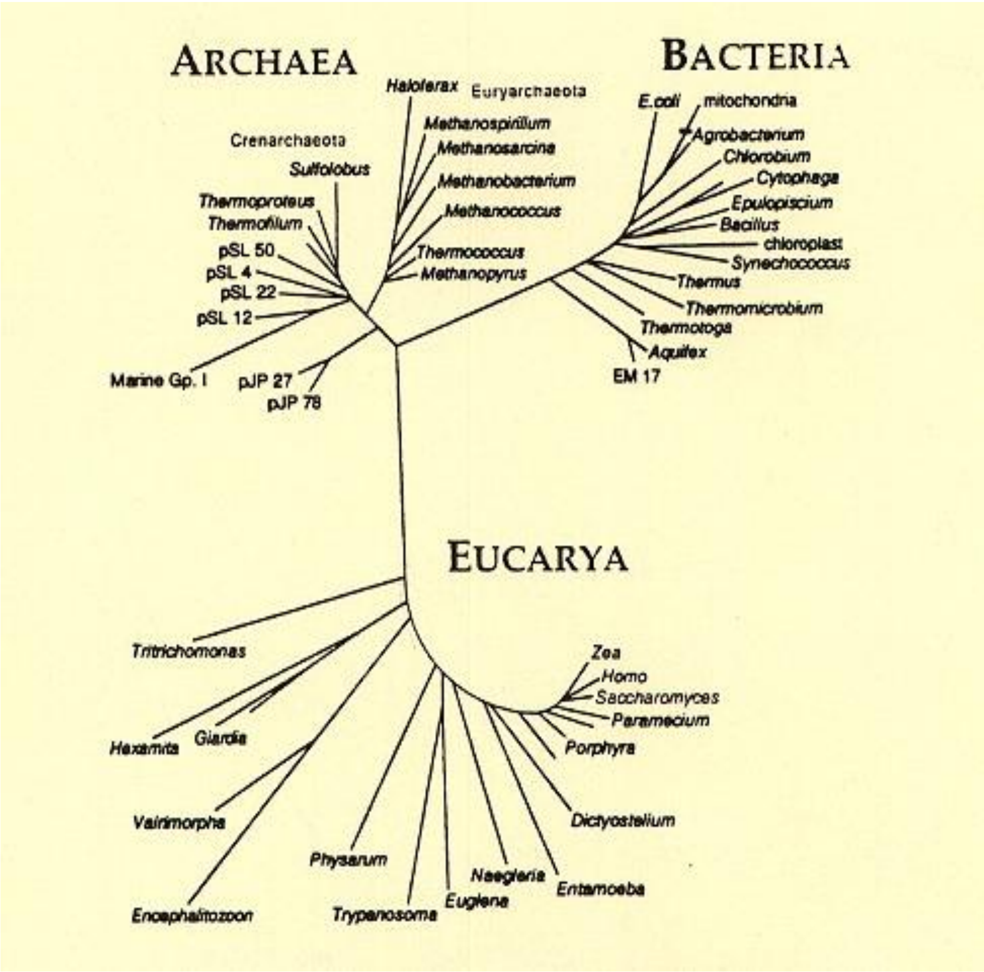


Figure (1): The three kingdoms

Different bacterial groups dominate almost every medium and found everywhere in air, soil and different water sources, on surfaces and inside other living organisms including human beings.

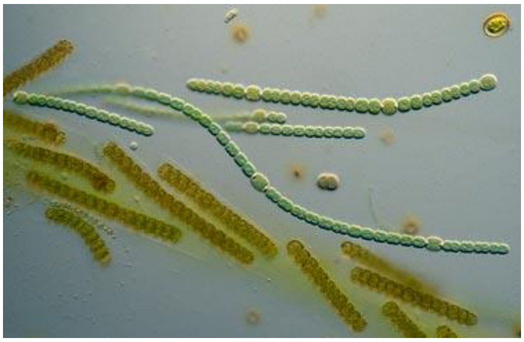


Figure (2): Cyanobacteria found in aquatitc enviroments

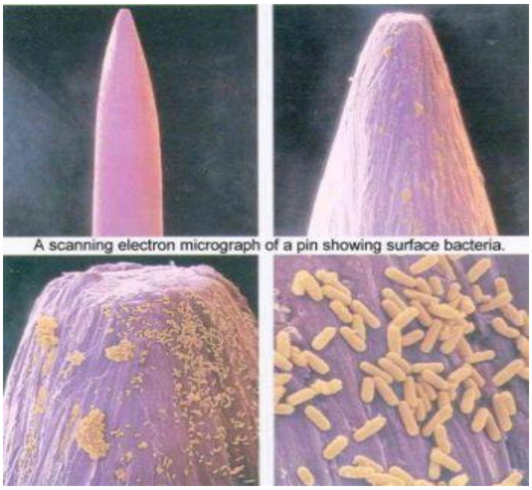


Figure (3): Bacteria found on a pin tip

Figure (4): Ancient fossil bacteria

BACTERIAL MORPHOLOGY & STRUCTURE

(1) MORPHOLOGY

The Bacteria is this group of prokaryotic microorganisms that can not be seen by the naked eye and its reproduction is by binary fission or budding. Morphological characteristics can be studied by different microscopy methods (e. g.: brightfield, phase- contrast, fluorescence and electron microscopy techniques). It is possible also to study the structural components of bacterial cells but it would be more difficult than studying morphological characters because of the minute size and weight of the bacterial cells. Bacterial morphology is divided into two categories 1) forms and groupings of cells: bacilli, cocci, spirilli, filamentous and involution forms (that are irregular forms develop in extreme conditions such as depletion of nutrients, etc) and 2) shapes of colonies.

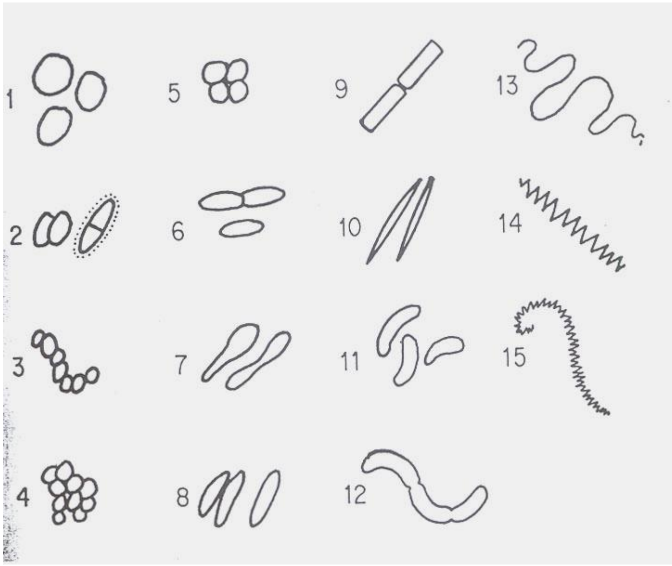


Figure (5): Fundamental shapes of bacteria: 1- single cocci, 2- diplococci, 3- streptococci, 4- staphylococci, 5- tetrads, 6- coccobacilli, 7- club- shped bacilli, 8- bacilli with rounded ends, 9- bacilli with flat ends, 10- fusiform bacilli, 11- vibrios, 12- *Spirillum*, 13- *Brrelia*, 14- *Treponema*, 15 *Leptospira*.

Groupings of bacterial cells

A) Groupings of cocci (according to the planes of binary fission):

- At one plane: diplococci or streptococci
- At two planes: staphylococci
- At two perpendicular planes: tetrads
- At three planes: packets of eights (sarcina)

B) Groupings of straight rods:

- Flat- ended rods: e.g. *Bacillus anthracis*
- Round- ended rods: e.g. *Escherichia coli*
- Clubbed (singly or in palisades) at various angles to one another: e.g. *Corynebacterium diphtheria*
- Fusiform (tapering): e.g. *Fusobacterium fusiform*

C) Groupings of curved rods:

- Curved rods are either flexible (*Spirochetes*) or rigid spirals (*Spirillum*)
- The spiral of one winding is called *Vibrio* (such as *Vibrio comma* and the genus *Desulfovibrio*).
- In *Vibrio* forms the flagella is on one end.
- In spiral forms the flagella is on two ends (e.g. *Spirillum*)
- The spiral may have its appendages within the body (not as flagella).
- The spirals may group to form S shape or serpentine form.

D) In Actinomycetes (e.g. *Streptomyces*) there are truly branched forms of bacteria.

The surface of the bacterial cell in relation to its activity

The ratio of surface area to the weight is important in determining the cell activity.

- Absorption takes place from the surface that is needed to be of large value as compared to its weight (the smaller the size of the cell, the greater is the ratio of surface/ weight).
- Although of this, there is a lower limit below which activities decrease or even stop. This limit is set by the space required to contain the enzymes for growth, respiration and reproduction (several thousands of enzymes per cell).

BACTERIAL STAINING

The visible light wavelength ranges from 4000 to 7000 Å. A particular wavelength of the visible light corresponds to a particular colour. These colours are called "corresponding colours" including violet, indigo, blue, blue green, green, yellow green, yellow, orange, red and deep red.

When a body absorbs a particular wavelength, the remaining part of the spectrum (the so-called complementary colour) is transmitted and stimulates the optical nerve to interpret the colour of the object. However, while the corresponding colour is a pure colour, complementary colour is not. The complementary colours for the corresponding colours of the spectrum are represented in Table (2).

Wavelength (Å)	Corresponding colours	Complementary colours
4000	Violet	Greenish yellow
4300	Indigo	Yellow
4600	Blue	Orange
4900	Blue green	Red
5100	Green	Deep red
5300	Yellow green	Violet
5600	Yellow	Indigo
6000	Orange	Blue
6400	Red	Blue green
7100	Deep red	Green

Table (2): Complementary and corresponding colours of the light spectrum

There are three light- sensitive regions in the eye 1) for 4400 Å (Blue), 2) for 5500 Å (yellow) and 3) for 5900 Å or orange light. At each region, the response to its colour is strong while it is weak for other colours. Colour sensation is the sum of weak and strong responses that vary in individuals. There is also a relation between the absorption of certain wavelengths and the number of unsaturated centers of different molecules. Thus multiple unsaturation sites may give various colours and interfere with the original colours.

Definition of a stain

The stain or dye is defined as "any coloured compound that reacts with, absorbed by or dissolved in another phase and renders that phase coloured".

A simple dye or salt- forming dye has an "auxochrome group" that ionizes and forms salts (i. e. bearing charge) and a "chromophore group" that bears the colour.

Acidic and basic stains

Auxochrome in a dye may ionize as a weak acid (negative ion) or as a base (positive ion). When the weak acid dye reacts with a simple cation it forms an acidic stain. While, for the basic dye reacting with a simple anion, the result is a basic stain. Thus the term "acidic" or "basic" refers only to the charge of the auxochrome group in the dye.

The staining process

First of all, a bacterial film or smear must be prepared before staining takes place. A drop or loopful of the appropriate culture is spread on a clean glass slide, left to dry and fixed by passing the slide into the flame (avoiding excess heating of the slide).

The staining process is a result of the interaction between stain and the proteins and nucleic acids in the cell. Amino acids of the protein are "amphoteric" compounds (ionize as acids or bases according to the hydrogen ion concentration of the medium).

- In the basic medium they ionize as acids:

$CH_2\text{-}NH_2.\ COOH \longleftrightarrow CH_2\text{-}NH_2.\ COO^- + H^+$

- In acidic medium they ionize as bases:

$CH_2\text{-}NH_2.COOH + H_2O \longleftrightarrow CH_2\text{-}NH_3^+.\ COOH + OH$

Amino acids may give equal amounts of acidic and basic ions at the "isoelectric point".

Certain factors can intensify staining such as acids, bases, aniline, phenol and temperature. These factors are called intensifiers or accentuators.

Mordants are chemical substances that have the power of making dye able to stain unstable materials or increase their affinity to certain dyes. Mordants have strong affinity for both the substrate and the dye, thus anchoring the dye to the substance. Iodine is usually used as a mordant in the Gram staining method.

<u>Mechanism of staining</u>

There are two interpretations for the mechanisms involved in this process 1) physical with no new compound are formed as a result of staining but only absorption of the stain on the cell surface and 2) chemical in which the stain reacts with cell constituents and new compounds result from this reaction. However, there is uncertainty about the exact mechanism(s) involved in the process whether it is physical, chemical or both.

- Staining with a basic stain in a basic medium will take place as follows:

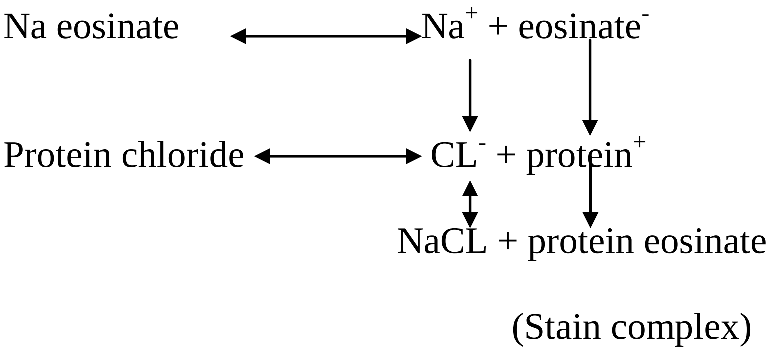
Methylene blue chloride (MBCL) ⟷ MB^{+} + CL^{-}

Na proteinate (bacterial cell) ⟷ $Proteinate^{-}$ + Na^{+}

MB proteinate + **NaCL**

(Stain complex)

- Staining with a acidic stain in acidic medium will take place as follows:



- The presence of the cell wall is essential for the staining process since the reaction is only given in the presence of the intact cell. In evidence of that, the broken cells of a Gram- positive bacterium give a Gram- negative reaction. Therefore, the role of the cell wall is to act as a barrier to the extraction of the dye complex from the cell by any organic solvent (decolourizing agent). Gram reaction is correlated to the major differences in the chemical composition and ultrastructure of the wall. The importance of Gram reaction in diagnosis is useful in the “Eubacteria” that have cell walls but not in eukaryotes (i. e. its significance applies only to prokaryotes).

Types of bacterial staining

(1) Simple staining: one stain is used to impart colour to the cells. Methods include the use of an acidic or a basic dye. In case of using a basic dye, this is called positive or direct staining in which the cell is stained. On the other hand, when applying an acidic stain, this is a negative or indirect staining giving colour to the background of the field. Negative staining is advantageous on the bases of that the cell constituents are not distorted (no film heating is involved) on the contrary of direct staining.

(2) Differential staining: Two different stains are involved such as in Gram's staining (Table 3).

Step	Function
Primary stain (a basic stain)	Staining the cell
Mordant	Anchoring the colour to the cell
Decolourizing agent (alcohol)	Extracting colour
Counter stain (a basic stain with different colou	Staining cells of differer characteristics

Table (3): Steps of Gram and other differential staining methods

As a result for Gram staining procedure, the Bacteria was divided into two groups, namely Gram positive and Gram negative (Gm +ve and Gm –ve) according to differences in cell walls and, consequently, to Gram's reaction.

(3) Structural staining: involving specific methods for staining various cell structures such as bacterial nucleus and endospores.

(4) Acid-fast staining: Some bacteria, such as mycobacteria (e. g.: *Mycobacterium tuberculosis* or T. B.), diphtheria- like microbes and generally actinomycetes, are stained with carbol fuchsin at temperature near boiling. The stain can not be removed even by washing with acidic alcohol. These bacteria are called acid- fast bacteria and the addition of counter stain will not impart colour to cells. If the stain is removed by acidic alcohol, the counter stain will impart colour to cells which called in this case non acid- fast. Acid fastness of the T. B. bacteria is thought to be due to the increase of fat and wax contents or the presence of mycolic acid in the waxy material of the microbe.

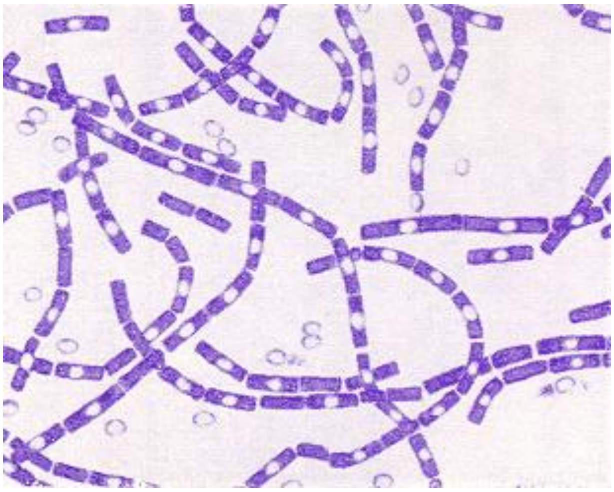


Figure (6): Gram stain of *Bacillus anthracis, the* cause of anthrax disease

End of Lecture (1)

Lecture (2)

THE BACTERIAL CELL

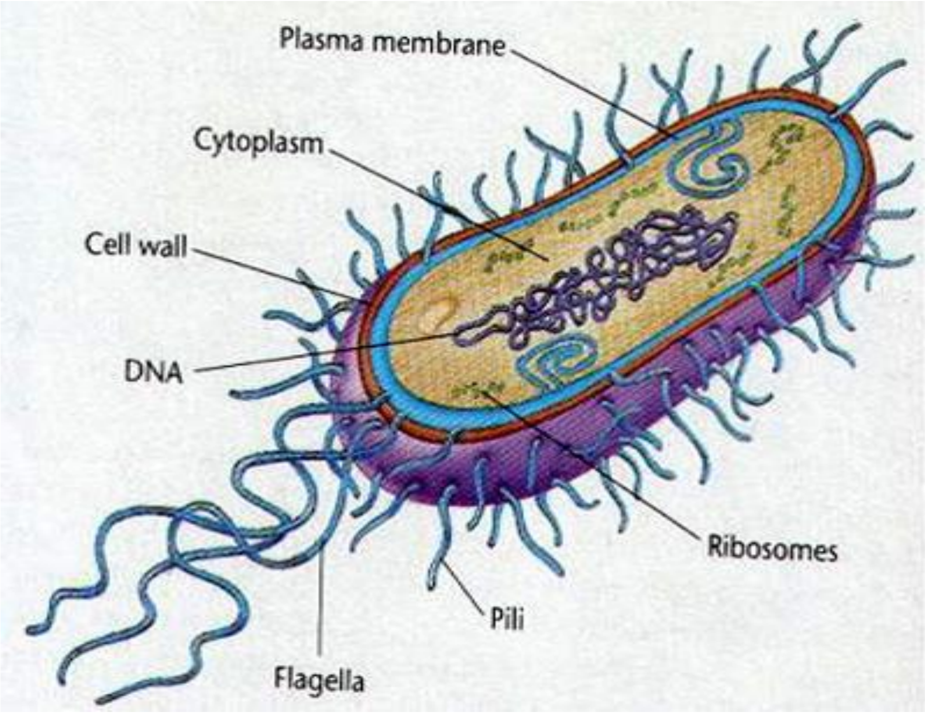


Figure (7): Diagrammatic illustration of a typical bacterial cell

The bacterial cell structure can be grouped into two categories:

(1) Surface structure: including the capsule and/ or slime layer, cell wall, flagella and pili, cell membrane (cytoplasmic membrane). The cytoplasmic membrane can be grouped into the internal structure because it may intrude in the cytoplasm giving membranous forms.

(2) The internal structure: may include the bacterial nucleus, the cytoplasm and cytoplasmic inclusions.

Table (4): Summary of characteristics for typical bacterial cell structure.

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

Slime layer and capsule

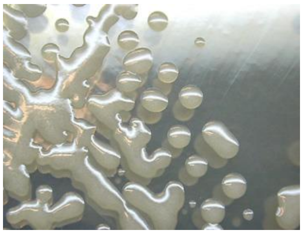


Figure (8): Colonies of *Bacillus anthracis*: The slimy or mucoid appearance of colonies is usually evidence of capsule production. In *B. anthracis,* the capsule is composed of poly-D-glutamate. The capsule is an evidence of virulence. Capsule protects the bacterium from phagocytosis and attack by the host immune system leading to successful infection.

In certain bacterial species, accumulation of a mucilaginous material occurs outside the cell. This layer differs in thickness according to the strain and the nutrient medium. When this layer is organized distinctively around the organism it is called “capsule”. Its substrate is transparent and its refractive index is not much different from the medium. The capsule is not easily stained and can not be seen under the dark field illumination and in the stained preparations it appears as unstained halos around the stained cell. True shapes and sizes of capsules are better observed in wet preparations in Indian ink or nigrosine (negative staining). From another point of view, some bacteriologists consider the capsule as a modified part of the cell wall formed by swelling of some components and their consequent gelatinization.

An organism may contain both slime layer and capsule. The slime layer, in this case, is just an accumulation of slime in which cell is embedded and the capsule has no distinct boundaries. For example, some strains of *Streptococcus salivarius*, in the presence of sucrose, show slime layers of levans (polymer of fructose) that is antigenetically distinct from capsule polysaccharides. Iron and sulphur bacteria have sheaths similar to the capsule.

Capsular material and the environment

1. Mucoid bacterial colonies in the rhizospheric region of desert plants increase the ability of these plants to resist drought and keep the moisture content of the root surface.
2. Cements water bacteria in films and facilitates its adhesion to solid surfaces.

3. The encapsulated bacteria of the oral microflora, in the presence of polysaccharides, stimulate dental caries processes.

4. Hinders the attack by bacteriophages specific for O- antigens and lipopolysaccharide regions of the cell wall.

5. The capsule may enclose a huge number of cells forming colonies called “zooglea”. Zooglea will form in concentrated sugar solutions in the sugar refinery plants. These zooglea cause problems in these factories by stopping the flow of sugar solution in the pipes.

6. Capsule formation may be responsible for considerable economic loss in dairy and other food industries. Carbohydrate-containing materials become “ropy” when encapsulated organisms grow on it.

7. Some organisms such as *Leuconostoc* species, are employed commercially in the production of dextran (polymer of glucose). Dextrans are used as plasma extenders in the treatment of chock resulting from blood loss.

Capsular structure

- Usually composed of polysaccharides and, in few cases, of polypeptides. For example: in streptococci the capsule consists of a polymer of the substances N- acetylglucosamine and glucuronic acid and the polymer of the two compounds is called hyaluronic acid (similar to the intercellular substance in animal cells). In *Shigella* the capsular material is composed of polysaccharides and phospholipids. The components of capsular material can be summarized as follows:

Table (5): Chemical composition of some bacterial capsules

Bacterium	Capsule composition	Structural subunits
Gram-positive Bacteria		
Bacillus anthracis	polypeptide (polyglutamic acid)	D-glutamic acid
Bacillus megaterium	polypeptide and polysaccharide	D-glutamic acid, amino sugars, sugars
Streptococcus mutans	polysaccharide	(dextran) glucose
Streptococcus pneumoniae	polysaccharides	sugars, amino sugars, uronic acids
Streptococcus pyogenes	polysaccharide (hyaluronic acid)	N-acetyl-glucosamine and glucuronic acid
Gram-negative Bacteria		
Acetobacter xylinum	polysaccharide	(cellulose) glucose
Escherichia coli	polysaccharide (colonic acid)	glucose, galactose, fucose glucuronic acid
Pseudomonas aeruginosa	polysaccharide	mannuronic acid
Azotobacter vinelandii	polysaccharide	glucuronic acid
Agrobacterium tumefaciens	polysaccharide	(glucan) glucose

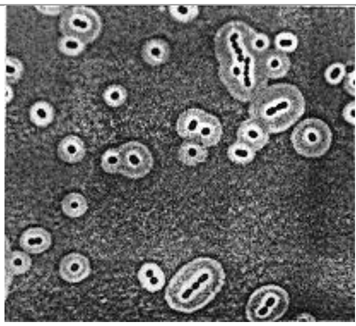


Figure (9): Light microscopy image of bacterial capsules: cells stained with Indian ink showing capsules as discrete layers of polysaccharide surrounding the cells. Sometimes bacterial cells are embedded more randomly in a polysaccharide matrix called a slime layer or biofilm. Polysaccharide films that cannot be detected visually, are called glycocalyx.

Relation of capsule to the growth of bacterial colonies

The appearance of capsulated bacteria ranges from smooth glistening colonies (S- form) to rough and wrinkled colonies (R- form) on solid media. S- form colonies form stable suspensions in liquid media while the rough colonies precipitate in liquid media. The R- form colonies are non- capsulated. Intermediate forms are

RS- forms and extremely rough forms (ER- forms). In some cases the formation of S and R-forms is due to the surrounding environmental conditions. An obvious example is those bacteria that form extracellular dextrans or levans (e.g.: *Leuconostoc mesentroides*). These particular polysaccharides are synthesized only from sucrose. However, all capsule- producing bacteria can mutate spontaneously to non- capsulated form (S- R mutation).

Relation of capsule to bacterial pathogenicity

If the smooth colonies are pathogenic (disease- causing), so the rough mutants are not. This means that the virulence is associated with the occurrence of the capsule.

The capsule confers resistance to the encapsulted cells. This is obvious because the capsule- free mutants are much more readily destroyed by phagocytes than are the encapsulated cells.

Most of bacterial antigens are proteins, but in pneumococcus (*Streptococcus pneumonia*), polysaccharides are also antigens resulting in specific antibodies produced by the host after infection. When these antibodies are mixed the pneumococcus cells, the capsules of the latters are largely swollen due to the precipitation of antibodies on the capsule. This swelling phenomenon is known as “Neufeld” reaction and most commonly “Quellung” reaction (Quellung in German = Swelling in English).

Flagella (in motile bacteria)

Flagella are unbranched filaments of uniform thickness (about 20 nm) throughout their length.

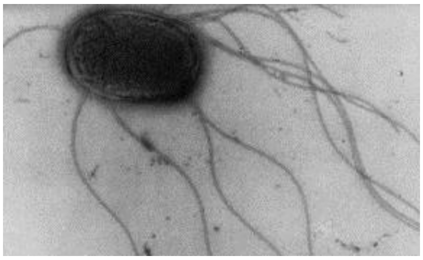


Figure (10): *Salmonella enteritidis* showing flagella: *Salmonella* is an enteric bacterium related to *E. coli*. The enterics are motile by means of peritriochous flagella (TEM image at 10,000X).

The ultrastructure of the flagellum of *E. coli* is illustrated in the Figure below. About 50 genes are required for flagellar synthesis and function. The flagellar apparatus consists of several distinct proteins: a system of rings embedded in the cell envelope (the basal body), a hook-like structure near the cell surface, and the flagellar filament. The innermost rings, the M and S rings, located in the plasma membrane, comprise the motor apparatus. The outermost rings, the P and L rings, located in the periplasm and the outer membrane respectively, function as bushings to support the rod where it is joined to the hook of the filament on the cell surface. As the M ring turns, powered by an influx of protons, the rotary motion is transferred to the filament which turns to drive the bacterium (Figure 11).

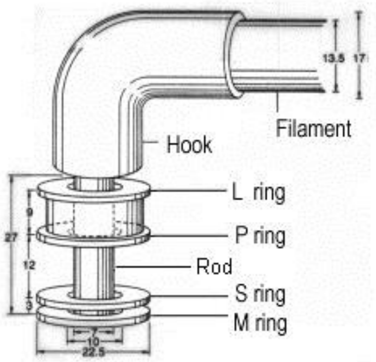


Figure (11): Structure of the flagellum

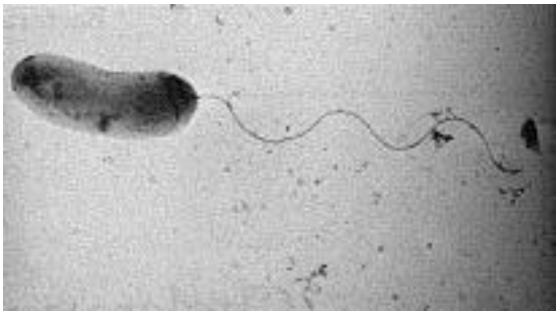


Figure (12): *Vibrio cholerae*: single polar flagellum for swimming movement (Electron Micrograph)

The presence or absence of flagella and their number and arrangement is a species characteristic and according to that, motile bacteria can be divided into the following categories:

(1) Atrichous: non- motile bacteria with no flagella.

(2) Monotrichous: with one polar flagellum at one end.

(3) Lophotrichous: with one group of polar flagella at one end.

(4) Amphitrichous: with polar flagellation at both ends.

(5) Peritrichous: the flagellation is distributed around the cell.

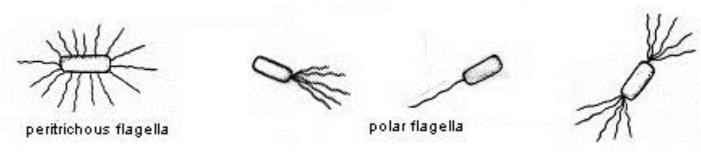


Figure (12): Different arrangements of bacterial flagella: Swimming motility, powered by flagella, occurs in half the bacilli and most of the spirilli.

There is a basal body to which (outside the wall) a short length of filament is attached termed the hook (about 0.05 µm long) and is thicker than the rest of the filament and different in its chemical composition. The rest of the filament can be seen by special staining techniques to increase its thickness or by electron microscopy, which is sometimes surrounded by a sheath. More than

98% of filament components is protein with high acidic amino acid content with some aromatic amino acids. The protein unit of filament (seen as beads under electron microscope) is called flagellin. Flagellin is synthesized within the cell and moves out through the hollow central core of the flagellum to its tip to be assembled there. The full length can be completed within 10 to 20 minutes.

- Another way of bacterial movement is present in the gliding bacteria that have flexible cell walls and their movement is a result of cell bending. It is essential then, for these bacteria to move, to be present in a solid/ liquid or liquid/ gas interface. Some form of attachment, to the surface on which they move, is also essential. Their movement is relatively slow compared to the flagellated bacteria.

Procaryotes are known to exhibit a variety of types of **tactic behavior**, i.e., the ability to move (swim) in response to environmental conditions. For example, during **chemotaxis** a bacterium can sense the quality and quantity of certain chemicals in its environment and swim towards them (if they are useful nutrients) or away from them (if they are harmful substances). Other types of tactic response in procaryotes include **phototaxis**, **aerotaxis** and **magnetotaxis**. The occurrence of tactic behavior provides evidence for the ecological (survival) advantage of flagella in bacteria and other procaryotes.

Detecting bacterial motility

Since motility is a primary criterion for the diagnosis and identification of bacteria, several techniques have been developed to demonstrate bacterial motility, directly or indirectly.

1. **Flagellar stains** outline flagella and show their pattern of distribution. If a bacterium possesses flagella, it is presumed to be motile. Since the bacterial flagellum is below the resolving power of the light microscope, although bacteria can be seen swimming in a microscope field, the organelles of movenent cannot be detected. Staining techniques such as Leifson's method utilize dyes and other components that precipitate along the protein filament to increase its effective diameter. Flagellar distribution is occasionally used to differentiate between morphologically related bacteria.

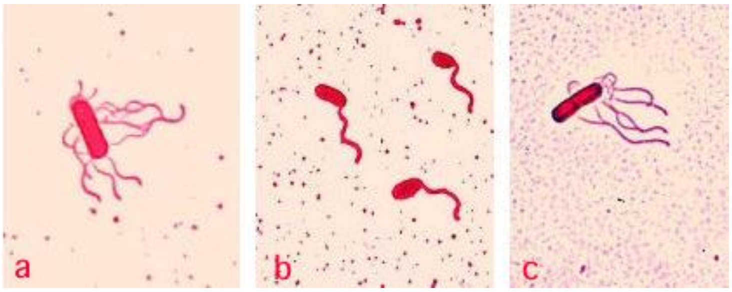


Figure (13): Flagellar staining of three bacteria: a. *Bacillus cereus* b. *Vibrio cholerae* c. *Bacillus brevis*.

2. **Motility test medium** A semisolid medium is inoculated with the bacteria in a straight- line stab with a needle. After incubation, if turbidity (cloudiness) due to bacterial growth can be observed away from the line of the stab, it is evidence that the bacteria are motile.

3. **Direct microscopic observation** of living bacteria in a wet mount usually by using a "hanging- drop slide". Most uincellular bacteria, because of their small size, will shake back and forth in a wet mount observed at 400X or 1000X. This is Brownian movement, due to random collisions between water, molecules and bacterial cells. True motility is confirmed by observing the bacterium swim from one side of the microscope field to the other side.

Pili or fimbriae: (from Latin for hair/ fringe)

Very fine and smaller filaments or appendages than flagella and found only in some freshly isolated Gram- negative bacteria (less than 10µm in diameter and one µm long). Sometimes there are many types of pili. They have a role in sexual conjugation of bacterial cells (make cells stick together). Their number vary between one to 400 per cell.

Common pili (almost always called **fimbriae**) are usually involved in specific adherence (attachment) of procaryotes to surfaces in nature. In medical situations, they are major determinants of bacterial virulence because they allow pathogens to attach to (colonize) tissues and/ or to resist attack by phagocytic white blood cells. For example, enterotoxigenic strains of *E. coli* adhere to the mucosal epithelium of the intestine by means of specific fimbriae; the M-protein and associated fimbriae of *Streptococcus pyogenes* are involved in adherence and to resistance to engulfment by phagocytes (Figure 14).

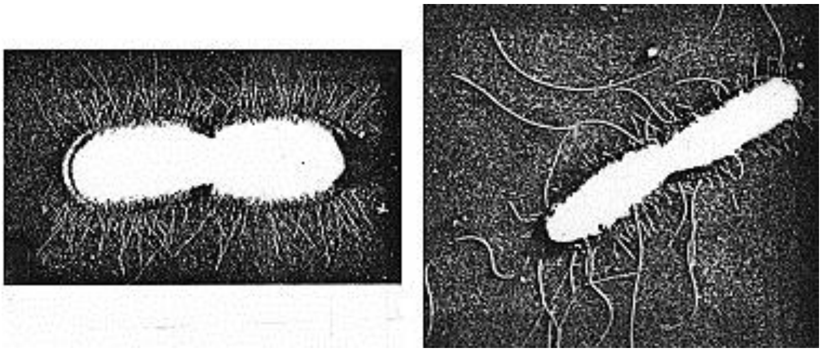


Figure (14): Fimbriae or pili and flagella on bacterial cell surface: **Left**: dividing *Shigella* enclosed in fimbriae. **Right**: dividing pair of *Salmonella* displaying both its peritrichous flagella and its fimbriae. The fimbriae are much shorter and slightly smaller in diameter than flagella. Both *Shigella* and *Salmonella* are enteric bacteria that cause different types of intestinal diarrheas.The bacteria can be differentiated by a motility test. *Salmonella* is motile; *Shigella* is nonmotile.

Table (6): Some properties of pili and fimbriae

Bacterial species where observed	**Typical number on cell**	**Distribution on cell surface**	**Function**
Escherichia coli (F or sex pilus)	1-4	uniform	mediates DNA transfer during conjugation
Escherichia coli (common pili or Type fimbriae)	100-200	uniform	surface adherence to epithelial cells of the GI tract
Neisseria gonorrhoeae	100-200	uniform	surface adherence to epithelial cells of the urogenital tract
Streptococcus pyogenes (fimbriae plus the M-protein)	?	uniform	adherence, resistance to phagocytosis; antigenic variability
Pseudomonas aeruginosa	10-20	polar	surface adherence
Sulfolobus acidocaldarius (an archean)	?	?	attachment to sulfur particles

End of Lecture (2)

THE CELL ENVELOPE

The “cell envelopes” is a descriptive term for the several layers of material that envelope or enclose the protoplasm of the cell. The cell protoplasm (cytoplasm) is surrounded by the plasma membrane, a cell wall and a capsule. The cell wall itself is a layered structure in Gram-negative bacteria. All cells have a membrane, which is the essential and definitive characteristic of a "cell". Almost all procaryotes have a cell wall to prevent damage to the underlying protoplast. Outside the cell wall, foremost as a surface structure, may be a polysaccharide capsule, or at least a glycocalyx.

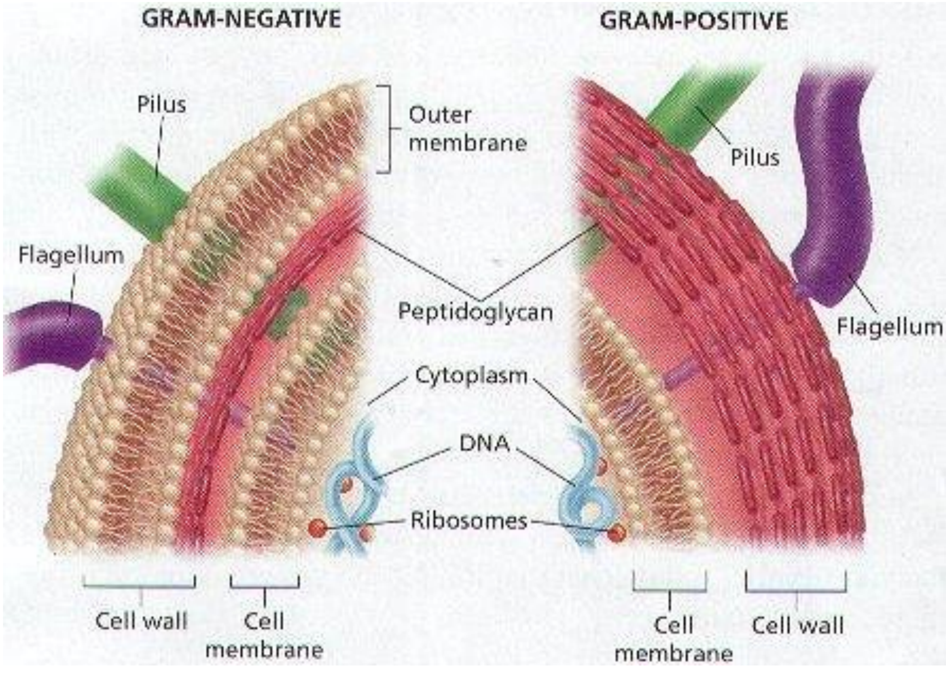


Figure (15): The cell envelope of Gram +ve and Gram -ve bacteria: The Gram-positive wall is a uniformly thick layer external to the plasma membrane. It is composed mainly of peptidoglycan (murein). The Gram-negative wall appears thin and multilayered. It consists of a relatively thin peptidoglycan sheet between the plasma membrane and a phospholipid-lipopolysaccharide outer membrane. The space between the inner (plasma) and outer membranes (wherein the peptidoglycan resides) is called the periplasm.

The bacterial cell wall

The wall is rigid but ductile. It gives the cell the required mechanical strength, determines the cell shape and encloses the cytoplasm. It may be elastic allowing cell expansion and contraction due to changes in turgor pressure. The characteristic antigens of each bacterium are also located on their cell walls.

The presence of the cell wall can be evidenced by many ways:

- Staining with special stains: mordant a heat- fixed film with tannic acid (5- 10%), washing with distilled water (protein is altered and will not take up the stain), the wall can be stained then with 0.2% aqueous crystal violet.
- Plasmolysis of the cell: cell contents will contract in hypertonic solution leaving the wall without contraction and then, can be stained easily.
- Cell destruction by various methods: by ultrasonic waves in the presence of powdered glass, or autolysis and digestion of the protoplasm without affecting the wall. Then wall can be separated from the destroyed cell by centrifugation. Investigation of wall preparations can be carried out by electron microscope.

The cell wall represents 20% of cell dry weight approximately (differs according to species, cell weight and age).

The cell walls of bacteria deserve special attention for several reasons:

1. They are an essential structure for viability, as described above.
2. They are composed of unique components found nowhere else in nature.

3. They are one of the most important sites for attack by antibiotics.

4. They provide ligands for adherence and receptor sites for drugs or viruses.

5. They cause symptoms of disease in animals.

6. They provide the immunological distinction and variation among strains of bacteria.

The cell walls of all **Bacteria** contain a unique type of **peptidoglycan** called **murein**. Peptidoglycan is a polymer of disaccharides (a glycan) cross-linked by short chains of amino acids (peptides), and many types of peptidoglycan exist. All **Bacterial** peptidoglycans contain **N-acetylmuramic acid**, which is the definitive component of **murein**. The cell walls of **Archaea** may be composed of protein, polysaccharides, or peptidoglycan-like molecules, but never contain murein. This feature distinguishes the **Bacteria** from the **Archaea**.

In the **Gram-positive Bacteria** (those that retain the purple crystal violet dye when subjected to the Gram-staining procedure) the cell wall is thick (15- 80 nanometers), consisting of several layers of peptidoglycan. In the **Gram-negative Bacteria** (which do not retain the crystal violet) the cell wall is relatively thin (10 nanometers) and is composed of a single layer of peptidoglycan surrounded by a membranous structure called the **outer membrane**. The outer membrane of Gram-negative bacteria invariably contains a unique component, **lipopolysaccharide** (**LPS** or **endotoxin**), which is toxic to animals.

Peptidoglycan structure and arrangement in *E. coli* is representative of all *Enterobacteriaceae*, and many other Gram-negative bacteria, as well. The glycan backbone is made up of alternating molecules of N-acetylglucosamine (G) and N-acetylmuramic acid (M) connected by a beta 1,4-glycoside bond. The 3-carbon of N-acetylmuramic acid (M) is substituted with a lactyl ether group derived from pyruvate. The lactyl ether connects the glycan backbone to a peptide side chain that contains L-alanine, (L-ala), D-glutamate (D-glu), Diaminopimelic acid (DAP), and D-alanine (D-ala). MurNAc is unique to bacterial cell walls, as is D-glu, DAP and D-ala. **Strands of murein** are assembled in the periplasm from about 10 muramic acid subunits. Then the strands are connected to form a continuous glycan molecule. The assembly of peptidoglycan on the outside of the plasma membrane is mediated by a group of periplasmic enzymes which are transglycosylases, transpeptidases and carboxypeptidases. The mechanism of action of penicillin and related beta-lactam antibiotics is to **block transpeptidase and carboxypeptidase enzymes** during their assembly of the murein cell wall. Hence, the beta lactam antibiotic are blocking cell wall synthesis in the bacteria.

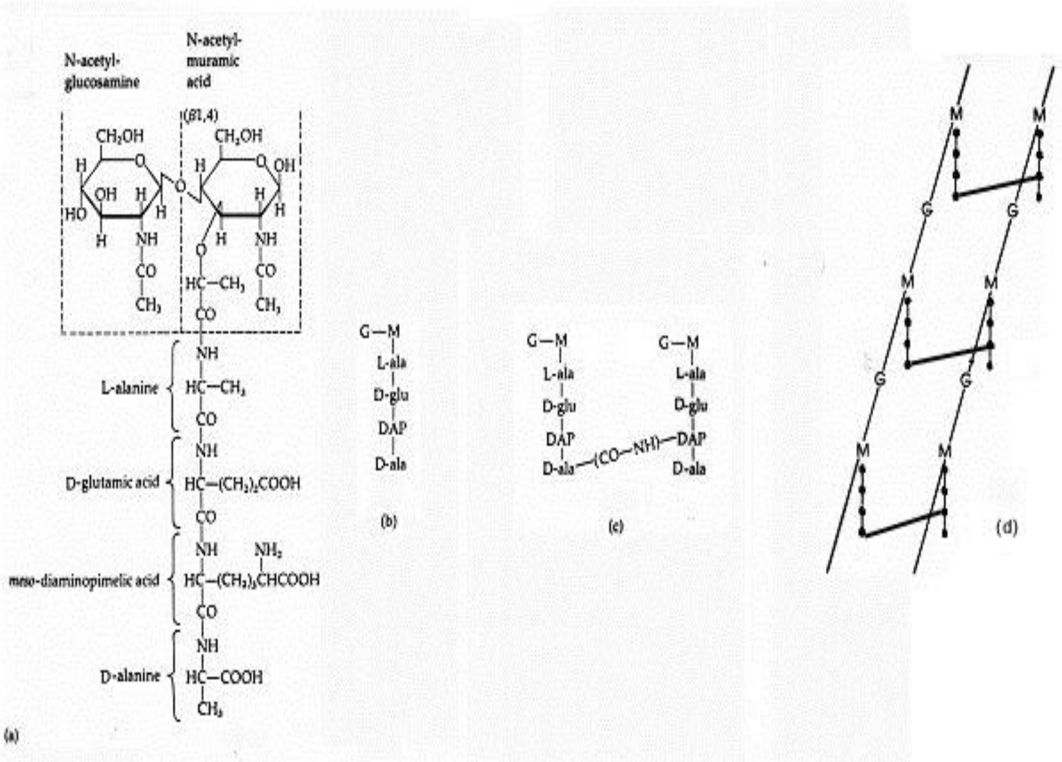


Figure (16): The structure of muramic acid subunit of the peptidoglycan of *Escherichia coli*: This is the type of murein found in most Gram-negative bacteria. The glycan backbone is a repeat polymer of two amino sugars, N-acetylglucosamine (G) and N-acetylmuramic acid (M). Attached to the N-acetylmuramic acid is a tetrapeptide consisting of L-ala-D-glu-DAP-D-ala.b. Nearby tetrapeptide side chains may be linked to one another by an interpeptide bond between DAP on one chain and D-ala on the other. d. The polymeric form of the molecule.

The glycan backbone of the peptidoglycan molecule can be cleaved by an enzyme called **lysozyme** that is present in animal serum, tissues and secretions, and in the phagocytic lysosome. The function of lysozyme is to lyse bacterial cells as a constitutive defense against bacterial pathogens. Some Gram-positive bacteria are very sensitive to lysozyme and the enzyme is quite active at low concentrations. Lachrymal secretions (tears) can be diluted 1:40,000 and retain the ability to lyse certain bacterial cells. Gram-negative bacteria are less vulnerable to attack by lysozyme because their peptidoglycan is shielded by the outer membrane.

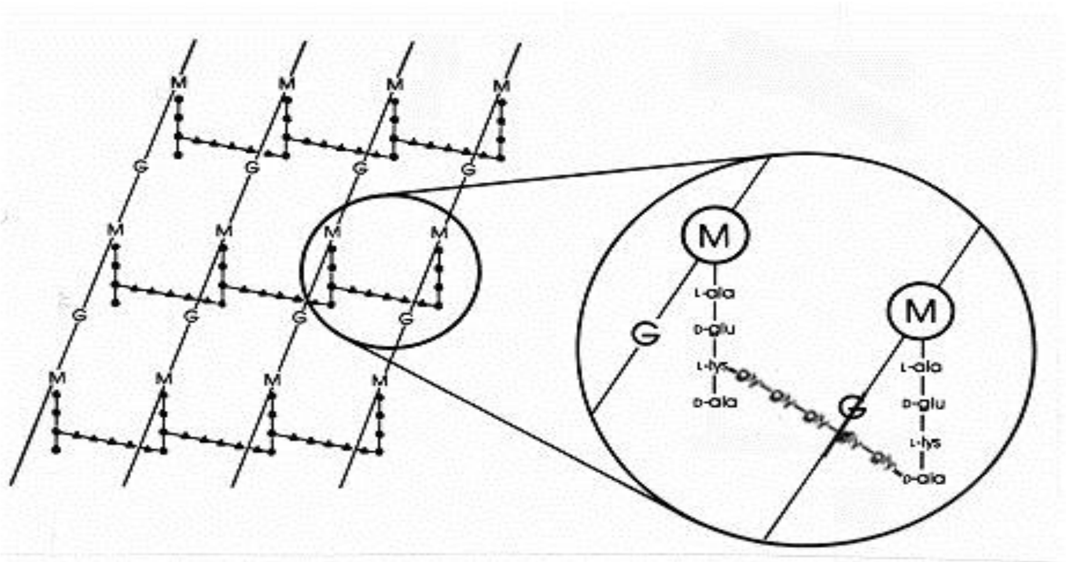


Figure (17): A diagram of the peptidoglycan sheet of *Staphylococcus aureus*: G = N-acetyl-glucosamine; M = N-acetyl-muramic acid; L-ala = L-alanine; D-ala = D-alanine; D-glu = D-glutamic acid; L-lys = L-lysine. This is one type of murein found in Gram-positive bacteria. Compared to the *E. coli* peptidoglycan there is L-lys in place of DAP (diaminopimelic acid) in the tetrapeptide. The free amino group of L-lys is substituted with a glycine pentapeptide (gly-gly-gly-gly-gly-) which then becomes an interpeptide bridge forming a link with a carboxy group from D-ala in an adjacent tetrapeptide side chain. Gram-positive peptidoglycans differ from species to species, mainly in regards to the amino acids in the third position of the tetrapeptide side chain and in the amino acid composition of the interpeptide bridge.

The cell wall is an enormous bag- shaped macromolecule

- The cell wall macromolecule building unit is called peptydoglycan, mucopeptide, mucocomplex, murein and many other names. It consists of peptide and sugar units. The peptidoglycan is extensively cross- linked, giving rise to a single enormous bag-shaped macromolecule. Therefore, we can summarize the structure of the cell wall as follows:
- The building units of the peptidoglycan backbone are as follows:

(1) A disaccharide of N- acetylglucosamine (NAG) and N-acetyl muramic acid (NAM) which is bonded through ß- 1, 4- glycosidic linkages.

(2) A tetrapeptide consisting of L- alanine, D- glutamine, L- lysine and D- alanine.

(3) A pentaglycine bridge (a pentapeptide) of the amino acid glycine.

- The tetrapeptide (L- alanine, D- glutamine, L- lysine and D- alanine) in the peptidoglycan structure is unusual in two respects:

(1) It contains D- amino acids, which are never found in proteins.

(2) The D- glutamic residue forms a peptide linkage at its side chain (carboxyl groups).

- The pentaglycine peptide [$(Gly)_5$] cross links N- acetylmuramic acid residues on different polysaccharide strands. The amino group of $(Gly)_5$ forms a peptide bond with the carboxyl group of D- alanine, whereas the carboxyl group of $(Gly)_5$ forms a peptide bond with the side- chain amino group of L- lysine.

<u>Synthesis of peptidoglycan</u>:

(1) A peptide unit is built on NAM while the sugar is attached to uridine diphosphate.

(2) The NAM- peptide unit is transferred to a carrier lipid (C_{55}- isoprenoid alcohol).

(3) NAG and the pentaglycine bridge are added to the NAM- peptide unit while it is attached to the carrier lipid.

(4) The disaccharide peptide unit is transferred from the carrier lipid to a growing polysaccharide chain.

(5) Different polysaccharide strands are cross- linked by a transpeptidation reaction involving the pentaglycine bridges to form one enormous bag- shaped macromolecule.

- The carrier lipid is a hydrophobic alcohol that enables the nascent sugar- peptide to resist permeability of the cell membrane.
- This series of reactions occurs in both Gram positive and negative bacteria as peptidoglycan is contained in both. It is now the difference, between both kinds of bacteria, in the outer layers that cover each kind.

(1) Teichoic acid covers murein in Gram- positive bacteria

Teichoic acid is a polymer of glycerol (or another sugar such as ribitol) linked by phosphodiester bonds. The available hydroxyl groups are esterified to alanine or other sugars such as glucose. Teichoic acid is attached to the NAM- NAG backbone of peptidoglycan by a phosphodiester bond.

(2) Outer membrane in Gram negative bacteria

- An outer membrane that contains phospholipids, proteins and lipopolysaccharides surrounds the peptidoglycan layer of Gram negative bacteria (such as *E. coli* and *Salmonella typhimurium*). This membrane has a bilayer arrangement such as the plasma membrane. Thus, Gram negative cells have two membranes, whereas Gram positive cells have only one.
- The periplasmic space, between plasma membrane and peptidoglycan layer, contains many proteins that control the transport of sugars and other nutrients in participation with the plasma membrane.

• The lipopolysaccharides (LPS) consist of three regions namely; lipid A, core oligosaccharides and O- specific side chain.

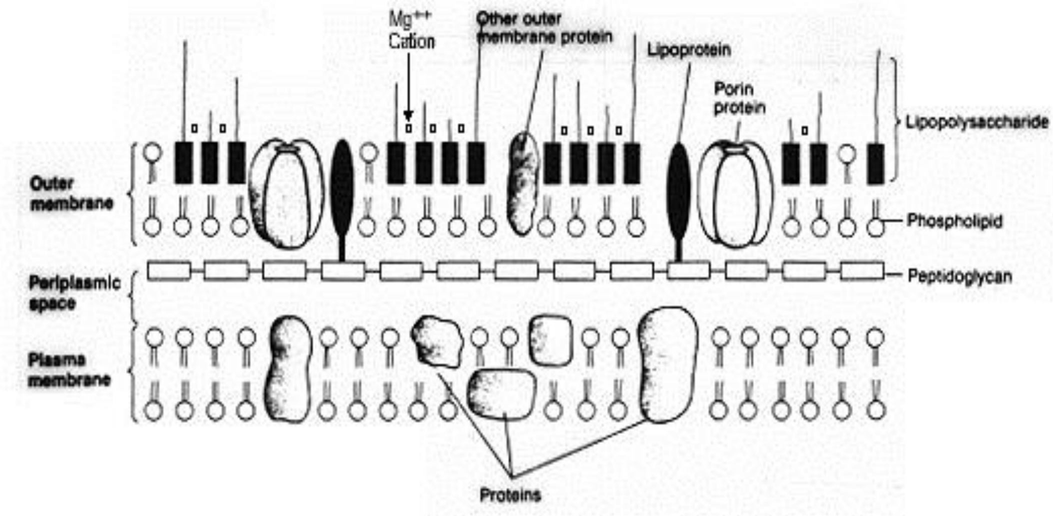


Figure (18): Schematic illustration of the outer membrane: cell wall and plasma membrane of a Gram-negative bacterium

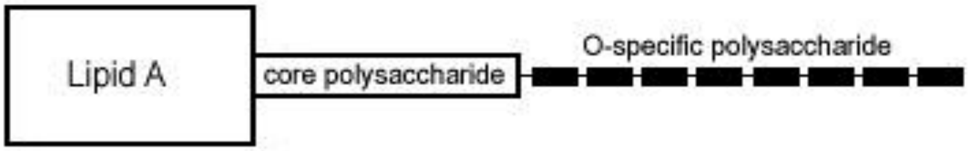


Figure (19): Structure of LPS

The lipid A moiety is the hydrophobic part of the molecule, while the core oligosaccharides and O- side chain are highly hydrophilic.

• The lipid A contains six saturated fatty acid chains linked to two glucosamine residues.

• The oligosaccharide region is composed of ten sugar units that project outwards followed by the O- side chain that is made up of many repeating tetrasaccharide units. The two sugar regions contain several sugars that are rarely present elsewhere in nature. Example of these sugars are 2- keto- 3- deoxyoctonate (KDO), an eight carbon sugar; heptose, a seven- carbon sugar, L- rhamnose and abequose (a six- carbon sugar with CH_3 instead of CH_2 OH at C_6.

- The lipopolysaccharide molecule is negatively charged because several of its sugars are phosphorylated.
- The LPS are synthesized in the plasma membrane then transferred to the outer membrane. The sequence of synthesis starts with lipid A, core oligosaccharides and, finally, O- side chain is added to the tip of the molecule.
- The core oligosaccharide is synthesized by sequential addition of sugars from activated donors such as UDP- glucose and UDP- galactose. While the O- side chain synthesis is similar to murein. Its repeating tetrasaccharide units are built on the inner leaflet of the plasma membrane and transferred to the growing chain by the same carrier lipid (C_{55}).

How Gram –ve bacteria counteracts host defenses

- The saturated fatty acid chains contribute to the barrier role of the outer membrane. Periplasmic proteins are kept in and most harmful molecules are kept out (penicillin does not enter Gram –ve cells).
- The lipid A may also confer rigidity on the outer membrane, while O- side chains are not essential for viability (some *E. coli* species do not have it).
- The outer coat of polysaccharide makes the bacterial surface very hydrophilic which renders it less susceptible to phagocytosis by host cells.

- The O- specific chains of LPS are highly diverse. Gram negative bacteria can mutate rapidly to alter the nature of these chains. A host population that has not been exposed to the new surface structure will have a low level of antibody against the novel O- side chains. Thus, varying the O- side chains is a way of staying one step ahead of the host's defense system.
- The genetic information for the alteration of O- side chains sometimes comes from a temperate phage hidden by the Gram negative bacterium. For example, phage P_{22} contributes a gene that adds glucose to the repeated tetrasaccharide unit of the O- side chain. This kind of alteration is called phage conversion.

Gram-Negative Envelope

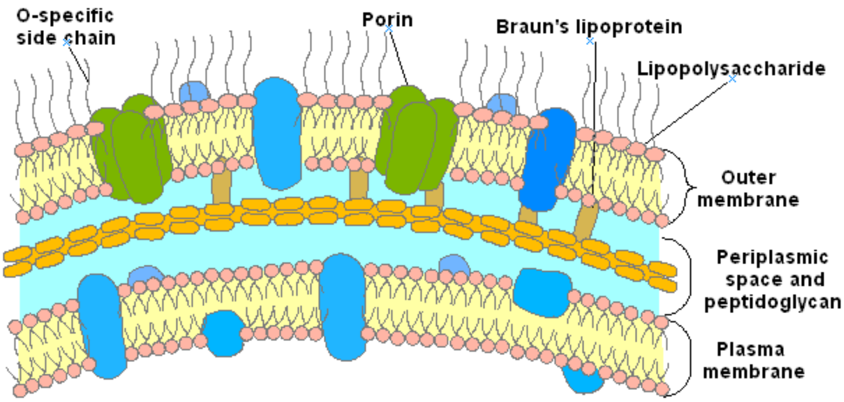


Figure (20)

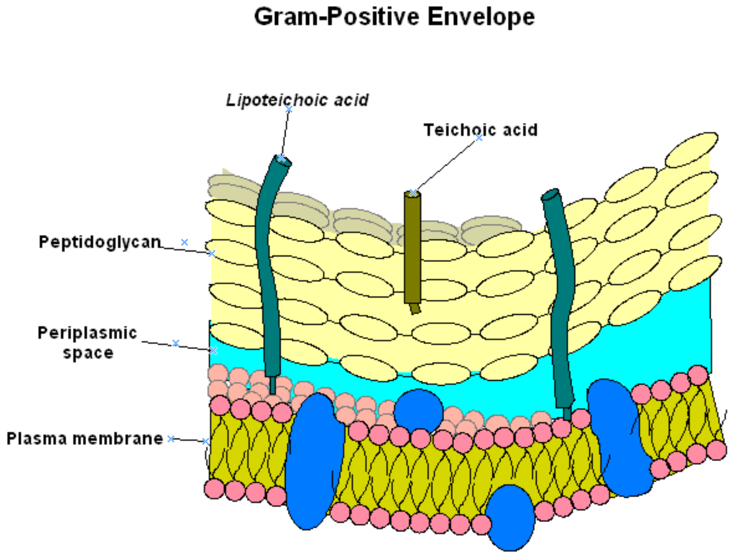
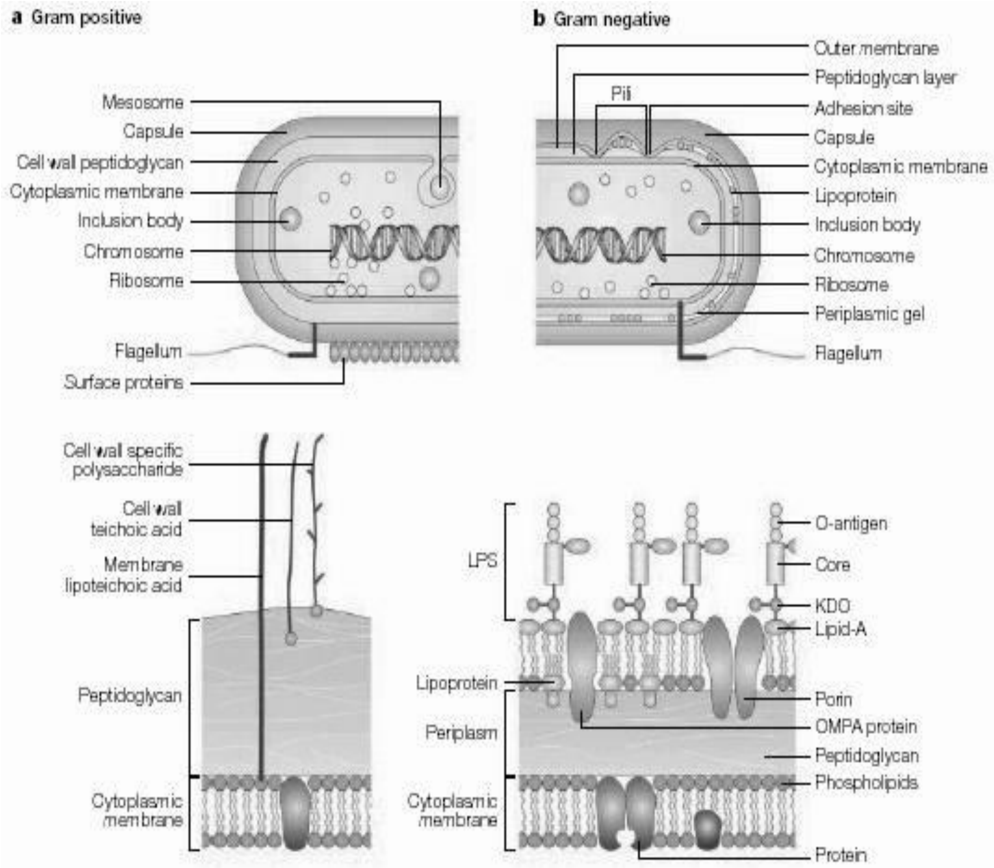


Figure (21)

Table (7): Correlation of Grams stain with other properties of Bacteria.

Property	**Gram-posi**	**Gram-neg**
Thickness of wall	thick (20-80	thin (10 r
Number of layers	1	2
Peptidoglycan content	>50%	10-20%
Teichoic acids in wall	present	absent
Lipid and lipoprotein content	0-3%	58%
Protein content	0	9%
Lipopolysaccharide content	0	13%
Sensitivity to Penicillin G	yes	no (1)
Sensitivity to lysozyme	yes	no (2)

Figure (22): Comparison between the cell wall of the two different groups of bacteria



____ ***End of Lecture (3)***

Plasma membrane (cytoplasmic membrane)

- Cytoplasmic membrane is a bi- layered membrane that is stabilized by the hydrophobic forces between the fatty acid residues and electrostatic forces between the hydrophilic heads.

The **plasma membrane** is the most dynamic structure of a procaryotic cell. Its main function is as a **selective permeability barrier** that regulates the passage of substances into and out of the cell. The bacterial membrane allows passage of water and uncharged molecules but does not allow passage of larger molecules or any charged substances except by means special membrane **transport processes** and **transport systems**.

Since procaryotes lack any intracellular organelles for processes such as respiration or photosynthesis or secretion, the plasma membrane carry out 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. The predominant functions of bacterial membranes are listed in Table (8).

Table (8): 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 and sensing functions)
9. Location of specialized enzyme system

Bacterial membranes are composed of 40 percent phospholipid and 60 percent protein. The phospholipids are amphoteric 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. 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. The arrangement of proteins and lipids to form a membrane is called the fluid mosaic model.

The membranes of Bacteria are structurally similar to the cell membranes of eukaryotes, 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. The structure of archaeal membranes is thought to be an adaptation to their existence and survival in extreme environments.

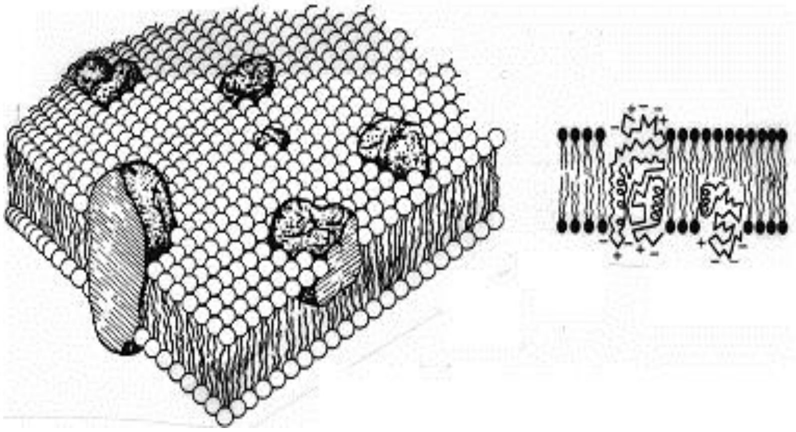


Figure (23): Mosaic model of a biological membrane: In aqueous environments membrane phospholipids arrange themselves to form a fluid bilayer. Membrane proteins, which may be structural or functional, may be permanently or transiently associated with one side or the other of the membrane, or even be permanently built into the bilayer, while other proteins may form transport channels through the membrane.

It is considered as soft, elastic fluid structure and the active site of transportation or substrate permeability system. The influx and outflux of substances is mediated by membrane proteins.

The plasma membrane of procaryotes may invaginate into the cytoplasm or form stacks or vesicles attached to the inner membrane surface. These structures are sometimes referred to as **mesosomes.** Such internal membrane systems may be analogous to the cristae of mitochondria or the thylakoids of chloroplasts which increase the surface area of membranes to which enzymes are bound for specific enzymatic functions. The photosynthetic apparatus (light harvesting pigments and ATPase) of photosynthetic procaryotes is contained in these types of membranous structures. Mesosomes may also represent specialized membrane regions involved in DNA replication and segregation, cell wall synthesis, or increased enzymatic activity. There are a few antibiotics (e.g. polymyxin), hydrophobic agents (e.g. bile salts), and proteins that can damage bacterial membranes.

Transport processes

The proteins that mediate the passage of solutes through membranes are referred to variously as **transport systems**, **carrier proteins**, **porters**, and **permeases**. Transport systems operate by one of three **transport processes** as described below in the Figure. In a **uniport** process, a solute passes through the membrane unidirectionally. In **symport** processes (also called **cotransport**) two solutes must be transported in the same direction at the same time; in **antiport** processes (also called **exchange diffusion**), one solute is transported in one direction simultaneously as a second solute is transported in the opposite direction.

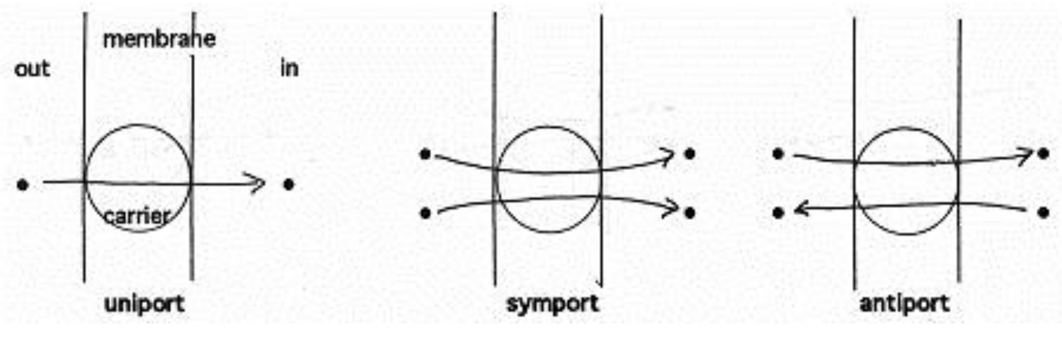


Figure (24): Transport processes

Types of transport systems

Bacteria have a variety of types of transport systems which can be used alternatively in various environmental situations. **Facilitated diffusion** is a carrier-mediated system that does not require energy and does not concentrate solutes against a gradient. Active transport systems such as Ion-driven transport and Binding protein-dependent transport, use energy and concentrate molecules against a concentration gradient. There are two types of **active transport system** in bacteria: **ion driven transport systems** (IDT) and **binding-protein dependent transport systems** (BPDT). The definitive feature of an active transport system is the accumulation of the solute in the cytoplasm at concentrations far in excess of the environment. According to the laws of physical chemistry, this type of process requires energy.

There are four types of carrier-mediated transport systems in procaryotes. The **carrier** is a protein (or group of proteins) that functions in the passage of a small molecule from one side of a membrane to the other side. A transport system may be a single transmembranous protein that forms a channel facilitating the passage of a specific solute, or it may be a coordinated system of proteins that binds and sequentially passes a small molecule through the membrane. Transport systems have the property of **specificity for the solute** transported. Some transport systems will transport structurally related molecules, although at reduced efficiency compared to their primary substrate. Most transport systems transport specific sugars, amino acids, anions or cations that are of nutritional value to the bacterium.

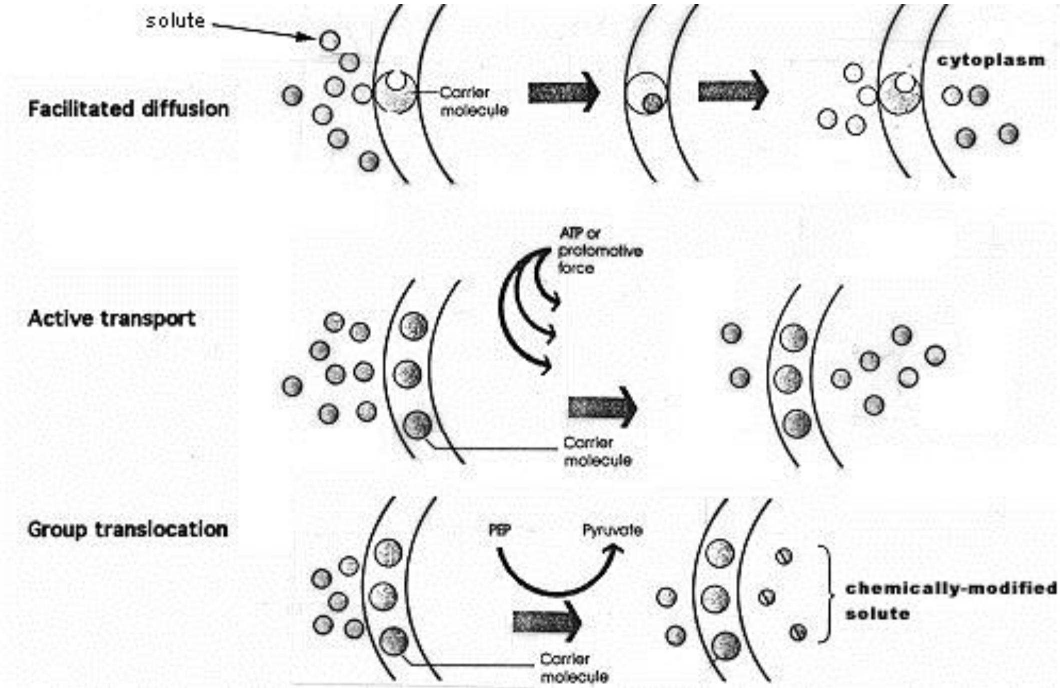


Figure (25): Operation of bacterial transport systems: Bacterial transport systems are operated by transport proteins (sometimes called carriers, porters or permeases) in the plasma membrane.

<u>The cytoplasm</u>

The cytoplasmic constituents of procaryotic cells invariably include the **procaryotic chromosome** and **ribosomes**. The chromosome is typically one large circular molecule of **DNA**, more or less free in the cytoplasm. Procaryotes sometimes possess smaller extrachromosomal pieces of DNA called **plasmids**. The total DNA content of a procaryote is referred to as the cell **genome**. During cell growth and division, the procaryotic chromosome is replicated distribution to progeny cells. However, the eukaryotic processes of meiosis and mitosis are absent in procaryotes. Replication and segregation of procaryotic DNA is coordinated by the membrane, and possibly, by mesosomes.

The distinct granular appearance of procaryotic cytoplasm is due to the presence and distribution of **ribosomes.** The ribosomes of procaryotes are smaller than cytoplasmic ribosomes of eukaryotes. Procaryotic ribosomes are 70S (Svedberg unit) in size, being composed of 30S and 50S subunits. The 80S ribosomes of eukaryotes are made up of 40S and 60S subunits. Ribosomes are involved in the process of translation (protein synthesis), but some details of their activities differ in eukaryotes, Bacteria and Archaea. Protein synthesis using 70S ribosomes occurs in eukaryotic mitochondria and chloroplasts.

Table (9): Small molecules found in a growing bacterial cell

Molecule	**Approximate number**
Amino acids, their precursors and derivatives	120
Nucleotides, their precursors and derivatives	100
Fatty acids and their precursors	50
Sugars, carbohydrates and their precursors or derivatives	250
Quinones, porphyrins, vitamins, coenzymes and prosthetic groups and their precursors	300

Table (10): Inorganic ions found in a growing bacterial cell

Ion	**Function**
K^+	Maintenance of ionic strength; cofactor for certain enzymes
NH_4^+	Principal form of inorganic N for assimilation
Ca^{++}	Cofactor for certain enzymes
Fe^{++}	Present in cytochromes and other metalloenzymes
Mg^{++}	Cofactor for many enzymes; stabilization of outer membrane of

	Gram-negative bacteria
Mn^{++}	Present in certain metalloenzymes
Co^{++}	Trace element constituent of vitamin B12 and its coenzyme derivatives and found in certain metalloenzymes
Cu^{++}	Trace element present in certain metalloenzymes
Mo^{++}	Trace element present in certain metalloenzymes
Ni^{++}	Trace element present in certain metalloenzymes
Zn^{++}	Trace element present in certain metalloenzymes
SO_4^{--}	Principal form of inorganic S for assimilation
PO_4^{---}	Principal form of P for assimilation and a participant in many metabolic reactions

Cytoplasmic inclusions

Often contained in the cytoplasm of procaryotic cells is one or another of some type of inclusion granule. Inclusions are distinct granules that may occupy a substantial part of the cytoplasm. Inclusion granules are usually reserve materials of some sort. For example, carbon and energy reserves may be stored as glycogen (a polymer of glucose) or as poly ß- hydroxybutyric acid (a type of fat) granules. Polyphosphate inclusions are reserves of PO_4 and possibly energy; elemental sulfur (sulfur globules) are stored by some phototrophic and some lithotrophic procaryotes as reserves of energy or electrons. Some inclusion bodies are actually membranous vesicles or intrusions into the cytoplasm which contain photosynthetic pigments or enzymes. Gas vacuoles are present in cyanobacteria to adjust its floating characteristics and exposure to light in aqueous environments.

Table (11): Some inclusions in the bacterial cells

Cytoplasmic inclusions	**Where found**	**Composition**	**Function**
glycogen	many bacteria e.g. *E. coli*	polyglucose	reserve carbon and energy source
Polybetahy droxyutyric acid (PHB)	many bacteria e.g. *Pseudomonas*	polymerized hydroxy butyrate	reserve carbon and energy source
polyphosphate (volutin granules)	many bacteria e.g. *Corynebacterium*	linear or cyclical polymers of PO_4	reserve phosphate;possibly a reserve of high energy phosphate
sulfur globules	phototrophic purple and green sulfur bacteria and lithotrophic colorless sulfur bacteria	elemental sulfur	reserve of electrons (reducing source) in phototrophs; reserve energy source in lithotrophs
gas vesicles	aquatic bacteria especially cyanobacteria	protein hulls or shells inflated with gases	buoyancy (floatation) in the vertical water column
parasporal crystals	endospore-forming bacilli (genus *Bacillus*)	protein	unknown but toxic to certain insects
magnetosomes	certain aquatic bacteria	magnetite (iron oxide) Fe3O4	orienting and migrating along geo- magnetic field lines
carboxysomes	many autotrophic bacteria	enzymes for autotr-ophic CO2 fixation	site of CO2 fixation
phycobilisomes	cyanobacteria	phycobiliproteins	light-harvesting pigments
chlorosomes	Green bacteria	lipid and protein and bacteriochlorophyll	light-harvesting pigments and antennae

BACTERIAL ENDOSPORES

Endospores are formed by a few groups of **Bacteria** as intracellular structures, but ultimately they are released as free endospores. Endospores exhibit no signs of life, being described as **cryptobiotic**. They are highly resistant to environmental stresses such as high temperature (some endospores can be boiled for hours and retain their viability), irradiation, strong acids, disinfectants, etc. They are probably the most durable cell produced in nature. Although cryptobiotic, they retain viability indefinitely such that under appropriate environmental conditions, they germinate back into vegetative cells. Endospores are formed by vegetative cells in response to environmental signals that indicate a limiting factor for vegetative growth, such as exhaustion of an essential nutrient. They germinate and become vegetative cells when the environmental stress is relieved. Hence, endospore-formation is a mechanism of survival rather than a mechanism of reproduction.

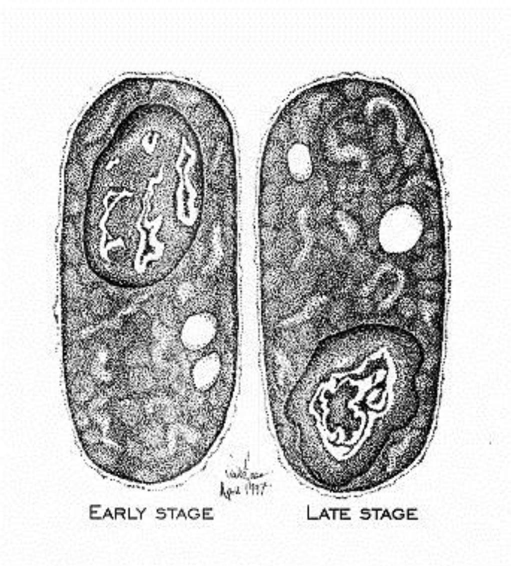
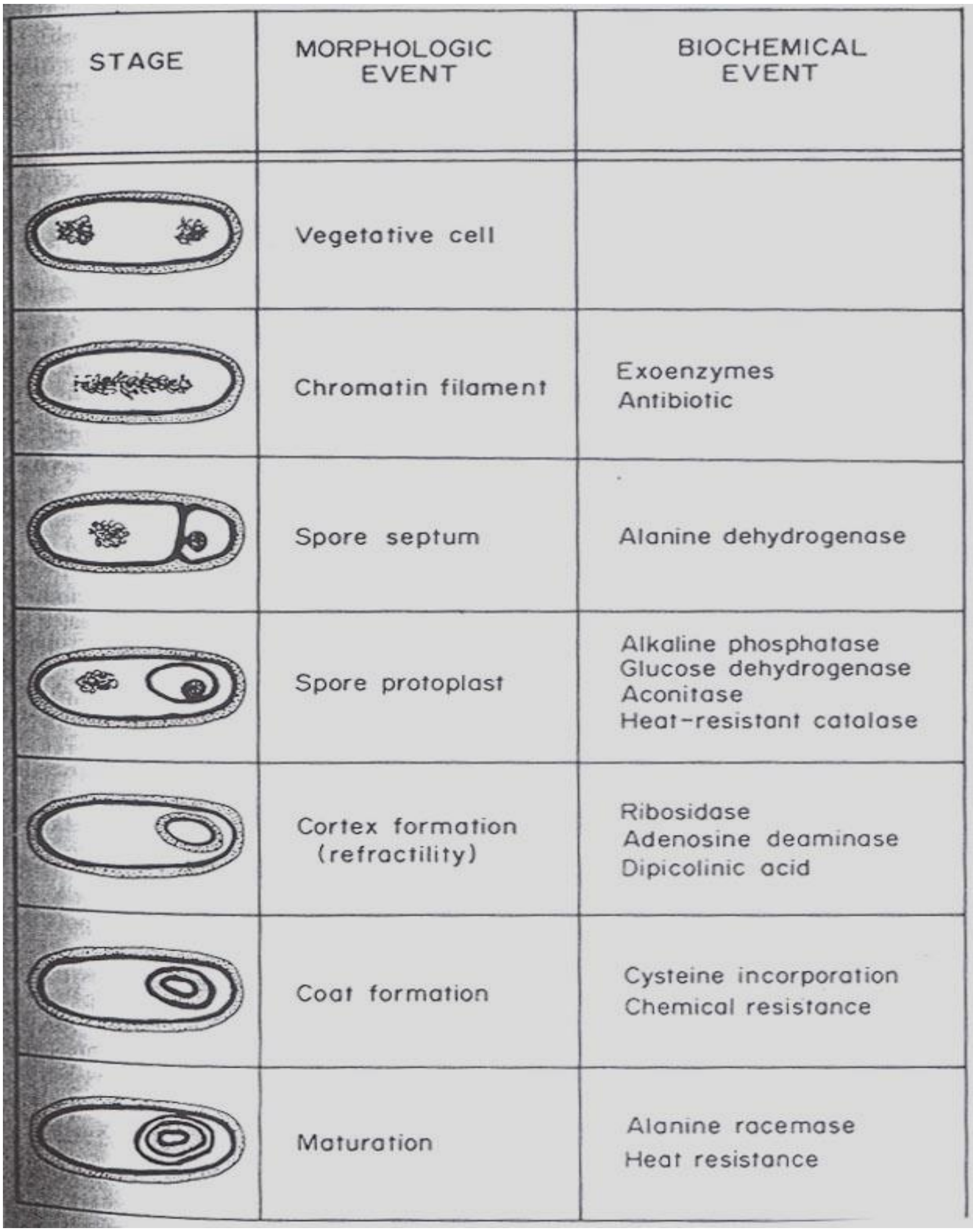


Figure (26): Early and late stages of endospore formation

During endospore formation, a vegetative cell is converted to a heat-resistant spore through many stages starting from the exponential phase where the chromosomal content is doubled. The following Figure the morphological and biochemical events until a spore is formed.



STAGE	MORPHOLOGIC EVENT	BIOCHEMICAL EVENT
	Vegetative cell	
	Chromatin filament	Exoenzymes Antibiotic
	Spore septum	Alanine dehydrogenase
	Spore protoplast	Alkaline phosphatase Glucose dehydrogenase Aconitase Heat-resistant catalase
	Cortex formation (refractility)	Ribosidase Adenosine deaminase Dipicolinic acid
	Coat formation	Cysteine incorporation Chemical resistance
	Maturation	Alanine racemase Heat resistance

Figure (27): Steps of endospore formation

Table (12): Differences between endospores and vegetative cells

Property	Vegetative cells	Endospores
Surface coats	Typical Gram-positive mur wall polymer	Thick spore coat, cortex, and peptidoglycan core wall
Microscopic appearance	Nonrefractile	Refractile
Calcium dipicolinic acid	Absent	Present in core
Cytoplasmic water activity	High	Very low
Enzymatic activity	Present	Absent
Macromolecular synthesis	Present	Absent
Heat resistance	Low	High
Resistance to chemicals and acids	Low	High
Radiation resistance	Low	High
Sensitivity to lysozyme	Sensitive	Resistant
Sensitivity to dyes and staining	Sensitive	Resistant

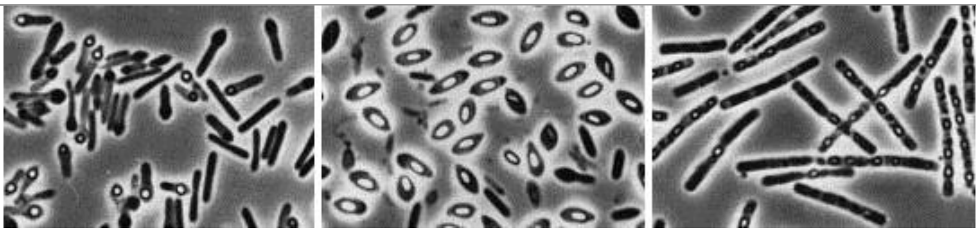


Figure (28): Bacterial endospores, phase- contrst microscopy image: sporulating bacteria demonstrates the refractility of endospores, as well as characteristic spore shapes and locations within the mother cell.

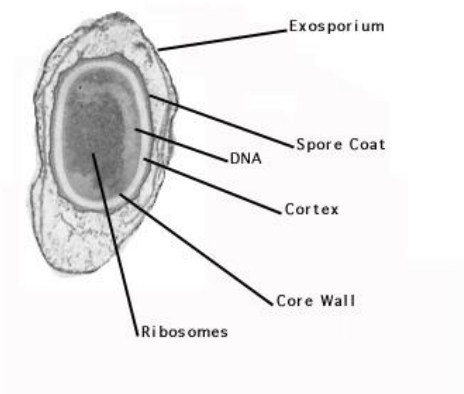


Figure (29): Electron micrograph of a bacterial endospore: The spore has a core wall of unique peptidoglycan surrounded by several layers, including the cortex, the spore coat and the exosporium. The dehydrated core contains the bacterial chromosome and a few ribosomes and enzymes to start protein synthesis and metabolism during germination.

End of Lecture (4)

BACTERIAL GROWTH AND REPRODUCTION

Bacterial growth can be defined as the increase in cell mass and individual constituents leading to cell division (reproduction).

Growth essential requirements are: nutrients, energy, water, optimum temperature, pH, oxygen level and, sometimes, specific vitamins and growth factors. Interaction may occur between different factors and these factors may affect each other in a way or another. We are going now to discuss some of the important growth factors.

(1) Nutrients

Nutrients are essential for cell growth, maintenance and division. Bacterial requirements for different nutrients are variable. In other words, there is no generalization for nutritional requirements for all bacteria but this is only group or species- dependent. This, in turn, is depending on the group of enzymes present in the bacterial cell.

Some major compounds or elements are needed for all the bacteria such as sources of nitrogen, carbon, sulphur, phosphorus, etc. These major requirements are called macroelements or macronutrients (Table 13). Some other elements are needed in very small amounts called microelements or micronutrients such as cobalt, zinc, nickel, etc.

Table (13): Major elements, their sources and functions in bacterial cells

Element	% of dry w	Source	Function
Carbon	50	organic compounds or CO_2	Main constituent of cellular material
Oxygen	20	H_2O, organic compounds, CO_2,	Constituent of cell material and cell water; O_2 is electron acceptor in aerobic respiration
Nitrogen	14	NH_3, NO_3, organic compounds,	Constituent of amino acids, nucleic acids nucleotides, and coenzymes
Hydrogen	8	H_2O, organic compounds, H_2	Main constituent of organic compounds and cell water
Phosphorus	3	inorganic phosphates (PO_4)	Constituent of nucleic acids, nucleotides, phospholipids, LPS, teichoic acids
Sulfur	1	SO_4, H_2S, S^o, organic sulfur compounds	Constituent of cysteine, methionine, glutathione, several coenzymes
Potassium	1	Potassium salts	Main cellular inorganic cation and cofactor for certain enzymes
Magnesium	0.5	Magnesium salts	Inorganic cellular cation, cofactor for certain enzymatic reactions
Calcium	0.5	Calcium salts	Inorganic cellular cation, cofactor for certain enzymes and a component of endospores
Iron	0.2	Iron salts	Component of cytochromes and certain nonheme iron-proteins and a cofactor for some enzymatic reactions

(2) Energy

Energy is required for carrying out all the metabolic reactions, motility and nutrient uptake. Bacterial cells derive their energy from the surrounding environmental sources. This energy may be stored in the cell in the form of high- energy compounds such as ATP (adinosine triphosphate). Examples are phototrophic bacteria that derive their energy from light sources (light- dependent energy) and chemotrophic bacteria that derive their energy from chemical reactions.

According to the mechanism of energy conversion into ATP, there are two types of organisms:

(1) Phototrophic organisms that use light as a source of energy and can be divided into oxygenic (oxygen is produced in the process) and anoxygenic phototrophs (do not produce oxygen).

(2) Chemotrophic organisms that us energy from chemical reactions (oxidation- reduction reactions).

(3) <u>Hydrogen donors and carbon sources</u>

(1) Organotrophs: bacteria that use organic compounds as a hydrogen donor.

(2) Lithotrophs: bacteria that use inorganic hydrogen donor.

(3) Autotrophy and heterotrophy refers only to the use of carbon source. Autotrophs are those bacteria that fix carbon dioxide or utilize it as a sole carbon source to build up macromolecule while heterotrophs are those bacteria that use organic compounds for biosynthesis.

Table (14): Classification of cellular organisms according to carbon and energy source

Carbon / Energy	**AUTOTROPHIC (Lithotrophic)** Principal carbon source inorganic (carbon dioxide)	**HETEROTROPHIC (Organotrophic)** Principal carbon source organic
Phototrophic (Photosynthetic) Use light energy	**Photoautotrophic (Photolithotrophic)** Includes all green plants (plants with chlorophyll), green and purple sulphur bacteria.	**Photoheterotrophic (Photoorganotrophic)** Includes few organisms (e.g. purple nonsulphur bacteria and some algal flagellates)
Chemotrophic Use energy from chemical processes	**Chemoautotrophic (Chemosynthetic or Chemolithotro** A few bacteria	**Chemoheterotrophic (Chemoorganotrophic)** Includes the great bulk of non-photosynthetic organisms, all animals and fungi, most bacteria and some parasitic flowering plants.

(4) Growth Factors

An organism, whether it is an autotroph or a heterotroph, may require small amounts of certain organic compounds for growth because they are essential substances that the organism is unable to synthesize from available nutrients. Such compounds are called **growth factors**. **Growth factors** are required in small amounts by cells because they fulfill specific roles in biosynthesis. The need for a growth factor results from either a blocked or missing metabolic pathway in the cells. Growth factors are organized into three categories.

1. **purines and pyrimidines**: required for synthesis of nucleic acids (DNA and RNA)
2. **amino acids**: required for the synthesis of proteins
3. **vitamins**: needed as coenzymes and functional groups of certain enzymes

Some bacteria (e.g *E. coli*) do not require any growth factors: they can synthesize all essential purines, pyrimidines, amino acids and vitamins, starting with their carbon source, as part of their own intermediary metabolism. Certain other bacteria (e.g. *Lactobacillus*) require purines, pyrimidines, vitamins and several amino acids in order to grow. These compounds must be added to culture media of these bacteria. The growth factors are not metabolized directly as sources of carbon or energy, rather they are assimilated by cells to fulfill their specific role in metabolism. Mutant strains of bacteria that require some growth factor not needed by the wild type (parent) strain are referred to as **auxotrophs**. Thus, a strain of *E. coli* that requires the amino acid tryptophan in order to grow would be called a tryptophan auxotroph and would be designated *E. coli* ***trp.***

Table (15): Common vitamins required in the nutrition of certain bacteria.

Vitamin	**Coenzyme form**	**Function**
p-Aminobenzoic (PABA)	--	Precursor for the biosynthesis of folic acid
Folic acid	Tetrahydrofolate	Transfer of one-carbon units and required for synthesis of thymine, purine bases, serine, methionine and pantothenate
Biotin	Biotin	Biosynthetic reactions that require CO_2 fixation
Lipoic acid	Lipoamide	Transfer of acyl groups in oxidation of keto acids
Mercaptoethane-sulfonic acid	Coenzyme M	CH_4 production by methanogens
Nicotinic acid	NAD (nicotinamide adenine dinucleotide) and NADP	Electron carrier in dehydrogenation reactions
Pantothenic acid	Coenzyme A and the Acyl Carrier Protein (ACP)	Oxidation of keto acids and acyl group carriers in metabolism
Pyridoxine (B_6)	Pyridoxal phosphate	Transamination, deamination, decarboxylation and racemation of amino acids
Riboflavin (B_2)	FMN (flavin mononucleotide) and FAD (flavin adenine dinucleotide)	Oxidoreduction reactions
Thiamine (B_1)	Thiamine pyrophosphate (TPP)	Decarboxylation of keto acids and transaminase reactions
Vitamin B_{12}	Cobalamine coupled to adenine nucleoside	Transfer of methyl groups
Vitamin K	Quinones and napthoquinones	Electron transport processes

Culture media for bacterial growth

For any bacterium to be propagated for any purpose it is necessary to provide the appropriate biochemical and biophysical environment. The biochemical (nutritional) environment is made available as a **culture medium**, and depending upon the special needs of particular bacteria (as well as particular investigators). A large variety and types of culture media have been developed with different purposes and uses. Culture media are employed in the isolation and maintenance of pure cultures of bacteria and are also used for identification of bacteria according to their biochemical and physiological properties.

Liquid media are used for growth of pure batch cultures, while solidified media are used widely for the isolation of pure cultures, for estimating viable bacterial populations, and a variety of other purposes. The usual gelling agent for solid or semisolid medium is agar, a hydrocolloid derived from red algae. Agar is used because of its unique physical properties (it melts at 100 degrees and remains liquid until cooled to 40 degrees, the temperature at which it gels) and because it cannot be metabolized by most bacteria. Hence as a medium component it is relatively inert.

Types of Culture Media

Culture media may be classified into several categories depending on their composition or use. A chemically- defined (synthetic) medium is one in which the exact chemical composition is known.

Table (16): Minimal medium for the growth of *Bacillus megaterium*: An example of a chemically-defined medium for growth of a heterotrophic bacterium.

Component	**Amount**	**Function of component**
sucrose	10.0 g	C and energy source
K_2HPO_4	2.5 g	pH buffer; P and K sourc
KH_2PO_4	2.5 g	pH buffer; P and K sourc
$(NH_4)2HPO_4$	1.0 g	pH buffer; N and P sourc
$MgSO_4$ $7H_2O$	0.20 g	S and Mg^{++} source
$FeSO_4$ $7H_2O$	0.01 g	Fe^{++} source
$MnSO_4$ $7H_2O$	0.007 g	Mn^{++} Source
water	985 ml	
pH 7.0		

Table (17): Defined enrichment medium for growing *Thiobacillus thiooxidans*: a lithoautotrophic bacterium.

Component	**Amount**	**Function of component**
NH_4Cl	0.52 g	N source
KH_2PO_4	0.28 g	P and K source
$MgSO_4$ $7H_2O$	0.25 g	S and Mg^{++} source
$CaCl_2$ $2H_2O$	0.07 g	Ca^{++} source
Elemental Sulfu	1.56 g	Energy source
CO_2	5%*	C source
water	1000 ml	
pH 3.0		

* Aerate medium intermittently with air containing 5% CO_2.

A complex (undefined) medium is one in which the exact chemical constitution of the medium is not known.

Table (18): Complex medium for the growth of fastidious bacteria

Component	Amount	Function of component
Beef extract	1.5 g	Source of vitamins and other growth
Yeast extract	3.0 g	Source of vitamins and other growth
Peptone	6.0 g	Source of amino acids, N, S, and P
Glucose	1.0 g	C and energy source
Agar	15.0 g	Inert solidifying agent
water	1000 ml	
pH 6.6		

Table (19): Selective enrichment medium for growth of extreme halophiles

Component	Amount	Function of component
Casamino acids	7.5 g	Source of amino acids, N, S and P
Yeast extract	10.0 g	Source of growth factors
Trisodium citrate	3.0 g	C and energy source
KCl	2.0 g	K^+ source
$MgSO_4$ 7 H_2O	20.0 g	S and Mg^{++} source
$FeCl_2$	0.023 g	Fe^{++} source
NaCl	250 g	Na^+ source for halophiles and inhibitc nonhalophiles
water	1000 ml	
pH 7.4		

Defined media are usually composed of pure biochemicals off the shelf; complex media usually contain complex materials of biological origin such as blood or milk or yeast extract or beef extract, the exact chemical composition of which is obviously undetermined. A defined medium is a minimal medium if it provides only the exact nutrients (including any growth factors) needed by the organism for growth. The use of defined minimal media requires the investigator to know the exact nutritional requirements of the organisms in question. Chemically- defined media are of value in studying the minimal nutritional requirements of microorganisms, for enrichment cultures, and for a wide variety of physiological studies. Complex media usually provide the full range of growth factors that may be required by an organism. Therefore, it is used to cultivate unknown bacteria or bacteria whose nutritional requirements are complex (i.e., organisms that require a lot of growth factors, known or unknown).

Most pathogenic bacteria of animals, which have adapted themselves to growth in animal tissues, require complex media for their growth. Blood, serum and tissue extracts are frequently added to culture media for the cultivation of pathogens.

Other concepts employed in the construction of culture media are the principles of selection and enrichment. A **selective medium** is one which has a component(s) added to it which will inhibit or prevent the growth of certain types or species of bacteria and/ or promote the growth of desired species. One can also adjust the physical conditions of a culture medium, such as pH and temperature, to render it selective for organisms that are able to grow under these certain conditions.

A culture medium may also be a **differential medium** if allows the investigator to distinguish between different types of bacteria based on some observable trait in their pattern of growth on the medium. Thus a **selective, differential medium** for the isolation of *Staphylococcus aureus,* the most common bacterial pathogen of humans, contains a very high concentration of salt (which the staph will tolerate) that inhibits most other bacteria, mannitol as a source of fermentable sugar, and a pH indicator dye. From clinical specimens, only staph will grow. *S. aureus* is differentiated from *S. epidermidis* (a nonpathogenic component of the normal flora) on the basis of its ability to ferment mannitol. Mannitol- fermenting colonies *(S. aureus*) produce acid which reacts with the indicator dye forming a colored halo around the colonies; mannitol non-fermenters (*S. epidermidis*) use other non- fermentative substrates in the medium for growth and do not form a halo around their colonies.

On the other hand, an **enrichment medium** (Tables 17 & 19 above) contains some component that permits the growth of specific types or species of bacteria, usually because they alone can utilize the component from their environment. However, an enrichment medium may have selective features. An enrichment medium for nonsymbiotic nitrogen- fixing bacteria omits a source of nitrogen from the medium. The medium is inoculated with a potential source of these bacteria (e.g. a soil sample) and incubated in the atmosphere wherein the only source of nitrogen available is N_2. A selective enrichment medium for growth of the extreme halophile

(*Halococcus*) contains nearly 25 percent salt (NaCl), which is required by the extreme halophile and which inhibits the growth of all other procaryotes.

Physical and nvironmental requirements for growth

The procaryotes exist in nature under an enormous range of physical conditions such as O_2 concentration, Hydrogen ion concentration (pH) and temperature.Each group of bacteria has three ranges for every factor (i. e. optimum, minimum and maximum). Therefore, each group will have its optimum growth in different ranges from the other groups. According to this, thermophiles grow at high temperatures, acidophiles grow at low pH, osmophiles grow at high solute concentration, and so on.

The Effect of Oxygen

Oxygen is a universal component of cells and is always provided in large amounts by H_2O. However, procaryotes display a wide range of responses to molecular oxygen O_2.

Obligate aerobes require O_2 for growth; they use O_2 as a final electron acceptor in aerobic respiration.

Obligate anaerobes do not need or use O_2 as a nutrient. In fact, O_2 is a toxic substance, which either kills or inhibits their growth. Obligate anaerobic procaryotes may live by fermentation, anaerobic respiration, bacterial photosynthesis, or the novel process of methanogenesis.

Facultative anaerobes (or **facultative aerobes**) are organisms that can switch between aerobic and anaerobic types of metabolism. Under anaerobic conditions (no O_2) they grow by fermentation or anaerobic respiration, but in the presence of O_2 they switch to aerobic respiration.

Aerotolerant anaerobes are bacteria with an exclusively anaerobic (fermentative) type of metabolism but they are insensitive to the presence of O_2. They live by fermentation alone whether or not O_2 is present in their environment.

Terminology for O_2 relations of microorganisms

	Environment		
Group	**Aerobic**	**Anaerobic**	**O_2 Effect**
Obligate Aerobe	Growth	No growth	Required (utilized for aerobic respiration)
Microaerophile	Growth if level not too high	No growth	Required but at levels below 0.2 atm
Obligate Anaerobe	No growth	Growth Toxic	
Facultative Anaerobe (Facultative Aerobe)	Growth	Growth	Not required for growth but utilized when available
Aerotolerant Anaerobe	Growth	Growth	Not required and not utilized

The response of an organism to O_2 in its environment depends upon the occurrence and distribution of various enzymes which react with O_2 and various oxygen radicals that are invariably generated by cells in the presence of O_2. All cells contain enzymes capable of reacting with O_2. For example, oxidations of flavoproteins by O_2 invariably result in the formation of H_2O_2 (peroxide) as one major product and small quantities of an even more toxic free radical, superoxide or O_2. Also, chlorophyll and other pigments in cells can react with O_2 in the presence of light and generate singlet oxygen, another radical form of oxygen which is a potent oxidizing agent in biological systems.

In aerobes and aerotolerant anaerobes the potential for lethal accumulation of superoxide is prevented by the enzyme superoxide dismutase. All organisms which can live in the presence of O_2 (whether or not they utilize it in their metabolism) contain superoxide dismutase. Nearly all organisms contain the enzyme catalase, which decomposes H_2O_2. Even though certain aerotolerant bacteria such as the lactic acid bacteria lack catalase, they decompose H_2O_2 by means of peroxidase enzymes which derive electrons from $NADH_2$ to reduce peroxide to H_2O. Obligate anaerobes lack superoxide dismutase and catalase and/ or peroxidase, and therefore undergo lethal oxidations by various oxygen radicals when they are exposed to O_2.

All photosynthetic (and some nonphotosynthetic) organisms are protected from lethal oxidations of singlet oxygen by their possession of carotenoid pigments which physically react with the singlet oxygen radical and lower it to its nontoxic "ground" (triplet) state. Carotenoids are said to "quench" singlet oxygen radicals.

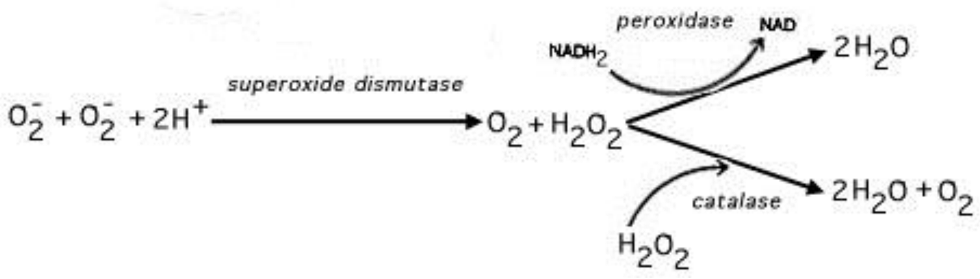


Figure (30): The action of superoxide dismutase, catalase and peroxidase: These enzymes detoxify oxygen radicals that are inevitably generated by living systems in the presence of O_2.

Table (21): Distribution of superoxide dismutase, catalase and peroxidase in procaryotes with different O_2 tolerances

Group	Superoxide dismutase	Catalase	Peroxidase
Obligate aerobes and most facultative anaerobes (e.g. Enterics)	+	+	-
Most aerotolerant anaerobes (e.g. Streptococci)	+	-	+
Obligate anaerobes (e.g. Clostridia, Methanogens, Bacteroides)	-	-	-

The Effect of pH

The pH, or hydrogen ion concentration, [H^+], of natural environments varies from about 0.5 in the most acidic soils to about 10.5 in the most alkaline lakes. The range of pH over which an organism grows is defined by **three cardinal points**: the **minimum pH**, below which the organism cannot grow, the **maximum pH**, above which the organism cannot grow, and the **optimum pH**, at which the organism grows best. For most bacteria there is an orderly increase in growth rate between the minimum and the optimum and a corresponding orderly decrease in growth rate between the optimum and the maximum pH, reflecting the general effect of changing [H^+] on the rates of enzymatic reaction.

Microorganisms which grow at an optimum pH well below neutrality (7.0) are called **acidophiles**. Those which grow best at neutral pH are called **neutrophiles** and those that grow best under alkaline conditions are called **alkaliphiles**. Obligate acidophiles, such as some *Thiobacillus* species, actually require a low pH for growth since their membranes dissolve and the cells lyse at neutrality. Several genera of Archaea, including *Sulfolobus* and *Thermoplasma*, are obligate acidophiles.

In the construction and use of culture media, one must always consider the optimum pH for growth of a desired organism and incorporate **buffers** in order to maintain the pH of the medium in the changing environment of bacterial waste products that accumulate during growth. Many pathogenic bacteria exhibit a relatively narrow range of pH over which they will grow. Most diagnostic media for the growth and identification of human pathogens have a pH near 7.

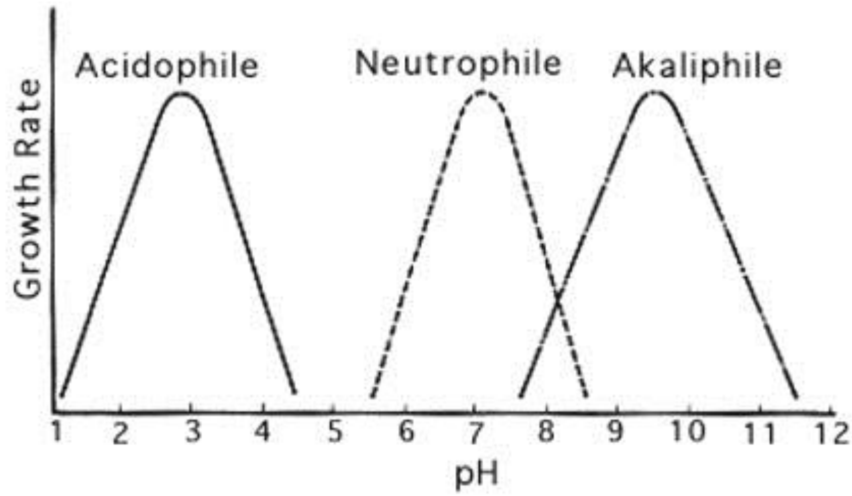


Figure (31): Growth rate vs pH for three environmental classes of prokaryotes

Table (22): Minimum, maximum and optimum pH for growth of certain procaryotes

Organism	Minimum pH	Optimum pH	Maximum pH
Thiobacillus thiooxidans	0.5	2.0-2.8	4.0-6.0
Sulfolobus acidocaldarius	1.0	2.0-3.0	5.0
Bacillus acidocaldarius	2.0	4.0	6.0
Zymomonas lindneri	3.5	5.5-6.0	7.5
Lactobacillus acidophilus	4.0-4.6	5.8-6.6	6.8
Staphylococcus aureus	4.2	7.0-7.5	9.3
Escherichia coli	4.4	6.0-7.0	9.0
Clostridium sporogenes	5.0-5.8	6.0-7.6	8.5-9.0
Erwinia caratovora	5.6	7.1	9.3
Pseudomonas aeruginosa	5.6	6.6-7.0	8.0
Thiobacillus novellus	5.7	7.0	9.0
Streptococcus pneumoniae	6.5	7.8	8.3
Nitrobacter sp	6.6	7.6-8.6	10.0

The effect of temperature

Microorganisms have been found growing in virtually all environments where there is liquid water, regardless of its temperature. Procaryotes have been detected growing at temperatures at least as high as 120 degrees. Microorganisms have been found growing at very low temperatures as well. In supercooled solutions of H_2O as low as -20 degrees, certain organisms can extract water for growth, and many forms of life flourish in the icy waters of the Antarctic, as well as household refrigerators, near zero degrees. A particular microorganism will exhibit a range of temperature over which it can grow, defined by three cardinal points in the same manner as pH. For example, organisms with an optimum temperature near 37 degrees (the body temperature of warm- blooded animals) are called **mesophiles**. Organisms with an optimum T between about 45 degrees and 70 degrees are **thermophiles**. Some Archaea with an optimum T of 80 degrees or higher and a maximum T as high as 115 degrees, are referred to as **extreme thermophiles** or **hyperthermophiles**. The cold- loving organisms are **psychrophiles** defined by their ability to grow at 0 degrees. A variant of a psychrophile (which usually has an optimum T of 10- 15 degrees) is a **psychrotroph**, which grows at 0 degrees but displays an optimum T in the mesophile range, nearer room temperature. Psychrotrophs are responsible for food spoilage in refrigerators since they are invariably brought in from their mesophilic habitats and continue to grow in the refrigerated environment. Of course, they grow slower at 2 degrees than at 25 degrees.

Psychrophilic bacteria are adapted to their cool environment by having largely unsaturated fatty acids in their plasma membranes. The degree of unsaturation of a fatty acid correlates with its solidification T or thermal transition stage (i.e., the temperature at which the lipid melts or solidifies); unsaturated fatty acids remain liquid at low T but are also denatured at moderate T. Saturated fatty acids, as in the membranes of thermophilic bacteria, are stable at high temperatures, but they also solidify at relatively high T. Thus, saturated fatty acids (like butter) are solid at room temperature while unsaturated fatty acids (like safflower oil) remain liquid in the refrigerator. Whether fatty acids in a membrane are in a liquid or a solid phase affects the fluidity of the membrane, which directly affects its ability to function. Psychrophiles also have enzymes that continue to function, albeit at a reduced rate, at temperatures at or near 0 degrees.

Thermophiles are adapted to temperatures above 60 degrees in a variety of ways. Often thermophiles have a high G + C content in their DNA such that the melting point of the DNA (the temperature at which the strands of the double helix separate) is at least as high as the organism's maximum T for growth. But this is not always the case, and the correlation is far from perfect, so thermophile DNA must be stabilized in these cells by other means. The membrane fatty acids of thermophilic bacteria are highly saturated allowing their membranes to remain stable and functional at high temperatures. The membranes of hyperthermophiles, virtually all of which are Archaea, are not composed of fatty acids but of repeating subunits of phytane, a branched, saturated, "isoprenoid" substance, which contributes heavily to the ability of these bacteria to live in superheated environments. The structural proteins (e.g. ribosomal proteins, transport proteins (permeases) and enzymes of thermophiles and hyperthermophiles are very heat stable compared with their mesophilic counterparts. The proteins are modified in a number of ways including dehydration and through slight changes in their primary structure, which accounts for their thermal stability.

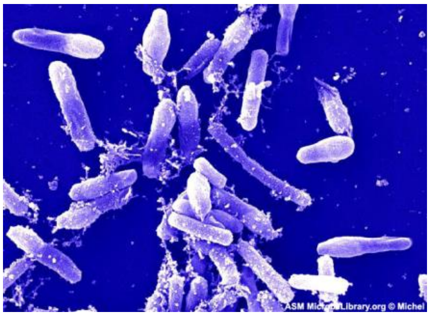


Figure (32): SEM of a thermophilic *Bacillus* sp. from compost: isolated from a compost pile at 55° C with terminal endospores in a slightly-swollen sporangium.

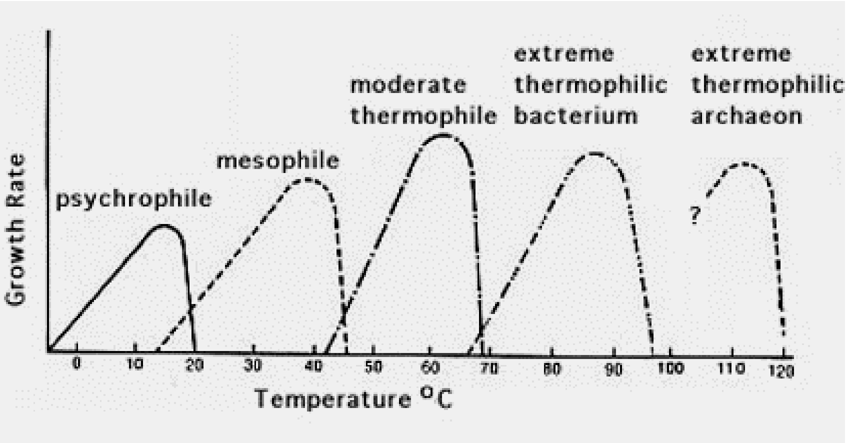


Figure (33): Growth rate vs temperature for five environmental classes of procaryotes: Most procaryotes will grow over a temperature range of about 30 degrees. The curves exhibit three cardinal points: minimum, optimum and maximum temperatures for growth.

Table (23): Temperatures for growth of different bacterial groups (degrees °C)

Group	**Minimum**	**Optimum**	**Maximum**	**Comments**
Psychrophile	Below 0	10-15	Below 20	Grow best at relatively low T
Psychrotroph	0	15-30	Above 25	Able to grow at low T but prefer moderate T
Mesophile	10-15	30-40	Below 45	Most bacteria esp. those living in association with warm-blooded animals
Thermophile*	45	50-85	Above 100 (boiling)	Among all thermophiles is wide variation in optimum and maximum T

*For "degrees" of thermophily see text and graphs above

Table (24): Required temperature ranges for growth of certain bacteria and archaea

Bacterium	**Minimum**	**Optimum**	**Maximum**
Listeria monocytogenes	1	30-37	45
Vibrio marinus	4	15	30
Pseudomonas maltophilia	4	35	41
Thiobacillus novellus	5	25-30	42
Staphylococcus aureus	10	30-37	45
Escherichia coli	10	37	45
Clostridium kluyveri	19	35	37
Streptococcus pyogenes	20	37	40
Streptococcus pneumoniae	25	37	42
Bacillus flavothermus	30	60	72
Thermus aquaticus	40	70-72	79
Methanococcus jannaschii	60	85	90
Sulfolobus acidocaldarius	70	75-85	90
Pyrobacterium brockii	80	102-105	115

Table (25): Optimum growth temperature of some procaryotes

Genus and species	**Optimal growth temp (degrees °C**
Vibrio cholerae	18-37
Photobacterium phosphoreum	20
Rhizobium leguminosarum	20
Streptomyces griseus	25
Rhodobacter sphaeroides	25-30
Pseudomonas fluorescens	25-30
Erwinia amylovora	27-30
Staphylococcus aureus	30-37
Escherichia coli	37
Mycobacterium tuberculosis	37
Pseudomonas aeruginosa	37
Streptococcus pyogenes	37

Treponema pallidum	37
Thermoplasma acidophilum	59
Thermus aquaticus	70
Bacillus caldolyticus	72
Pyrococcus furiosus	100

Table (26): Temperatures for growth of hyperthermophilic Archaea (degrees °C)

Genus	Minimum	Optimum	Maximum	Optimum pH
Sulfolobus	55	75-85	87	2-3
Desulfurococcus	60	85	93	6
Methanothermus	60	83	88	6-7
Pyrodictium	82	105	113	6
Methanopyrus	85	100	110	7

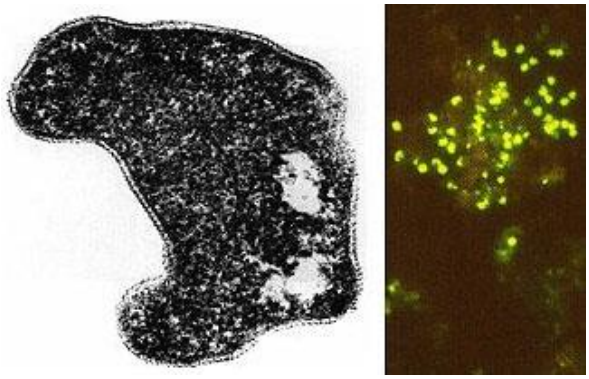


Figure (34): *Sulfolobus acidocaldarius*: an extreme thermophile and acidophile found in heated acid springs and other soils with temperatures from 60 to 95 degrees C, and a pH of 1 to 5. **Left**: irregular spheres that are often lobed (Electron micrograph of a thin section, 85,000X). **Right**: Fluorescent photomicrograph of cells attached to a sulfur crystal.

Water availability

Water is the solvent in which the molecules of life are dissolved, and the availability of water is therefore a critical factor that affects the growth of all cells. The availability of water for a cell depends upon its presence in the atmosphere (relative humidity) or its presence in solution or a substance (**water activity**). The water activity (A_w) of pure H_2O is 1.0 (100% water). Water activity is affected by the presence of solutes such as salts or sugars, dissolved in the water. The higher the solute concentration of a substance, the lower is the water activity and vise- versa. Microorganisms live over a range of A_w from 1.0 to 0.7. The A_w of human blood is 0.99; seawater = 0.98. Water activities in agricultural soils range between 0.9 and 1.0.

The only common solute in nature that occurs over a wide concentration range is salt (NaCl), and some microorganisms are named based on their growth response to salt. Microorganisms that require some NaCl for growth are **halophiles**. **Mild halophiles** require 1- 6% salt, **moderate halophiles** require 6- 15% salt. **Extreme halophiles** that require 15- 30% NaCl for growth are found among the archaea. Bacteria that are able to grow at moderate salt concentrations, but they grow best in the absence of NaCl, are called **halotolerant**. Although halophiles are "osmophiles" (and halotolerant organisms are "osmotolerant") the term **osmophiles** is usually reserved for organisms that are able to live in environments high in sugar. Organisms which live in dry environments (i. e. lack of water) are called **xerophiles**.

The concept of lowering water activity in order to prevent bacterial growth is the basis for preservation of foods by drying (in sunlight or by evaporation) or by addition of high concentrations of salt or sugar.

Table (27): Limiting water activities (A_w) for growth of certain procaryotes

Organism	Minimum A_w for growth
Caulobacter	1.00
Spirillum	1.00
Pseudomonas	.91
Salmonella/E. coli	.91
Lactobacillus	.90
Bacillus	.90
Staphylococcus	.85
Halococcus	.75

MEASUREMENT OF BACTERIAL GROWTH

Growth depends upon the ability of the cell to form new protoplasm from nutrients available in the environment. In most bacteria, growth involves increase in cell mass and number of ribosomes, duplication of the bacterial chromosome, synthesis of new cell wall and plasma membrane, partitioning of the two chromosomes, septum formation, and cell division. This asexual process of reproduction is called **binary fission**. For unicellular organisms such as the bacteria, growth can be measured in terms of two different parameters: changes in **cell mass** and changes in **cell numbers**.

1- Methods for measuring cell mass

Methods for measurement of the cell mass involve both direct and indirect techniques.

1. Direct **physical measurement** of dry weight, wet weight, or volume of cells after centrifugation (packed cell volume).

2. Direct **chemical measurement** of some chemical component of the cells such as total N, total protein, or total DNA content.

3. Indirect **measurement of chemical activity** such as rate of O_2 production or consumption, CO_2 production or consumption, etc.

4. **Turbidity measurements** employ a variety of instruments to determine the amount of light scattered by a suspension of cells. Particulate objects such as bacteria scatter light in proportion to their numbers. The turbidity or **optical density** of a suspension of cells is directly related to cell mass or cell number, after construction and calibration of a standard curve. The method is simple and nondestructive, but the sensitivity is limited to about 10^7 cells per ml for most bacteria.

2- <u>Methods for Measuring Cell Numbers</u>

Measuring techniques involve direct counts, visually or instrumentally, and indirect viable cell counts.

1. **Direct microscopic counts** using special slides known as counting chambers. Dead cells cannot be distinguished from living ones. Only dense suspensions can be counted (>10^7 cells per ml), but samples can be concentrated by centrifugation or filtration to increase sensitivity. A variation of the direct microscopic count has been used to observe and measure growth of bacteria in natural environments (Figure 36).

2. **Electronic counting chambers** count numbers and measure size distribution of cells. For cells the size of bacteria the suspening medium must be very clean. Such elecronic devices are more often used to count eukaryotic cells such as blood cells.

A typical counting chamber (haemocytometer), The instrument, seen from one side *at* (a), consists of a rectangular glass block in which the central plateau lies precisely 0.1 mm below the level of the shoulder's on either side.

The central plateau is separated from each shoulder by a trough, and is itself divided into two parts by a shallow trough (seen at (b))- On the surface of each part of the central plateau is an etched grid (c) consisting of a square which is divided into 400 small squares, each 1/400 mm^2. A glass cover-slip is positioned as shown at (b) and is pressed firmly onto the shoulders of the chamber. Proper (close) contact is indicated by the appearance of a pattern of coloured lines (Newton's rings), shown in black and white at (b).

<u>Using the chamber</u>. A small volume of a bacterial suspension is picked up in a Pasteur pipette by capillary attraction; the thread of liquid in the pipette should not be more than 10 mm.

The pipette is then placed as shown in (b), i.e. with the opening of the pipette in contact with the central plateau, and the side of the pipette against the cover-slip. With the pipette in this position, liquid is automatically drawn by capillary attr action into the space bounded by the cover-slip and part of the central plateau, the liquid should not overflow into the trough. A second sample can be examined, if required, in the other half of the counting chamber. The chamber is left for 30 minutes to allow the cells to settle, and counting is then carried out under a high power of the microscope-which is focused on the grid of the chamber. Since the volume between grid and cover-slip is accurately known, the count of cells per unit volume can be calculated.

<u>A worked example</u>. Each small square in the grid is 1/400 mm^2. As the distance between grid and cover-slip is 1/10 mm, the volume of liquid over each small square is 1/4000 mm^3-i.e. 1/4000000 ml. Suppose, for example, that on scanning all 400 small squares. 500 cells were counted, this would give an average of 500/ 400 (= 1.25) cells per small square, i.e. 1.25 cells per 1/4000000 ml. The sample therefore contains 1.25 x 4000000 cells/ml, i.e. 5×10^6 cells/ ml.

N.B, The chamber described above is the *Thoma chamber*; in a *Helber chamber* the distance between central plateau and cover-slip is 0.02 mm,

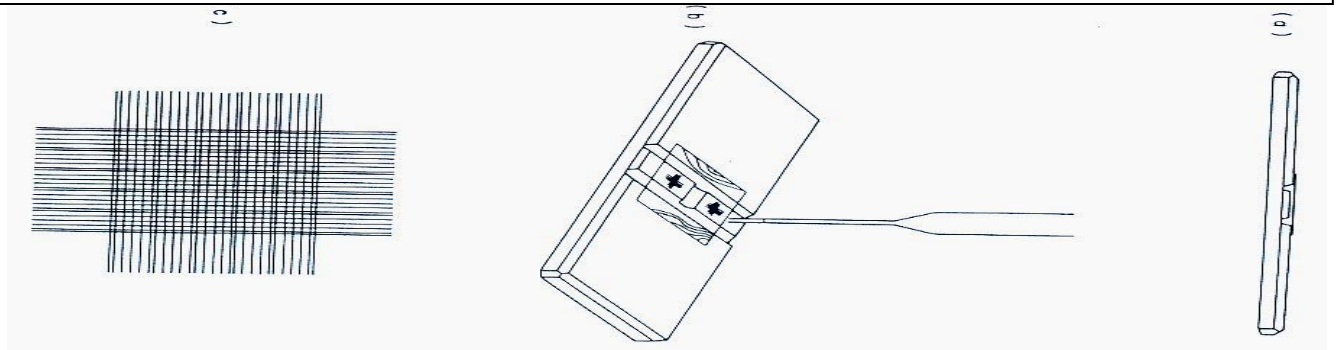


Figure (36): Counting slide or chamber (haemocytometer)

3. **Indirect viable cell counts**, also called **plate counts**, involve plating out (spreading) a sample of a culture on a nutrient agar surface. The sample or cell suspension can be diluted in a nontoxic diluent (e.g. water or saline) before plating. If plated on a suitable medium, each viable unit grows and forms a colony. Each colony that can be counted is called a **colony forming unit (CFU)** and the number of cfu's is related to the viable number of bacteria in the sample.

Advantages of the technique are its sensitivity (theoretically, a single cell can be detected), and it allows for inspection and positive identification of the organism counted. **Disadvantages** are (1) only living cells develop colonies that are counted; (2) clumps or chains of cells develop into a single colony; (3) colonies develop only from those organisms for which the cultural conditions are suitable for growth. The latter makes the technique virtually useless to characterize or count the **total number of bacteria** in complex microbial ecosystems such as soil or the animal rumen or gastrointestinal tract. Genetic probes can be used to demonstrate the diversity and relative abundance of procaryotes in such an environment, but many species identified by genetic techniques have so far proven unculturable.

Table (28): Some methods used to measure bacterial growth

Method	Application	Comments
Direct microscopic count	Enumeration of bacteria in milk or cellular vaccines	Cannot distinguish living from nonliving cells
Viable cell count (colony counts)	Enumeration of bacteria in milk, foods, soil, water, laboratory culturs, etc.	Very sensitive if plating Conditions are optimal
Turbidity measurement	Estimations of large numbers of bacteria in clear liquid media and broths	Fast and nondestructive, but cannot detect cell densities less than 10^7 cells
Measurement of total N or protein	Measurement of total cell yield from very dense cultures	only practical application is in the research laboratory
Measurement of Biochemical activity e.g. O2 uptake CO2 production, ATP production, etc.	Microbiological assays	Requires a fixed standard to relate chemical activity to cell mass and/or cell numbers
Measurement of dry weight or wet weight of cells or volume of cells after centrifugation	Measurement of total cell yield in cultures	probably more sensitive than total N or total protein measurements

BACTERIAL GROWTH CURVE

In the optimum conditions, division of a bacterial cell may occur every 20 minutes. The number of generations “n” gives a number of cells = 2^n as follows:

Generatio	Number of cells
0	$2^0 = 1$
1	$2^1 = 2$
2	$2^2 = 4$
3	$2^3 = 8$
n	2^n

Therefore, if this happen, the surface of the earth will be covered by a layer of about 30 cm thick of bacteria within 36 hours starting from one cell. Actually, this is not what happens because many factors will interfere. When a fresh medium is inoculated with a given number of cells, and the population growth is monitored over a period of time, plotting the data will yield a typical bacterial growth curve as follows:

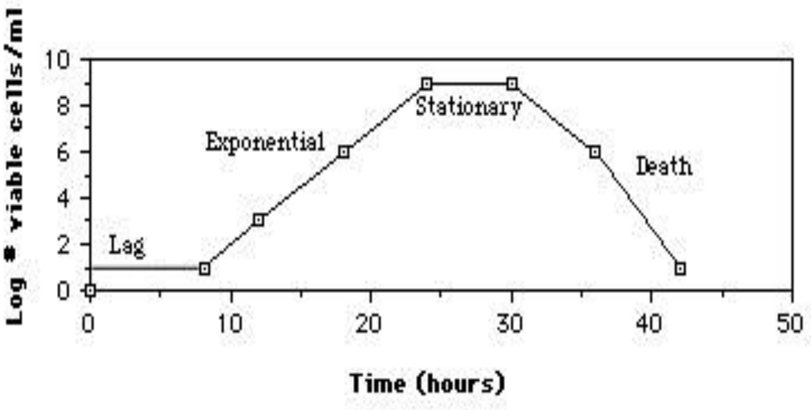


Figure (37): The typical bacterial growth curve: When bacteria are grown in a closed system (also called a batch culture), like a test tube, the population of cells almost always exhibits these growth dynamics.

Different growth phases can be distinguished from each other by the generation time:

1. **<u>Stationary or lag phase:</u>** no increase in cell number and the duration of this phase is depending on the bacterial inoculum quantity, age of inoculum, nutrient medium, incubation temperature and all the other factors that are required for adaptation. Generation time here is unlimited (because there are no new cells and no division occurs.

2. **<u>Phase of accelerated growth:</u>** cells start to divide and generation time decreases (not shown in the Figure).

3. **<u>Exponential or logarithmic phase:</u>** the generation time becomes fixed (the shortest generation time) and if we plot the relation between the log number of cells *Vs* time it would be a linear relationship.

4. **<u>Phase of declining acceleration:</u>** generation time increases while growth decreases (not shown in the Figure).

5. **<u>Maximum stationary phase:</u>** maximum number of cells and the number of dying cells is equal to the number dividing cells and generation time increases.

6. **<u>Phase of decline or death:</u>** cell enzymes start to degrade the dying cells. This does not mean that all the cells are dead, by the sudden end according to that, but few cells can continue to divide for sometime (unlimited period according to various conditions including species, nutrients, etc.).

MODES OF CELL DIVISION

(1) Division by forming a septum (fission):

a) **Binary fission** resulting in two identical cells.

b) **Asymmetrical binary fission** (non- identical).

c) **Multiple fission** (repeated binary fission resulting in bag-shaped colonies) as in cyanobacteria.

d) **Ternary fission** resulting in three cells.

(2) Budding: Outgrowth of daughter cell from mother cell like buds in higher plants.

Doubling time (generation time)

It is defined as "The time required for one complete cell cycle". In other words, it means growth in dimensions, synthesis of cell envelopes, DNA replication (chromosome), septum formation and finally, separation of daughter cell. Doubling time is species-dependent and also depends on growth conditions. Therefore, optimum growth conditions will result in minimum doubling 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.

Table (29): Generation times for some common bacteria under optimal growth conditions

Bacterium	**Medium**	**Generation Time (minu**
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 acidophilu	Milk	66-87
Rhizobium japonicum	Mannitol-salts-yeast ext	344-461
Mycobacterium tubercul	Synthetic	792-932
Treponema pallidum	Rabbit testes	1980

Batch culture

When growth from lag phase to death phase occurs in the same batch of medium it is called a “batch culture”. This includes growth under normal incubating conditions and is known as balanced growth giving rise to a normal growth curve.

Continuous culture

It is called also “continuous- flow culture” or “open culture”. This is because the bacteria is grown in an apparatus called the chemostat that is producing a continuous flow of fresh, sterile medium with simultaneous outflow of the old medium. Therefore, cells are kept in balanced growth conditions or optimum growth for an extended period of time (Figure 38).

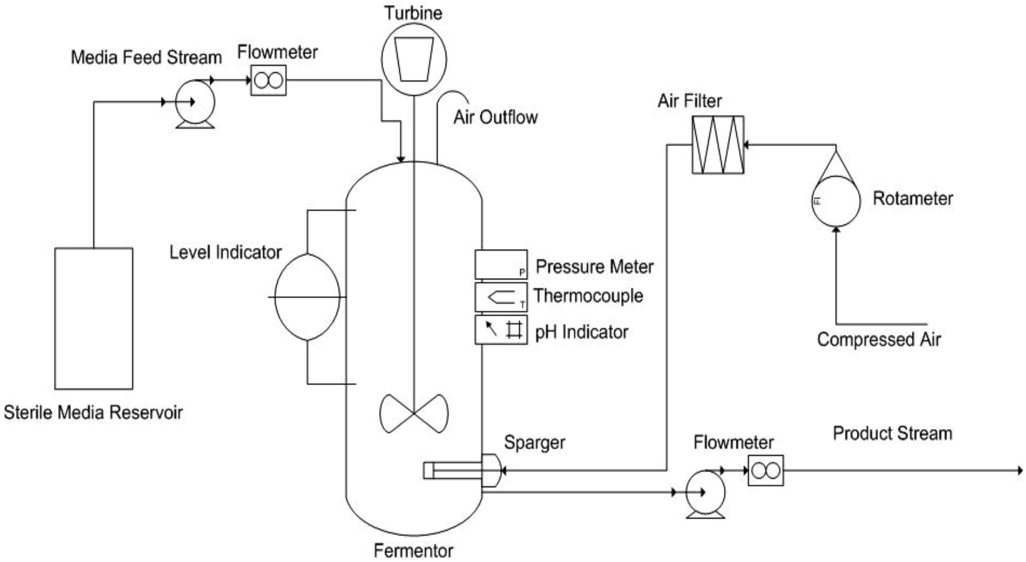


Figure (38): Schematic diagram of a chemostat or fermentor

Synchronous growth

Synchronized growth is obtaining synchronized cell division (division at the same time) approximately by mechanical or chemical ways. For example, this can be done mechanically by filtration through membrane filter that retain all the cells bigger than its pore size. The passing cells will be of the same size and, therefore, will grow and divide synchronously.

Diauxic growth

In a mixture of two different nutrients (carbon sources) cells of some species prefer to start with one of them and then, growth slows down or stops then, starts again on the second source. This advantage can be useful for culture maintaining purposes (i. e. for longer time).____________ ***End of Lecture (5)***

BACTERIAL GROWTH INHIBITION

A number of chemical substances slow the growth of microorganisms or inhibit it at all. In case of stopping or slowing down the process of growth the active substance is called "**bacteriostatic**", while if its effect is unrecoverable stopping for growth it is called "**bactericidal**".

Damage to cell growth can be carried out through the damage of biosynthesis by one of the following mechanisms:

(1) Interference with the energy source: e.g. poisons for oxidation reactions. Because oxidation liberates energy and the cell can not utilize it.

(2) Interference with precursors or intermediates: such as vitamins (can not be replaced by another substance).

(3) Interference with biosynthesis itself: such as interference with protein and nucleic acid synthesis.

(4) Damaging the cell wall or cell membrane: Therefore, all the intake, uptake and growth in cell size and mass processes will be damaged and stopped.

Inhibitors are used for controlling growth, or in general, stopping and destroying dangerous and pathogenic bacteria. This can be achieved by chemical, physical and mechanical methods.

Sterilization, therefore, is the processes that result in complete destruction and killing of all the microorganisms in the sterilized medium including endospores and viruses.

(1) Physical methods

a) Sterilization by heat.

Different species of bacteria differ in their susceptibility to heat. Spores of spore- forming bacteria also differ in their resistance to heat. Therefore, the process depends on both the temperature and time. Sterilization by heat involve the use of hot- air ovens (e.g.: for soil and glassware) and incineration of different tools such as the triangular spreader and inoculation loops.

Table (30): Methods for using heat to control bacterial growth in different materials

Treatment	**Temperature**	**Effectiveness**
Incineration	>500^{o}C	Vaporizes organic material on nonflammable surfaces but may destroy many substances in the process
Boiling	100^{o}C	30 minutes of boiling kills microbial pathogens and vegetative forms of bacteria but may not kill bacterial endospores
Intermittent boiling	100^{o}C	Three 30-minute intervals of boiling, followed by periods of cooling kills bacterial endospores
Autoclave and pressure cooker (steam under pressure)	121^{o}C/15 minutes at 15# pressure	kills all forms of life including bacterial endospores. The substance being sterilized must be maintained at the effective T for the full time
Dry heat (hot air oven)	160^{o}C/2 hours	For materials that must remain dry and which are not destroyed at T between 121^o and 170^o Good for glassware, metal, not plastic or rubber items
Dry heat (hot air oven)	170^{o}C/1 hour	Same as above. Note increasing T by 10 degrees shortens the sterilizing time by 50 percent
Pasteurization (batch method)	63^{o}C/30 minutes	kills most vegetative bacterial cells including pathogens such as streptococci, staphylococci and Mycobacterium tuberculosis
Pasteurization (flash method)	72^o/15 seconds	Effect on bacterial cells similar to batch method; for milk, this method is more conducive to industry and has fewer undesirable effects on quality or taste

b) <u>Sterilization by moist heat.</u>

This is carried out by using an instrument called the autoclave. Inside the autoclave a steam is generated from a heated water tank and the hot steam will penetrate the bottles and other vessels to kill all the vegetative and sporulated organisms. The steam pressure can be controlled through valves and also temperature. The most common conditions for autoclaving is at 121°C and 1.5 atmospheric pressure for 20 minutes. Different vessels and glassware require different sterilization times according to their volume. Sometimes this is not enough for killing some species of microorganisms and for sterilization of soil, for example. The time and temperature then can be raised and in some cases it requires three successive sterilization cycles such as for soil (Figure 39).

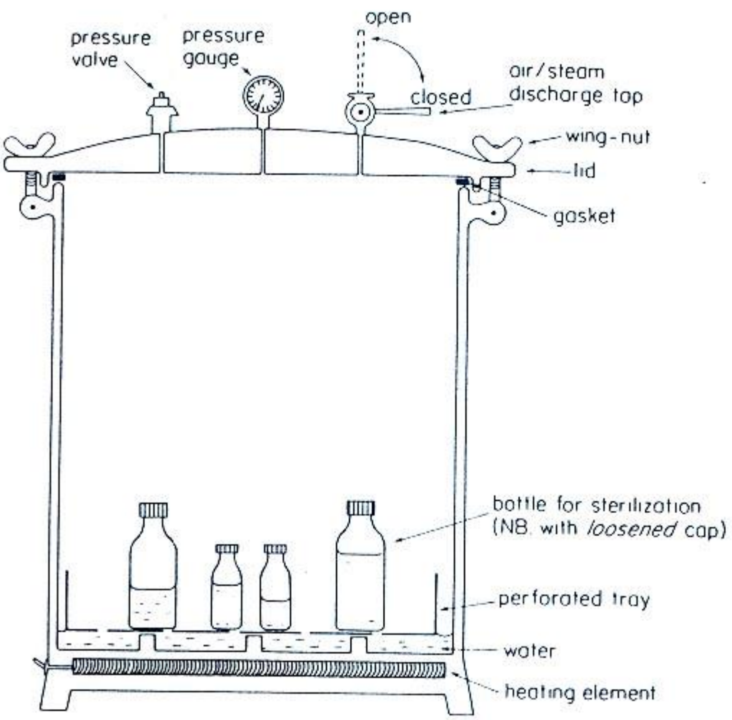


Figure (39): The autoclave

A comparison of the minimum sterilization times, by dry and moist heat, at various temperatures is represented in the following Table.

Table (31): Different temperature regimes for both dry and moist heat (autoclaving)

	Moist Heat		Dry Heat
Temperature	Time (min)	Pressure	Time (min)
121C	15	15	—
126C	10	20	—
134C	3	30	—
140C	—	—	180
150C	—	—	150
160C	—	—	120
170C	—	—	60

c) Irradiation.

Different kinds of radiation are used including X- ray, UV, and gamma rays. UV is used for surface sterilization purposes only because it can not penetrate inside surfaces. The bacterial cells that have been exposed to UV or X- rays can be reactivated. In case of UV the reactivation is carried out by visible light and the process is called "photoreactivation". The X- ray effect can be reversed in a different way. Exposing the bacteria to a sub- optimum temperature carries out the process.

(2) Mechanical methods

Heat- sensitive solutions and chemicals such as vitamins and amino acids can be sterilized by filtration. Filtration is carried out by different methods:

(a) Membrane filtration: This is the most effective filtration method for solutions because membrane filters can be obtained in different pore sizes. The most commonly used pore sizes are 0.2 and 0.45 µm. Most of the membrane filter materials are biologically inert such as cellulose acetate or cellulose nitrate.

(b) Seitz filters: These are another 0.45 µm pore- sized, asbestos filters with a stainless steel holder. The whole system is autoclaved.

(c) Sintered- glass filters: A disk of sintered glass in a funnel of different pore sizes (grades). Normally grade 5 or G5 is the recommended size for bacteriology because it has a pore size of 0.45 µm.

(3) Chemical methods (antimicrobial agents)

Many chemical compounds are used for this purpose depending on the medium that needs to be sterilized. For example ethylene oxide is used for sterilization of preserved food, pharmaceuticals and instruments. It kills vegetative cells and spores but only effective in the presence of water. Other chemicals that are used for different purposes include calcium hypochlorite, silver nitrate, osmium tetroxide and many others. Antimicrobial agents are chemicals that kill or inhibit the growth microorganisms. Antimicrobial agents include chemical preservatives and antiseptics, as well as drugs used in the treatment of infectious diseases of plants and animals. Antimicrobial agents may be of natural or synthetic origin, and they may have a static or cidal effect on microorganisms.

Types of antimicrobial agents

Antiseptics: microbicidal agents harmless enough to be applied to the skin and mucous membrane; should not be taken internally. Examples: mercurials, silver nitrate, iodine solution, alcohols, detergents.

Disinfectants: Agents that kill microorganisms, but not necessarily their spores, not safe for application to living tissues; they are used on sterilize objects such as tables, floors, utensils, etc. Examples: chlorine, hypochlorites, chlorine compounds, copper sulfate, quaternary ammonium compounds.

Note that disinfectants and antiseptics are distinguished on the basis of whether they are safe for application to mucous membranes. Often, safety depends on the concentration of the compound. For example, sodium hypochlorite (chlorine), as added to water is safe for drinking, but “chlorox” (5% hypochlorite), an excellent disinfectant, is hardly safe to drink.

Table (32): Common antiseptics and disinfectants

Chemical	**Action**	**Uses**
Ethanol (50-70%)	Denatures proteins and solubilizes lipids	Antiseptic used on skin
Isopropanol (50-70%)	Denatures proteins and solubilizes lipids	Antiseptic used on skin
Formaldehyde (8%)	Reacts with NH_2, SH and COOH groups	Disinfectant, kills endospores
Tincture of Iodine (2% I2 in 70% alcohol)	Inactivates proteins	Antiseptic used on skin
Chlorine (Cl_2) gas	Forms hypochlorous acid (HClO), a strong oxidizing agent	Disinfect drinking water; general disinfectant
Silver nitrate ($AgNO_3$)	Precipitates proteins	General antiseptic and used in the eyes of newborns
Mercuric chloride	Inactivates proteins by reacting with sulfide groups	Disinfectant, although occasionally used as an antiseptic on skin
Detergents (e.g. quaternary ammonium compounds)	Disrupts cell membranes	Skin antiseptics and disinfectants
Phenolic compounds (e.g. carboloic acid, lysol, hexylresorcinol, hexachlorophene)	Denature proteins and disrupt cell membranes	Antiseptics at low concentrations; disinfectants at high concentrations
Ethylene oxide gas	Alkylating agent	Disinfectant used to sterilize heat-sensitive objects such as rubber and plastics

Preservatives: static agents used to inhibit the growth of microorganisms, most often in foods. If eaten they should be nontoxic. Examples; calcium propionate, sodium benzoate, formaldehyde, nitrate and sulfur dioxide.

Chemotherapeutic agents: antimicrobial agents of synthetic origin useful in the treatment of microbial or viral disease. Examples: sulfonilamides and different antibiotics.

Table (33): Common food preservatives and their uses

Preservative	**Effective Concentration**	**Uses**
Propionic acid and propionates	0.32%	Antifungal agent in breads, cake, Swiss cheeses
Sorbic acid and sorbates	0.2%	Antifungal agent in cheeses, jellies, syrups, cakes
Benzoic acid and benzoates	0.1%	Antifungal agent in margarine, cider, relishes, soft drinks
Sodium diacetate	0.32%	Antifungal agent in breads
Lactic acid	unknown	Antimicrobial agent in cheeses, buttermilk, yogurt and pickled foods
Sulfur dioxide, sulfites	200-300 ppm	Antimicrobial agent in dried fruits, grapes, molasses
Sodium nitrite	200 ppm	Antibacterial agent in cured meats, fish
Sodium chloride	unknown	Prevents microbial spoilage of meats, fish, etc.
Sugar	unknown	Prevents microbial spoilage of preserves, jams, syrups, jellies, etc.
Wood smoke	unknown	Prevents microbial spoilage of meats, fish, etc.

Examples of bacterial growth inhibitors

(1) Sulfhydryl poisons:

Many enzymes depend on the group C- SH for their activity (such as in urease for which this group is important for splitting NH_3 from urea). Therefore, they are inactivated when the SH group is combined with another compound or captured by this compound. Many metals, especially heavy metals, form insoluble sulphides such as silver, mercury, copper and lead. They combine with –SH and inactivate the enzyme. An example for emphasizing the mechanism of these compounds is the action of mercury on *E. coli*. This species is grown well on ammonium lactate medium and when it is supplemented with $HgCl_2$ (8×10^{-6} M) growth is completely stopped. If the amino acid cysteine (that contains –SH group) is added growth will start again. This is because the added mercuric chloride combined with –SH groups essential for growth and the added SH groups in cysteine has again reactivated growth.

(2) <u>Surface active substances:</u>

Usually these substances are bacteriostatic and rarely, bactericidal to bacteria. They include phenols, soap and detergents, salts of some organic acids and some amines. Their action is expressed as "phenol coefficient" in comparison to the same effect of phenol. These substances reduce the surface tension of bacterial cells. Therefore, the surface will be wet and the substance will foam with some other effects in few cases. Their action may be due to adsorption to cell surface and then make cells leak their solutes into the medium (open pores in cell membranes).

(3) <u>Dyes:</u>

In general, dyes inhibit growth of bacteria but Gram positive are more resistant to their effect than Gram negative. Their action may be due to combination with cell proteins or nucleic acids. The effect is then related to the concentration of dye, the species and the used dye itself.

(4) <u>Sulfonamides:</u>

A dye was discovered to inhibit growth of both Gram negative and Gram positive bacteria. This dye's name is prontosil or sulfanilamide that converts into animal body to p- amino-benzenesulfonamide.

The most useful sulfonamides are sulfadiazine, sulfapyridine and sulfathiazole. An important component of the vitamin folic acid is p- aminobenzoic acid. The sulfonamide action is to replace p-aminobenzoic acid and, hence, the folic acid molecule is not formed.

(5) <u>Antibiotics:</u>

An antibiotic is an antimicrobial agent produced by a living microorganism that inhibit growth of another organism. Antibiotics are low molecular-weight (non-protein) molecules produced as secondary metabolites, mainly by microorganisms that live in the soil. Most of these microorganisms form some type of a spore or other dormant cell. Among the molds, the notable antibiotic producers are *Penicillium* and *Cephalosporium*, which are the main source of the beta-lactam antibiotics (penicillin and its relatives). In the Bacteria, the Actinomycetes, notably *Streptomyces* species, produce a variety of types of antibiotics including the aminoglycosides (e.g. streptomycin), macrolides (e.g. erythromycin), and the tetracyclines. Endospore- forming *Bacillus* species produce polypeptide antibiotics such as polymyxin and bacitracin. According to their action against bacterial populations they are divided into two groups:

1- Bactericidal antibiotics: Having a lethal action such as penicillin, streptomycin, cephalosporin, neomycin, and polymyxin. Erythromycin is also lethal in high concentrations.

2- Bacteriostatic antibiotics: These inhibit growth and their action is depending on the organism and the drug concentration. Examples include tetracycline and chloramphenicol. Sulfonamides are bacteriostatic also although they are not antibiotics.

The modern era of antimicrobial chemotherapy began in 1929 with Fleming's discovery of the powerful bactericidal substance penicillin, and Domagk's discovery in 1935 of synthetic chemicals (sulfonamides) with broad antimicrobial activity. In the early 1940's, spurred partially by the need for antibacterial agents in WW II, penicillin was isolated, purified and injected into experimental animals, where it was found to not only cure infections but also to possess incredibly low toxicity for the animals. An intense search for similar antimicrobial agents, of low toxicity to animals, that might prove useful in the treatment of infectious disease. The rapid isolation of streptomycin, chloramphenicol and tetracycline soon followed, and by the 1950's, these and several other antibiotics were in clinical usage.

Mode of action of antibiotics

Penicillin and other antibiotics such as bacitracin and cephalosporins interfere with the synthesis of cell wall and consequently cause bacterial lysis. Chloramphenicol interferes with protein synthesis and inhibit it. Tetracycline also inhibits protein synthesis and also interfere with oxidative phosphorylation and synthesis of nucleic acids and cell wall. Streptomycin also interferes with nucleic acid synthesis and metabolism of ribosomes. Polymyxin damages cell membrane.

Range of action of antibiotics

- Active against Gram positive bacteria: penicillin.
- Active against Gram negative bacteria: streptomycin, polymyxin and neomycin.
- Wide range (wide- spectrum) antibiotics (active against both Gram negative and positive bacteria): tetracyclines, chloramphenicol, ampicillin and cephalosporin. Sulfonamides also are classified as wide spectrum drugs according to their action. The action of these antibiotics is not very sharp with respect to Gram positive and negative bacteria. Therefore, there are some exceptions such as some Gram negative bacteria are sensitive to penicillin.

The most important property of a clinically-useful antimicrobial agent, especially from the patient's point of view, is its **selective toxicity**, i.e., that the agent acts in some way that inhibits or kills bacterial pathogens but has little or no toxic effect on the host taking the drug This implies that the biochemical processes in the bacteria are in some way different from those in the animal cells, and that the advantage of this difference can be taken in chemotherapy. The range of bacteria or other microorganisms that are affected by a certain antibiotic are is expressed as its **spectrum of action**. Antibiotics effective against procaryotes which kill or inhibit a wide range of Gram-positive and Gram-negative bacteria are said to be **broad spectrum**. If effective mainly against Gram-positive or Gram-negative bacteria, they are **narrow spectrum**. If effective against a single organism or disease, they are referred to as **limited spectrum**.

Therefore, antibiotics can be divided according to their modes of action as follows:

1- <u>Cell wall synthesis inhibitors:</u> Cell wall synthesis inhibitors generally inhibit some step in the synthesis of bacterial peptidoglycan. Generally they exert their selective toxicity against eubacteria because human cells lack cell walls.

<u>Beta lactam antibiotics</u>**:** Chemically, these antibiotics contain a 4-membered beta lactam ring. They are the products of two groups of fungi, *Penicillium* and *Cephalosporium* molds, and are correspondingly represented by the penicillins and cephalosporins.

The beta lactam antibiotics inhibit the last step in peptidoglycan synthesis, the final cross-linking between peptide side chains. Beta lactam antibiotics are normally bactericidal and require that cells be actively growing in order to exert their toxicity.

<u>Natural penicillins</u>, such as Penicillin G or Penicillin V, are produced by fermentation of *Penicillium chrysogenum*. They are effective against *Streptococcus*, *Gonococcus* and *Staphylococcus*, except where resistance has developed. They are considered narrow spectrum since they are not effective against Gram-negative rods.

<u>Semisynthetic penicillins</u> first appeared in 1959. A mold produces the main part of the molecule (6-aminopenicillanic acid) which can be modified chemically by the addition of side shains. Many of these compounds have been developed to have distinct benefits or

advantages over penicillin G, such as increased spectrum of activity (effectiveness against Gram-negative rods), resistance to penicillinase, effectiveness when administered orally, etc. Amoxycillin and Ampicillin have broadened spectra against Gram-negatives and are effective orally; Methicillin is penicillinase-resistant.

Clavulanic acid is a chemical sometimes added to a semisynthetic penicillin preparation. Thus, amoxycillin plus clavulanate is clavamox or augmentin. The clavulanate is not an antimicrobial agent. It inhibits beta lactamase enzymes and has given extended life to penicillinase-sensitive beta lactams.

Although nontoxic, penicillins occasionally cause death when administered to persons who are allergic to them. In the U.S. there are 300 – 500 deaths annually due to penicillin allergy.

Cephalolsporins are beta lactam antibiotics with a similar mode of action to penicillins that are produced by species of *Cephalosporium*. The have a low toxicity and a somewhat broader spectrum than natural penicillins. They are often used as penicillin substitutes, against Gram- negative bacteria, and in surgical prophylaxis. They are subject to degradation by some bacterial beta-lactamases, but they tend to be resistant to beta-lactamases from *S. aureus*.

Bacitracin is a polypeptide antibiotic produced by *Bacillus* species. It prevents cell wall growth by inhibiting the release of the mucopeptide subunits of peptidoglycan from the lipid carrier molecule that carries the subunit to the outside of the membrane.

Teichoic acid synthesis, which requires the same carrier, is also inhibited.

2. <u>Cell membrane inhibitors</u>: disorganize the structure or inhibit the function of bacterial membranes. The integrity of the cytoplasmic and outer membranes is vital to bacteria, and compounds that disorganize the membranes rapidly kill the cells. However, due to the similarities in phospholipids in eubacterial and eukaryotic membranes, this action is rarely specific enough to permit these compounds to be used systemically. The only antibacterial antibiotic of clinical importance that acts by this mechanism is <u>Polymyxin</u>, produced by *Bacillus polymyxis*. Polymyxin is effective mainly against Gram-negative bacteria and is usually limited to topical usage. Polymyxins bind to membrane phospholipids and thereby interfere with membrane function. Polymyxin is occasionally given for urinary tract infections caused by *Pseudomonas* that are gentamicin, carbenicillin and tobramycin resistant. The balance between effectiveness and damage to the kidney and other organs is dangerously close, and the drug should only be given under close supervision in the hospital.

3. <u>Protein synthesis inhibitors:</u> Many useful antibiotics action are due to inhibition of some step in the complex process of translation. Their attack is always at one of the events occurring on the ribosome and rather than the stage of amino acid activation or attachment to a particular tRNA. Most have an affinity or specificity for 70S (as opposed to 80S) ribosomes, and they achieve their selective toxicity in this manner. The most important antibiotics with this mode of action are the tetracyclines, chloramphenicol, the macrolides (e.g. erythromycin) and the aminoglycosides (e.g. streptomycin).

The aminoglycosides are products of *Streptomyces* species and are represented by streptomycin, kanamycin, tobramycin and gentamicin. These antibiotics exert their activity by binding to bacterial ribosomes and preventing the initiation of protein synthesis. Aminoglycosides have been used against a wide variety of bacterial infections caused by Gram-positive and Gram-negative bacteria. The tetracyclines consist of eight related antibiotics which are all natural products of *Streptomyces*, although some can now be produced semisynthetically. Tetracycline, chlortetracycline and doxycycline are the best known. The tetracyclines are broad-spectrum antibiotics with a wide range of activity against both Gram +ve and Gram –ve bacteria. The tetracyclines have a remarkably low toxicity and minimal side effects when taken by animals. The combination of their broad spectrum and low toxicity has led to their overuse and misuse by the medical community and the wide-spread development of resistance has reduced their effectiveness. Nonetheless, tetracyclines still have some important uses, such as in the treatment of Lyme disease.

Chloramphenicol has a broad spectrum of activity but it exerts a bacteriostatic effect. It is effective against intracellular parasites such as the rickettsiae. Unfortunately, aplastic anemia, which is dose related develops in a small proportion (1/50,000) of patients.

The Macrolides are a family of antibiotics whose structures contain large lactone rings linked through glycoside bonds with amino sugars. The most important members of the group are erythromycin and oleandomycin. Erythromycin is active against most Gram-positive bacteria, *Neisseria*, *Legionella* and *Haemophilus*, but not against the *Enterobacteriaceae*. Macrolides inhibit bacterial protein synthesis by binding to the 50S ribosomal subunit. Macrolides are bacteriostatic for most bacteria but are cidal for a few Gram-positive bacteria.

4. Inhibitors of nucleic acids: Some chemotherapeutic agents affect the synthesis of DNA or RNA, or can bind to DNA or RNA so that their messages cannot be read. Either case, of course, can block the growth of cells. The majorities of these drugs is unselective, however, and affect animal cells and bacterial cells alike and therefore have no therapeutic application. Two nucleic acid synthesis inhibitors which have selective activity against procaryotes and some medical utility are nalidixic acid and rifamycins. **Nalidixic acid** is a synthetic chemotherapeutic agent which has activity mainly against Gram-negative bacteria. Nalidixic acid belongs to a group of compounds called quinolones.

The rifamycins are also the products of *Streptomyces*. Rifampicin is a semisynthetic derivative of rifamycin that is active against Gram-positive bacteria (including *Mycobacterium tuberculosis*) and some Gram-negative bacteria. Rifampicin acts quite specifically on eubacterial RNA polymerase and is inactive towards RNA polymerase from animal cells or towards DNA polymerase. The antibiotic binds to the beta subunit of the polymerase and apparently blocks the entry of the first nucleotide which is necessary to activate the polymerase, thereby blocking mRNA synthesis. It has been found to have greater bactericidal effect against *M .tuberculosis* than other anti-tuberculosis drugs.

Competitive Inhibitors: The competitive inhibitors are mostly all synthetic chemotherapeutic agents. Most are "growth factor analogs" which are structurally similar to a bacterial growth factor but which do not fulfill its metabolic function in the cell. Some are bacteriostatic and some are bactericidal (sulfonamides were discussed above).

Bacterial resistance to antibiotics

Penicillin became generally available for treatment of bacterial infections, especially those caused by staphylococci and streptococci, about 1946. Initially, the antibiotic was effective against all sorts of infections caused by these two Gram-positive bacteria. Resistance to penicillin in some strains of staphylococci was recognized almost immediately. (Resistance to penicillin today occurs in as many as 80% of all strains of *Staphylococcus aureus*). Surprisingly, *Streptococcus pyogenes* have never fully developed resistance to penicillin and it remains a reasonable choice antibiotic for many types of streptococcal infections. Natural penicillins have never been effective against most Gram- negative pathogens (e.g. *Salmonella*, *Shigella*, *Bordetella pertussis*, *Yersinia pestis*, *Pseudomonas*) with the notable exception of *Neisseria gonorrhoeae*. Gram-negative bacteria are inherently resistant because their vulnerable cell wall is protected by an outer membrane that prevents permeation of the penicillin molecule.

The period of the late 1940s and early 1950s, streptomycin, chloramphenicol, and tetracycline, were dicovered and introduced as chemotherapy. These antibiotics were effective against the full array of bacterial pathogens including Gram-positive and Gram-negative bacteria, intracellular parasites, and the tuberculosis bacillus. However, by 1953, during a *Shigella* outbreak in Japan, a strain of the dysentery bacillus was isolated which was multiple drug resistant, exhibiting resistance to chloramphenicol, tetracycline, streptomycin, and the sulfanilamides. There was also evidence mounting that bacteria could pass genes for multiple drug resistance between strains and even between species. It was also apparent that *Mycobacterium tuberculosis* was capable of rapid development of resistance to streptomycin which had become a mainstay in tuberculosis therapy. By the 1960's it became apparent that some bacterial pathogens were developing resistance to antibiotic-after-antibiotic, at a rate faster than new antibiotics could be brought to market. A more conservative approach to the use of antibiotics has not been fully accepted by the medical and agricultural communities, and the problems of emerging multiple-drug resistant pathogens still emerge. The most important pathogens to emerge in multiple drug resistant forms so far have been *Mycobacterium tuberculosis* and *Staphylococcus aureus*.

The basis of resistance

The reasons of that some bacteria are resistant to some antibiotics may be summarized as follows:

a) Bacterial cells lack the target on which the antibiotic acts.

b) The production of specific enzymes by the bacterial species that inactivate these enzymes.

c) Acquired resistance through bacterial mutation.

Although large numbers of antibiotics have been developed recently, bacterial resistance to these new agents continues. This means that there is a continuous need to produce new antibiotics and a need to prevent or delay bacterial resistance by avoiding inappropriate use of antibiotics. These kinds of resistance are more explained below.

Inherent (Natural) Resistance: Bacteria may be inherently resistant to an antibiotic. For example, a streptomycete has some gene that is responsible for resistance to its own antibiotic; or a Gram- negative bacterium has an outer membrane that establishes a permeability barrier against the antibiotic; or an organism lacks a transport system for the antibiotic; or it lacks the target or reaction that is hit by the antibiotic.

Acquired Resistance: Bacteria can develop resistance to antibiotics, e.g. bacterial populations previously- sensitive to antibiotics become resistant. Acquired resistance is driven by two genetic processes in bacteria: (1) mutation and selection (sometimes referred to as vertical evolution); (2) exchange of genes between strains and species (sometimes called horizontal evolution).

Bacteria are able to exchange genes in nature by three processes: conjugation, transduction and transformation. Conjugation involves cell-to-cell contact as DNA crosses a sex pilus from donor to recipient. During transduction, a virus transfers the genes between mating bacteria. In transformation, DNA is acquired directly from the environment, having been released from another cell. Genetic recombination can follow the transfer of DNA from one cell to another leading to the emergence of a new genotype (recombinant). It is common for DNA to be transferred as plasmids between mating bacteria. The combined effects of fast growth rates, high concentrations of cells, genetic processes of mutation and selection, and the ability to exchange genes, account for the extraordinary rates of adaptation and evolution that can be observed in the bacteria. For these reasons bacterial adaptation (resistance) to the antibiotic environment seems to take place very rapidly.

Synergism and antagonism between antibiotics

Synergism is the inhibiting and inactivating action of two different antibiotics on an organism simultaneously. Antagonism is the action of an antibiotic against the action of another antibiotic. Some antibiotics act synergistically and the others antagonistically.

Antibiotic sensitivity tests

Tests are performed to determine the action of a range of antibiotics on bacteria. These tests are useful in selecting the appropriate antibiotic for specific disease (chemotherapy). The most common test is the "disc diffusion test" that is only suitable for fast– growing organisms. In this test a plate of the appropriate agar medium is inoculated with a suspension of a pure culture, of the tested bacteria, and the inoculum is spread all over the plate. Before incubating the plates a number of small absorbent paper discs, dipped in different antibiotics, are placed apart from each other in the plate. After that the antibiotics start to diffuse from each disc giving a growth- inhibition zone if that bacterium is sensitive to the antibiotic. These inhibition zones are measured and compared but the conditions should be standardized for the inoculum quantity and medium type, etc.

Figure (40): Antibiotic sensitivity test (disc diffusion method) showing the clearing zones

The lowest concentration of antimicrobial agent that inhibits the growth of the microorganism is the minimal inhibitory concentration (MIC). The MIC and the zone diameter of inhibition are inversely correlated (see Figure 41 below). In other words, the more susceptible the microorganism is to the antimicrobial agent, the lower the MIC and the larger the zone of inhibition. On the contrary, the more resistant microorganism require higher MIC and have smaller inhibition zone.

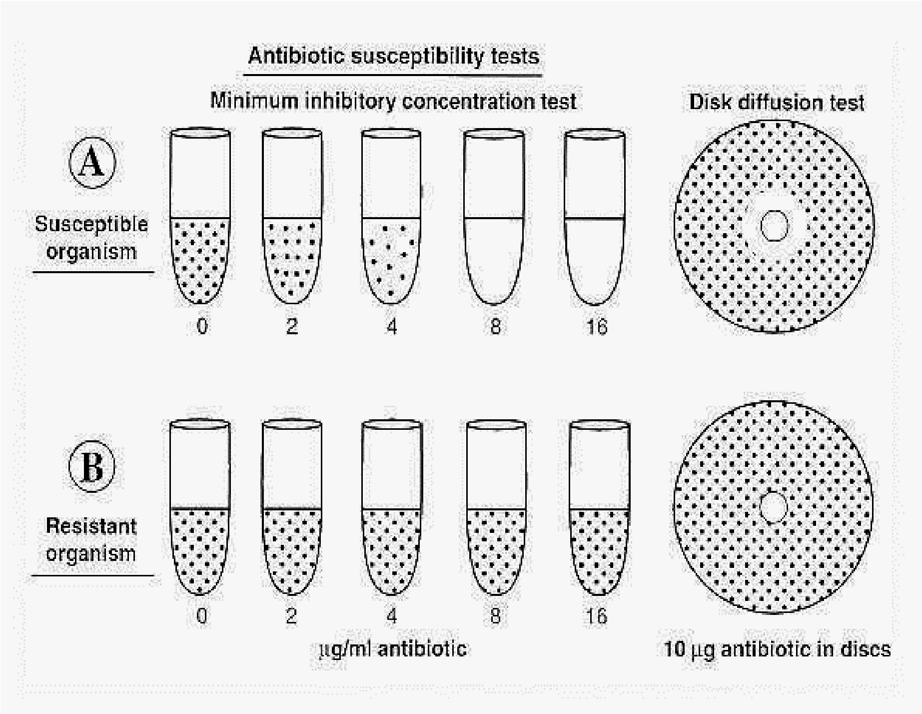


Figure (41): The minimum inhibitory concentration and disc diffusion method

____***End of Lecture (6)***

BACTERIAL METABOLISM

Introduction

Metabolism is the sum total of all the chemical transformations that occur in the cell. Metabolism includes **anabolism** (building up macromolecules) and **catabolism** (breaking down molecules). Biosynthesis (anabolism) is the portion of transformation involved in the synthesis of macromolecules from simpler molecules to build up the major portion of cell mass. An important part of metabolism of any organism involves the mobilization of chemical energy to derive biosynthesis. This is because many of the cell reactions require the activation of their reactants to an increased energy status. The common chemical form of energy used is adinosine triphosphate (ATP). Other energy- rich compounds are NAD (nicotinamide adenine dinucleotide), NADP, FAD (flavine adenine dinucleotide). Metabolic activity or reactions also involves specific proteins called **enzymes**.

Bacterial cells are made up basically of macromolecules (proteins, nucleic acids, lipids, polysaccharides, etc.). These components are always accompanied by a proportion of low molecular weight compounds that will become one of the following:

1. Part of the new generation macromolecules.
2. To catalyze macromolecular synthesis
3. Take part of the energy metabolism of the cell.

In practice all the atoms that end up in a daughter cell have to be taken up from the surrounding environment of the parental cells.

Fundamentally, most eukaryotes 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 eukaryotes.

1- Unique fermentations proceeding through the Embden-Meyerhof pathway.

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

3- Anaerobic respiration: respiration that uses substances other than 0_2 as a final electron acceptor.

4- Lithotrophy: use of inorganic substances as sources of energy.

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

6- Anoxygenic photosynthesis: photophosphorylation in the absence of O_2.

7- Methanogenesis: an ancient type of aracheon metabolism that uses H_2 as an energy source and produces methane.

8- Light-driven nonphotosynthetic photophosphorylation: unique aracheon 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 eukaryotes, the **CODH (acetyl CoA pathway)** and the **reverse TCA cycle**.

ENERGY- GENERATING METABOLISM

Metabolism energy- generating component is catabolism, and energy- consuming, biosynthetic component is 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 (42).

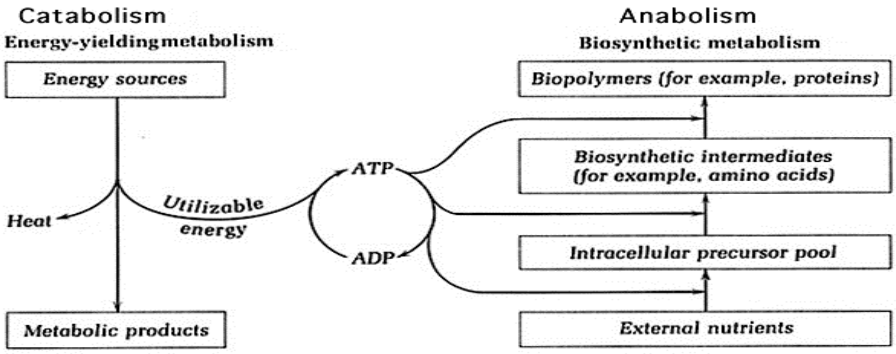


Figure (42): The relationship between catabolism and anabolism in a cell: During catabolism, energy is changed from one form to another and some energy is lost in the form of heat.

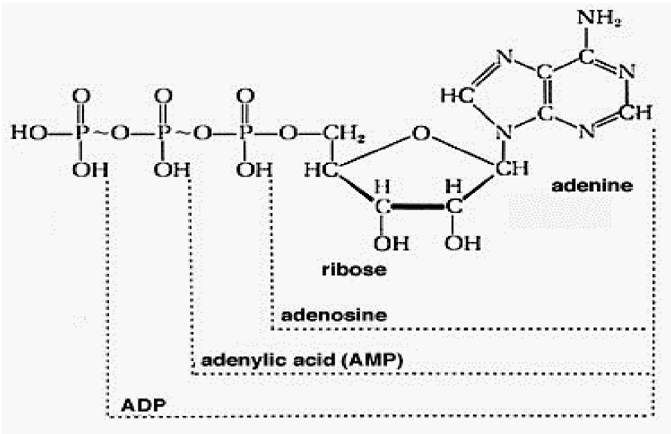


Figure (43): The structure of ATP: ATP is derived from the nucleotide adenosine monophosphate (AMP) or adenylic acid, to which two additional phosphate groups are attached through pyrophosphate bonds (~P). These two bonds are energy rich and their hydrolysis yields more energy than a covalent bond.

NAD

Another coenzyme commonly involved in energy- producing metabolism, derived from the vitamin niacin, is the pyridine nucleotide, **NAD** (**Nicotinamide Adenine Dinucleotide)**. The basis for chemical transformations of energy usually involves oxidation/ reduction reactions. For a biochemical to become oxidized, electrons must be removed by an oxidizing agent. The oxidizing agent is an electron acceptor that becomes reduced in the reaction. During the reaction, the oxidizing agent is converted to a reducing agent that can add its electrons to another chemical, thereby reducing it, and reoxidizing itself. The molecule that usually functions as the electron carrier in these types of **coupled oxidation-reduction reactions** in biological systems is NAD and its phosphorylated derivative, **NADP**. NAD or NADP can become alternately oxidized or reduced by the loss or gain of two electrons. The oxidized form of NAD is symbolized NAD; the reduced form is symbolized as NADH, $NADH_2$ or NADH + H^+.

Coenzyme A

Coenzyme A is another coenzyme frequently involved in energy-generating metabolism of procaryotes. Coenzyme A is involved in a type of ATP-generating reaction seen in some fermentative bacteria and in all respiratory organisms. The reaction occurs in association with the oxidation of keto acids such as pyruvic acid and alpha ketoglutaric acid. These substrates are central to glycolysis and the TCA cycle, respectively, and they are direct or indirect precursors of several essential macromolecules in a cell. The oxidations of pyruvate and alpha ketoglutatate, involving Coenzyme A, NAD, a dehydrogenation reaction and a decarboxylation reaction, are two of the most important, and complex, reactions in metabolism.

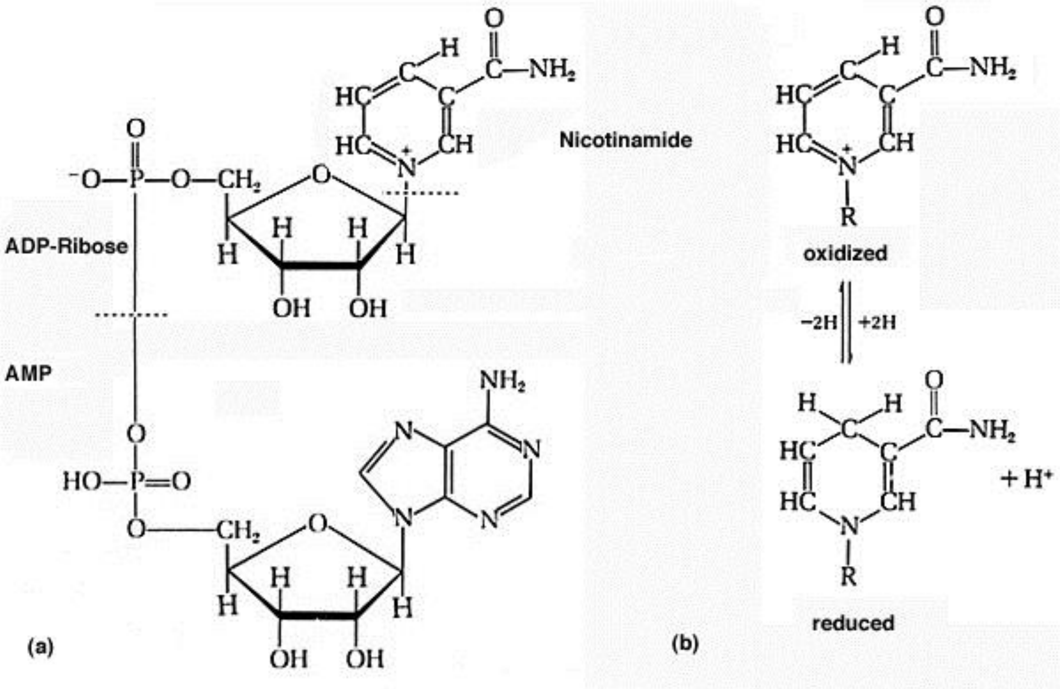


Figure (44): The Structure of NAD: (a) Nicotinamide Adenine Dinucleotide is composed of two nucleotide molecules: Adenosine monophosphate (adenine plus ribose-phosphate) and nicotinamide ribotide (nicotinamide plus ribose-phosphate). NADP has an identical structure except that it contains an additional phosphate group attached to one of the ribose residues. (b) The oxidized and reduced forms of of the nicotinamide moiety of NAD. Nicotinamide is the active part of the molecule where the reversible oxidation and reduction takes place.The oxidized form of NAD has one hydrogen atom less than the reduced form and, in addition, has a positive charge on the nitrogen atom which allows it to accept a second electron upon reduction. Thus the correct way to symbolize the reaction is NAD^+ + 2H----->NADH + H^+. However, for convenience we will hereafter use the symbols NAD and $NADH_2$.

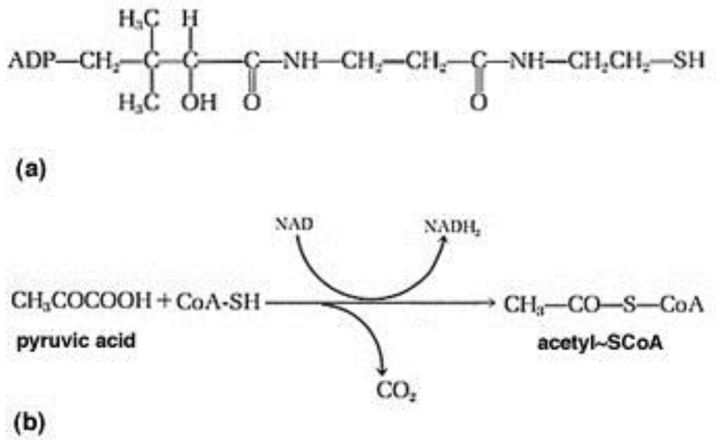


Figure (44): Coenzyme A: **(a)** The Structure of Coenzyme A. CoA-SH is a derivative of ADP. The molecule shown here attached to ADP is pantothenic acid carrying a terminal thiol (-S) group. **(b)** the oxidation of the keto acid, pyruvic acid, to acetyl~SCoA. This is the reaction that enters two carbons from pyruvate into the TCA cycle.

In the oxidation of keto acids, coenzyme A (CoA or CoASH) becomes attached through a thioester linkage (~S) to the carboxyl group of the oxidized product. Part of the energy released in the oxidation is conserved in the thioester bond. This bond energy can be subseqently used to synthesize ATP.

ATP Synthesis in procaryotes

The objective of a catabolic pathway is to make ATP: to transform either chemical energy or electromagnetic (light) energy into the chemical energy contained within the high- energy bonds of ATP. Cells fundamentally can produce ATP in two ways: **substrate level phosphorylation** and **electron transport phosphorylation**.

1- Substrate level phosphorylation (SLP): In a substrate level phosphorylation, ATP is made during the conversion of an organic molecule from one form to another. Energy released during the conversion is partially conserved during the synthesis of the high energy bond of ATP. SLP occurs during fermentations and respiration (the TCA cycle), and even during some lithotrophic transformations of inorganic substrates.

2- Electron Transport Phosphorylation (ETP): is much more complicated that evolved long after SLP. Electron Transport Phosphorylation takes place during respiration, photosynthesis, lithotrophy and possibly other types of bacterial metabolism.

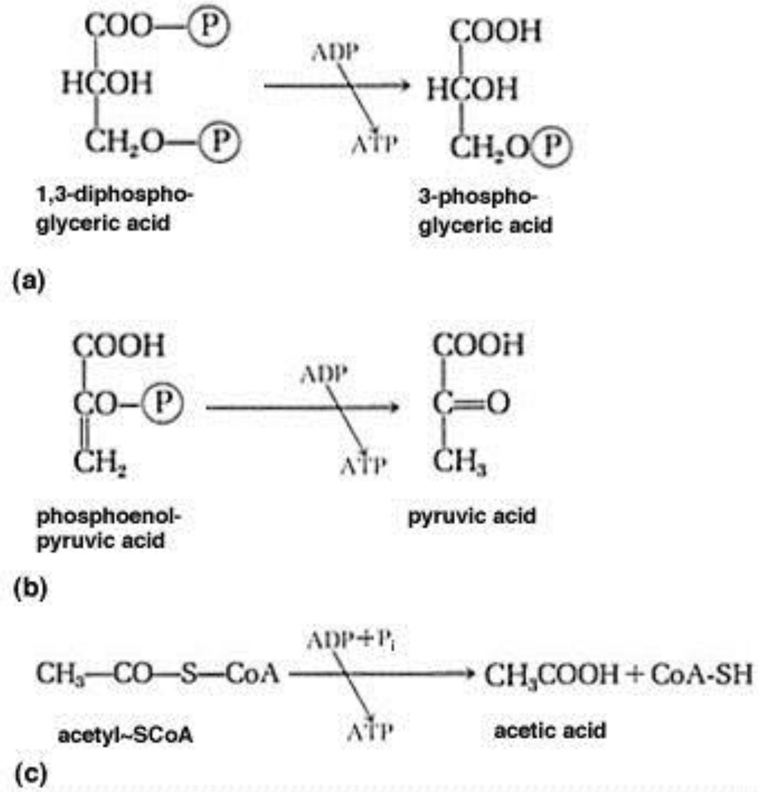


Figure (46): Three examples of substrate level phosphorylation. (a) and (b) are the two substrate level phosphorylations occur during the Embden Meyerhof pathway, but they occur in all other fermentation pathways which have an Embden Meyerhof component. (c) is a substrate level phosphorylation found in *Clostridium* and *Bifidobacterium.* These are two anaerobic (fermentative) bacteria that make one more ATP from glycolysis beyond the formation of pyruvate.

ETP requires that electrons removed from substrates be dumped into an electron transport system (ETS) contained within a membrane. The electrons are transferred through the ETS to some final electron acceptor in the membrane (like O_2 in aerobic respiration), while their traverse through the ETS results in the extrusion of protons and the establishment of a **proton motive force** (**pmf**) across the membrane. An essential component of the membrane for synthesis of ATP is a **membrane-bound ATPase** (ATP synthetase) enzyme. The ATPase enzyme transports protons, thereby utilizing the pmf (protons) during the synthesis of ATP. The idea in electron transport phosphorylation is to drive electrons through an ETS in the membrane, establish a pmf, and use the pmf to synthesize ATP. Obviously, ETP use more "machinery" than SLP, in the form of membranes, electron transport systems, ATPase enzymes, etc.

HETEROTROPHIC TYPES OF METABOLISM

Many **Bacteria** (but just a few **Archaea**) are heterotrophs, particularly those that live in associations with animals. Heterotrophic bacteria are the masters of decomposition and biodegradation in the environment. Heterotrophic metabolism is driven mainly by two metabolic processes: fermentations and respirations.

Fermentation

Fermentation is an ancient mode of metabolism, and it must have evolved with the appearance of organic material on the planet. Fermentation is metabolism in which energy is derived from the

partial oxidation of an organic compound using **organic intermediates as electron donors and electron acceptors**. No exogenous electron acceptors are involved; no membrane or electron transport system is required; all **ATP is produced by substrate level phosphorylation**.

Fermentation may be as simple as the two steps illustrated in the following model. Some amino acid fermentations by the clostridia are this simple. But the **pathways of fermentation** are more complex, usually involving several preliminary steps to prepare the energy source for oxidation and substrate level phosphorylations.

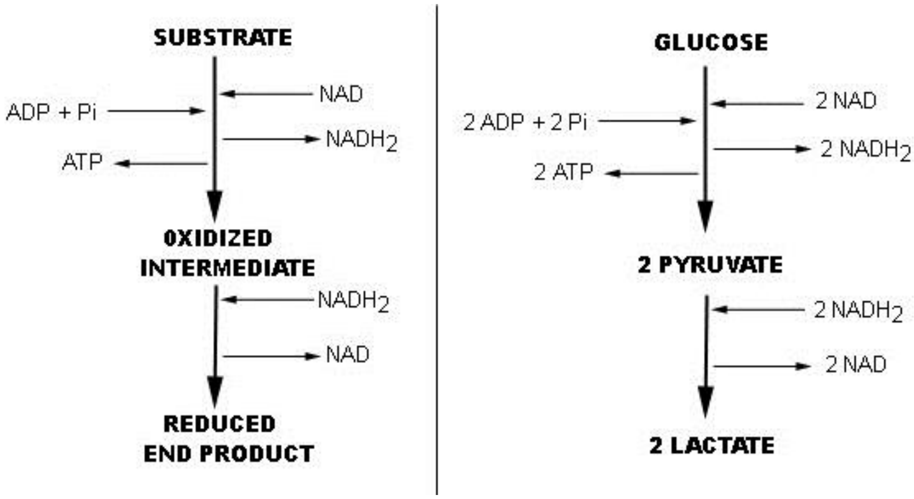


Figure (47): Model fermentation: **Left**: The substrate is oxidized to an organic intermediate; the usual oxidizing agent is NAD. Some of the energy released by the oxidation is conserved during the synthesis of ATP by the process of substrate level phosphorylation. Finally, the oxidized intermediate is reduced to end products. **Right:** In lactic fermentation by *Lactobacillus*, the substrate (glucose) is oxidized to pyruvate, and pyruvate becomes reduced to lactic acid. Redox balance is maintained by coupling oxidations to reductions within the pathway. For example, in lactic acid fermentation via the Embden-mererhof pathway, the oxidation of glyceraldehyde phosphate to phosphoglyceric acid is coupled to the reduction of pyruvic acid to lactic acid.

In biochemistry, fermentation pathways start with glucose. This is because it is the simplest molecule, requiring the fewest catalytic steps, to enter into a pathway of glycolysis and central metabolism. In procaryotes there exist three major pathways of glycolysis (the dissimilation of sugars): the classic **Embden-Meyerhof pathway**, which is also used by most eukaryotes, including yeast (*Saccharomyces*): the **phosphoketolase or heterolactic pathway** related to the hexose-pentose shunt; and the **Entner-Doudoroff pathway**. Whether or not a bacterium is a fermenter, it will likely dissimilate sugars through one or more of these pathways (See Table 1 below).

The Embden-Meyerhof Pathway

This is the pathway of glycolysis most familiar to biochemists and eukaryotic biologists, as well as to brewers, breadmakers and cheeseheads. The pathway is operated by *Saccharomyces* to produce ethanol and CO_2. The pathway is used by the homolactic acid bacteria to produce lactic acid, and it is used by many other bacteria to produce a variety of fatty acids, alcohols and gases. Some end products of Embden- Meyerhof fermentations are essential components of foods and beverages, and some are useful fuels and industrial solvents. Diagnostic microbiologists use bacterial fermentation profiles (e.g. testing an organism's ability to ferment certain sugars, or examining an organisms's array of end products) in order to identify them, down to the genus level.

The first three steps of the pathway phosphorylate and rearrange the hexose for cleavage into 2 trioses (glyceraldehyde- phosphate). **Fructose 1, 6-diphosphate aldolase** is the key (cleavage) enzyme in the EM pathway. Lactic acid bacteria reduce the pyruvate to lactic acid; yeast reduces the pyruvate to alcohol (ethanol) and CO_2 as shown in the Figure below.

The oxidation of glucose to lactate yields a total of 56 kcal per mole of glucose. Since the cells harvest 2 ATP (16 kcal) as useful energy, the efficiency of the lactate fermentation is about 29 percent (16/ 56). Ethanol fermentations have a similar efficiency.

See the Figure below (Figure 48) for a simplified glycolysis pathway.

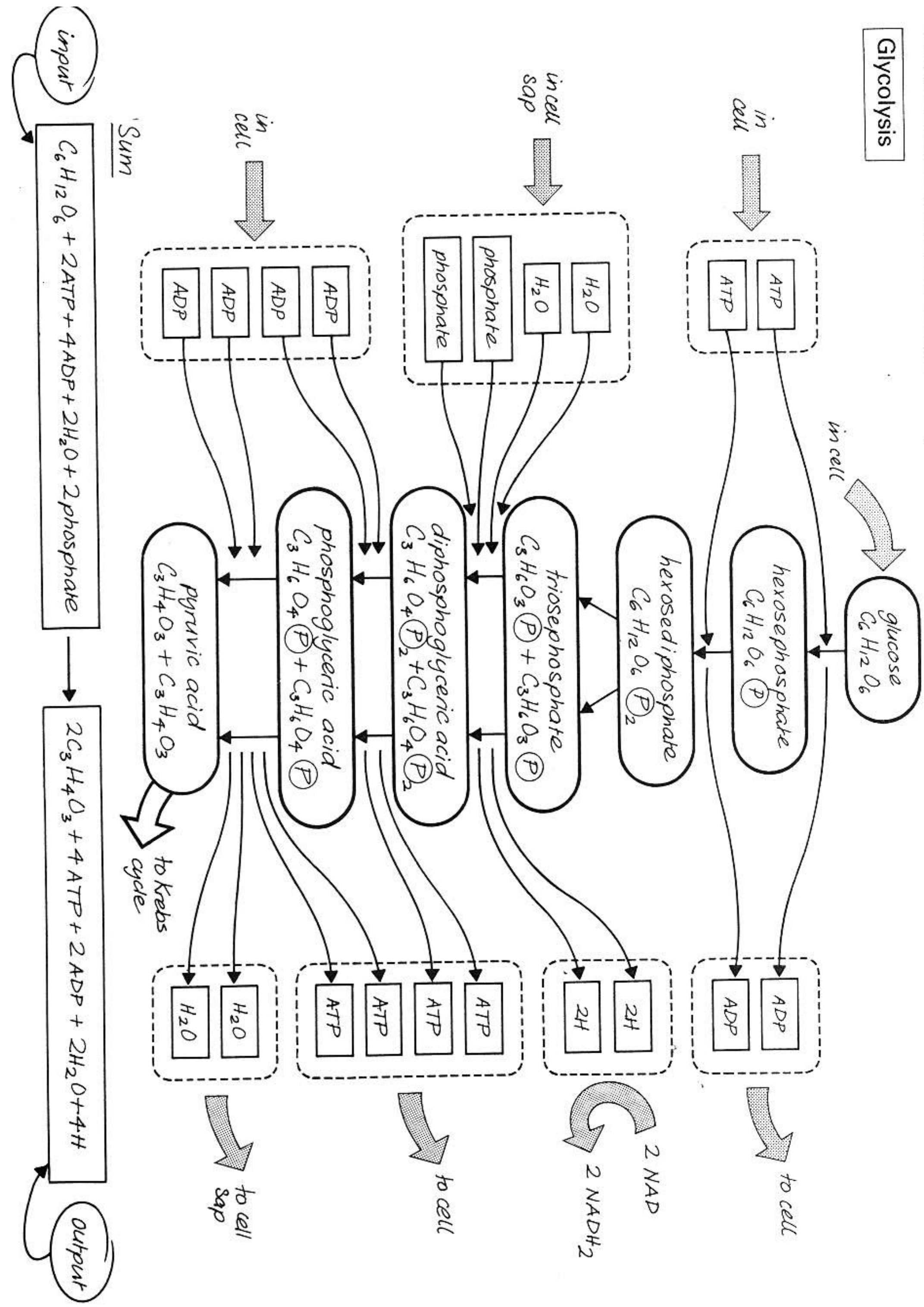


Figure (48): The Embden Meyerhof pathway (glycolysis): The overall reaction is the oxidation of glucose to 2 pyruvic acid molecules. The overall input and output of the process is illustrated.

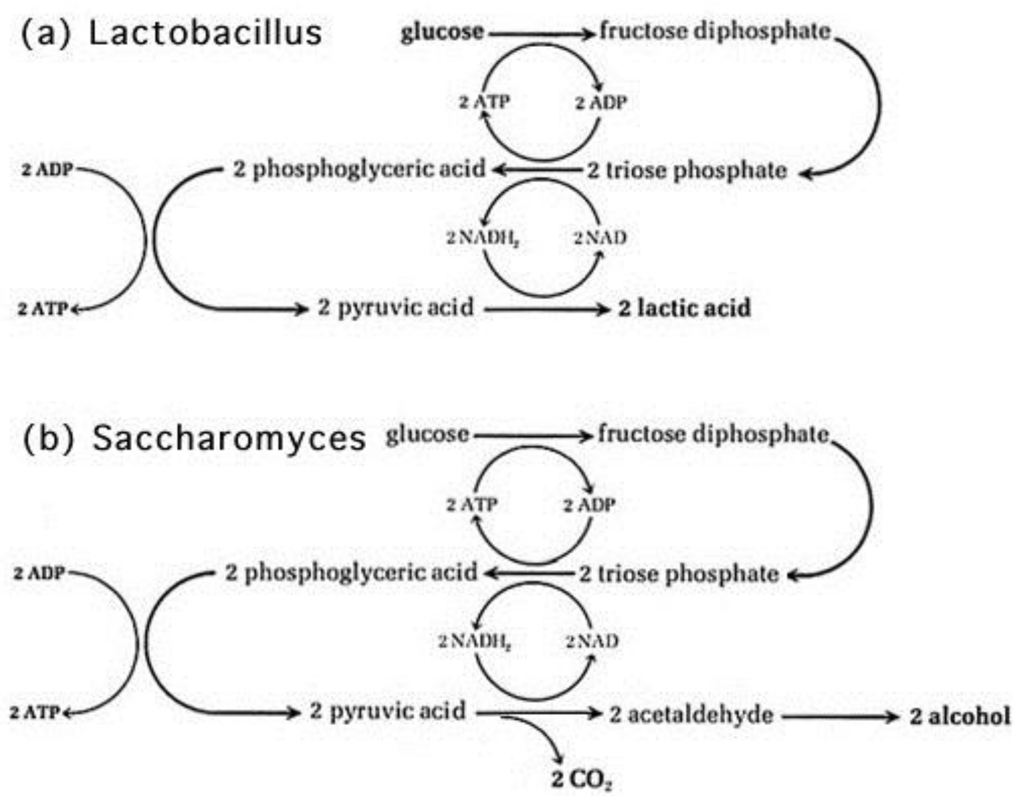


Figure (49): Homolactic and heterolactic fermentation modes: (a) The EM pathway of lactic acid fermentation in lactic acid bacteria (*Lactobacillus*) and (b) the EM pathway of alcohol fermentation in yeast (*Saccharomyces*). The pathways yield two moles of end products and two moles of ATP per mole of glucose fermented. The steps in the breakdown of glucose to pyruvate are identical. The difference is the manner of reducing pyruvic acid, giving rise to different end products.

Besides lactic acid, Embden-Meyerhof fermentations in bacteria can lead to a various end products depending on the reductive steps after the formation of pyruvic acid.

1. **Homolactic Fermentation**. Lactic acid is the sole end product. Pathway of the homolactic acid bacteria is found in *Lactobacillus* and most streptococci. The bacteria are used to ferment milk and milk products in the manufacture of yogurt, buttermilk, sour cream, cottage cheese, cheddar cheese, and most fermented dairy products.

2. **Mixed Acid Fermentations**. Mainly found in the pathway of the *Enterobacteriaceae.* End products are a mixture of **lactic acid**, **acetic acid**, **formic acid**, **succinate** and **ethanol**, with the possibility of gas formation (**CO_2** and **H_2**) if the bacterium possesses the enzyme formate dehydrogenase, which cleaves formate to the gases.

2a. **Butanediol Fermentation**. Forms mixed acids and gases as above, but, in addition, **2, 3- butanediol** from the condensation of 2 pyruvate. The use of the pathway decreases acid formation (butanediol is neutral) and causes the formation of a distinctive intermediate, **acetoin**. Water microbiologists have specific tests to detect low acid and acetoin in order to distinguish non fecal enteric bacteria (butanediol formers, such as *Klebsiella* and *Enterobacter*) from fecal enterics (mixed acid fermenters, such as *E. coli, Salmonella* and *Shigella*).

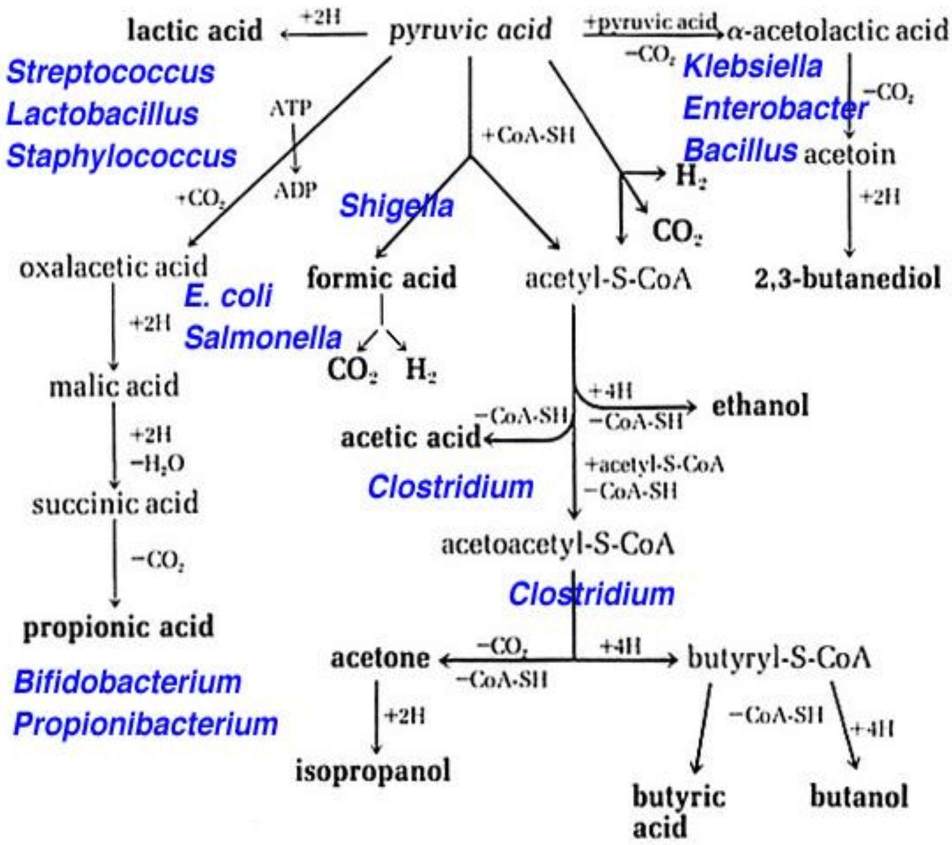
3. **Butyric acid fermentations**. Mode is similar to the butanol-acetone fermentation (below), run by the clostridia, the masters of fermentation. In addition to butyric acid, the clostridia form acetic acid, CO_2 and H_2 from the fermentation of sugars. Small amounts of ethanol and isopropanol may also be formed.

3a. **Butanol-acetone fermentation**. Butanol and acetone were discovered as the main end products of fermentation by *Clostridium acetobutylicum* during the World War I. This discovery solved a critical problem of explosives manufacture (acetone is required in the manufacture of gunpowder) and is said to have affected the outcome of the War. Acetone was distilled from the fermentation liquor of *Clostridium acetobutylicum*, because organic chemists did not figure out how to synthesize it chemically.

4. **Propionic acid fermentation**. This is an unusual fermentation carried out by the propionic acid bacteria which include corynebacteria, *Propionibacterium* and *Bifidobacterium*. Although sugars can be fermented straight through to propionate, propionic acid bacteria will ferment lactate (the end product of lactic acid fermentation) to acetic acid, CO_2 and propionic acid. The formation of propionate is a complex and indirect process involving 5 or 6 reactions. Overall, 3 moles of lactate are converted to 2 moles of propionate + 1 mole of acetate + 1mole of CO_2, and 1 mole of ATP is produced in the process. The propionic acid bacteria are used in the manufacture of Swiss cheese, which is distinguished by the distinct flavor of propionate and acetate, and holes caused by entrapment of CO_2.

The Embden- Meyerhof pathway for glucose dissimilation and the TCA cycle discussed below are two pathways that are at the center of metabolism in nearly all organisms. Not only do these pathways dissimilate organic compounds and provide energy but they also provide the precursors for biosynthesis of macromolecules that make up living systems. These are called **amphibolic pathways** since the have both an anabolic and a catabolic function.

Figure (50): Fermentations in bacteria that proceed through the Embden-Meyerhof pathway



The Heterolactic (Phosphoketolase) Pathway

The phosphoketolase pathway is distinguished by the key cleavage enzyme, **phosphoketolase**, which cleaves pentose phosphate into glyceraldehyde- 3- phosphate and acetyl phosphate. As a fermentation pathway, it is employed mainly by the **heterolactic acid bacteria**, which include some species of *Lactobacillus* and *Leuconostoc.* In this pathway, glucose- phosphate is oxidized to 6-phosphogluconic acid, which becomes oxidized and decarboxylated to form pentose phosphate. Pentose phosphate is subsequently cleaved to glyceraldehyde-3-phosphate (GAP) and acetyl phosphate. GAP is converted to lactic acid by the same enzymes as the E-M pathway. This branch of the pathway contains an oxidation coupled to a reduction while 2 ATP are produced by substrate level phosphorylation. Acetyl phosphate is reduced in two steps to ethanol, which balances the two oxidations before the cleavage but does not yield ATP. The overall reaction is Glucose ---------->1 lactic acid + 1 ethanol +1 CO_2 with a net gain of 1 ATP. The efficiency is about half that of the E-M pathway.

Heterolactic species of bacteria are occasionally used in the fermentation industry. For example, one type of fermented milk called kefir, analogous to yogurt which is produced by homolactic acid bacteria, is produced using a heterolactic *Lactobacillus* species. Likewise, sauerkraut (pickled cabbage salad) fermentations use *Leuconostoc* species of bacteria to complete the fermentation.

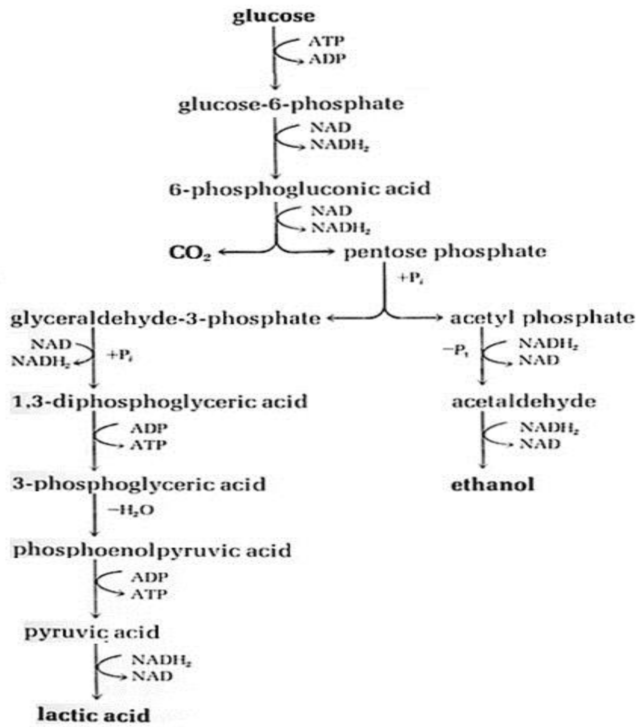


Figure (51): The heterolactic (phosphoketolase) pathway of fermentation: This pathway differs from the EM pathway in the early steps before the cleavage of the molecule.

The Entner-Doudoroff Pathway

Only a few bacteria, most notably *Zymomonas*, employ the Entner-Doudoroff pathway as a fermentation path. However, many bacteria, especially those grouped around the pseudomonads, use the pathway as a way to degrade carbohydrates for respiratory metabolism (see the Table below). The ED pathway yields 2 pyruvic acid from glucose (same as the EM pathway) but like the phosphoketolase pathway, oxidation occurs before the cleavage, and the net energy yield per mole of glucose utilized is one mole of ATP. The overall reaction is Glucose ---------->2 ethanol +2 CO2, and a net gain of 1 ATP. *Zymomonas* is a bacterium that lives on the surfaces of plants, including species of cactus which is indigenous to Mexico.

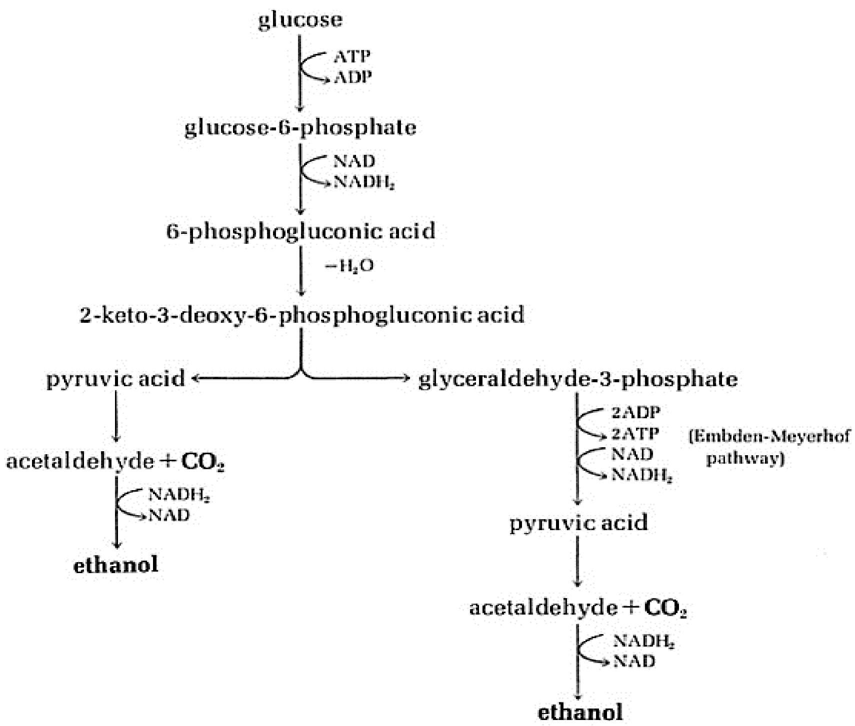


Figure (52): The Entner-Doudoroff Pathway of fermentation (ED)

Table (35): Oxidative pathways of glycolysis employed by various bacteria

Bacterium	EM pathway	Phosphoketolase pathway	ED pathway
Acetobacter aceti	-	+	-
Agrobacterium tumefaciens	-	-	+
Azotobacter vinelandii	-	-	+
Bacillus subtilis	major	minor	-
Escherichia coli	+	-	-
Lactobacillus acidophilus	+	-	-
Leuconostoc mesenteroides	-	+	-
Pseudomonas aeruginosa	-	-	+
Vibrio cholerae	minor	-	major
Zymomonas mobilis	-	-	+

Table (36): End product yields in microbial fermentations

Pathway	Key enzyme	Ethanol	Lactic Acid	CO_2	ATP
Embden-Meyerhof *Saccharomyces*	fructose 1,6-diP aldolase	2	0	2	2
Embden-Meyerhof *Lactobacillus*	fructose 1,6-diP aldolase	0	2	0	2
Heterolactic *Streptococcus*	phosphoketolase	1	1	1	1
Entner-Doudoroff *Zymomonas*	KDPG aldolase	2	0	2	1

RESPIRATION

Compared to fermentation as a means of oxidizing organic compounds, respiration is a lot more complicated. Respirations result in the **complete oxidation of the substrate** by an **outside electron acceptor**. In addition to a pathway of glycolysis, four essential structural or metabolic components are needed:

1. The **tricarboxylic acid (TCA) cycle** (also known as the citric acid cycle or the Kreb's cycle): when an organic compound is utilized as a substrate, the TCA cycle is used for the complete oxidation of the substrate. The end product that always results from the complete oxidation of an organic compound is CO_2.

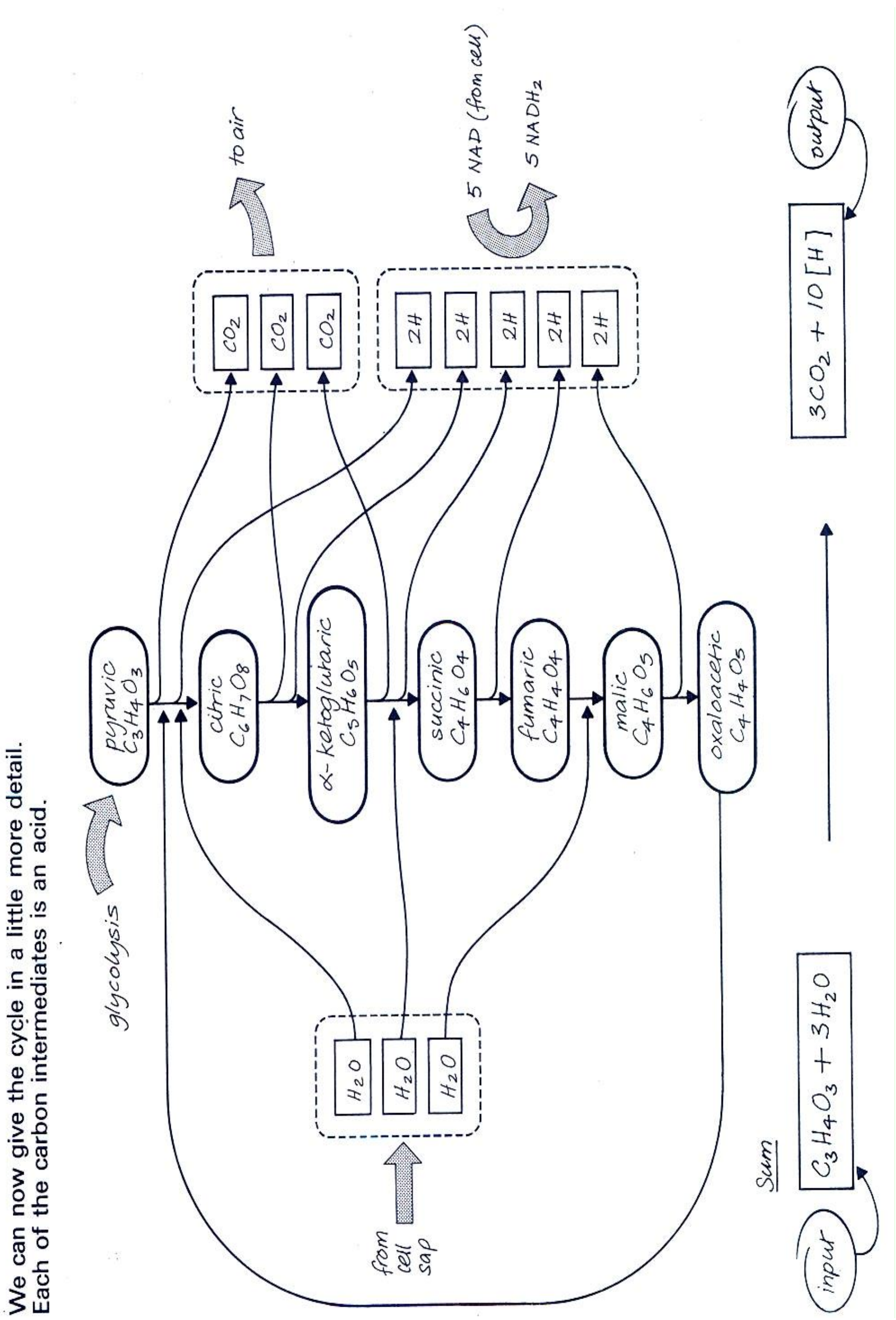


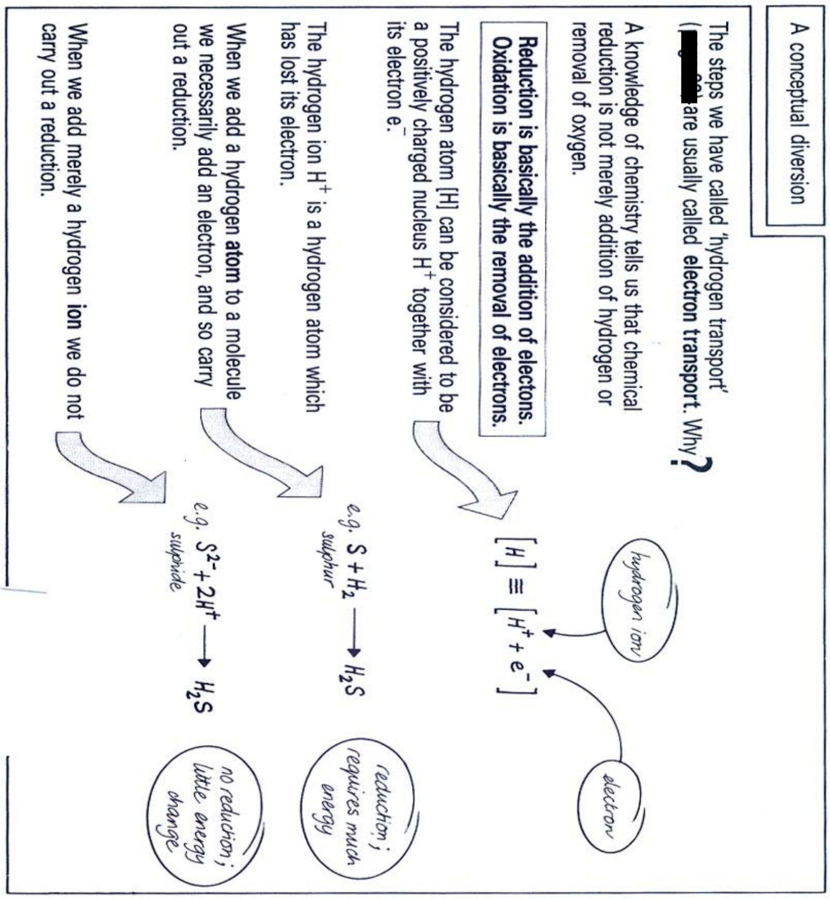
Figure (53): Krebs cycle simplified

2. A **membrane and an associated electron transport system (ETS)**. The ETS is a **sequence of electron carriers in the plasma**

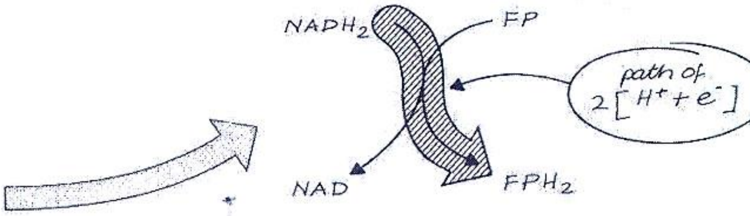
membrane that transports electrons taken from the substrate through the chain of carriers to a final electron acceptor.

3. An **exogenous electron acceptor** ("exogenous", meaning it is not internal to the pathway, as is pyruvate in a fermentation). For **aerobic respiration** the final electron acceptor is O_2. Molecular oxygen is reduced to H_20 in the last step of the electron transport system. But in the bacterial processes of **anaerobic respiration**, the final electron acceptors may be SO_4 or S or NO_3 or NO_2 or certain other inorganic compounds, or even an organic compound, such as fumarate. See the following Figures (Figure (54) for aerobic respiration.

Figure (54): A conceptual illustration of the reduction reaction



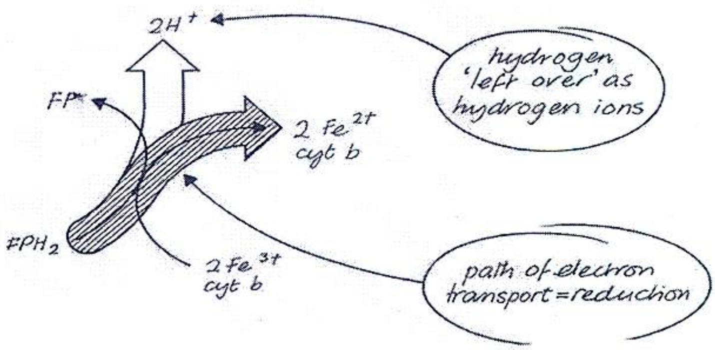
When an enzyme 'passes' the reducing power of $NADH_2$ on to flavoprotein it does so like this.



When FPH_2 has its reducing power passed on to cytochrome b **only the electrons are transported**, because cytochrome b (like all the cytochromes) contains an iron atom which can be either in the trivalent (more oxidised) state or in the divalent (more reduced) state

$$Fe^{3+} + e^- \longrightarrow Fe^{2+} \text{ reduction}$$

$$Fe^{2+} - e^- \longrightarrow Fe^{3+} \text{ oxidation}$$



This is continued in the next steps until the electrons are passed to oxygen, reducing it to water.

So the **electron transport chain** becomes:

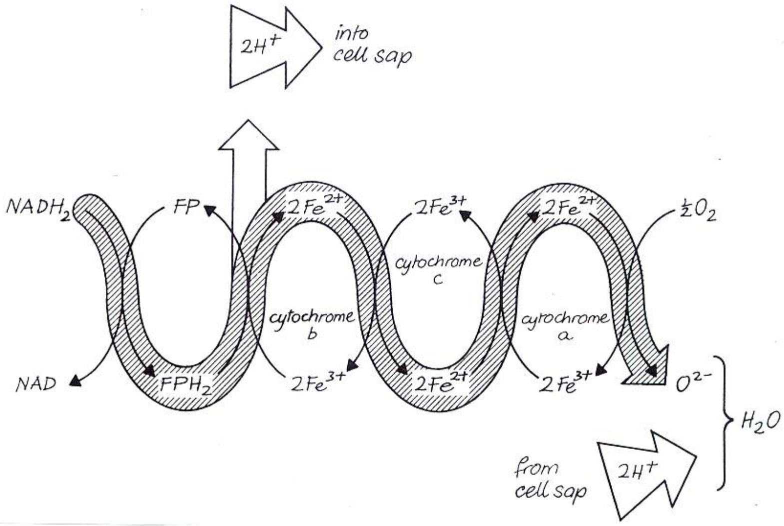


Figure (55): The electron transport system (ETS), a conceptual illustration

4. A transmembranous **ATPase enzyme** (ATP synthetase). This enzyme utilizes the proton motive force established on the membrane (by the operation of the ETS) to synthesize ATP in the process of **electron transport phosphorylation**.

Table (37): Electron acceptors for respiration and methanogenesis in procaryotes

electron acceptor	**reduced end product**	**name of process**	**organism**
O_2	H_2O	aerobic respiration	*Escherichia*, *Streptomyces*
NO_3	NO_2, N_2O or N_2	anaerobic respiration: denitrification	*Bacillus, Pseudomonas*
SO_4	S or H_2S	anaerobic respiration: sulfate reduction	*Desulfovibrio*
fumarate	succinate	anaerobic respiration: using an organic e- accept	*Escherichia*
CO_2	CH_4	methanogens	*Methanococcus*

Biological **methanogenesis** is the source of methane (natural gas) on the planet. Methane is preserved as a fossil fuel because it is produced and stored under anaerobic conditions, and oxygen is needed to oxidize the CH_4 molecule. Methanogenesis is not a form of anaerobic respiration, but it is a type of energy-generating metabolism that requires an exogenous electron acceptor in the form of CO_2. Methane is a significant greenhouse gas.

Anaerobic respiration

1- Nitrate respiration: Denitrification is an important process in agriculture because it removes NO_3 from the soil. NO_3 is a major source of nitrogen fertilizer in agriculture. Almost one- third the cost of some types of agriculture is in nitrate fertilizers. The use of nitrate as a respiratory electron acceptor is usually an alternative to the use of oxygen. Therefore, soil bacteria such as *Pseudomonas* will use O_2 as an electron acceptor if it is available, and disregard NO_3. This is the benefits of maintaining well- aerated soils by the agricultural practices of plowing and tilling. *E. coli* will utilize NO_3 (as well as fumarate) as a respiratory electron acceptor and so it may be able to continue to respire in the anaerobic intestinal habitat.

Among the products of denitrification, N_2O is of a major concern because it is a greenhouse gas with 300 -times the heat absorbing capacity of CO_2. Denitrifying bacteria, that respire using N_2O as an electron acceptor yielding N_2, therefore provide a sink for the N_2O.

2- Sulphate respiration: Sulfate reduction is not an alternative to the use of O_2 as an electron acceptor. It is an obligatory process that occurs only under anaerobic conditions. Methanogens and sulfate reducers may share habitat, especially in the anaerobic sediments of eutrophic lakes, where they evolve out methane and hydrogen sulfide at a surprising rate.

Anaerobic respiring bacteria and methanogens play an essential role in the biological cycles of carbon, nitrogen and sulfur. In general, they convert oxidized forms of the elements to a more reduced state. The lithotrophic procaryotes metabolize the reduced forms of nitrogen and sulfur to a more oxidized state in order to produce energy. The methanotrophic bacteria, which uniquely posses the enzyme methane monooxygenase, can oxidize methane as a source of energy.

LITHOTROPHIC TYPES OF METABOLISM

Lithotrophy is the use of an inorganic compound as a source of energy. Most lithotrophic bacteria are aerobic respirers that produce energy in the same manner as all aerobic respiring organisms: they remove electrons from a substrate and put them through an electron transport system that will produce ATP by electron transport phosphorylation. Lithotrophs just happen to get those electrons from an inorganic, rather than an organic compound.

Some lithotrophs are **facultative lithotrophs**, meaning they are able to use organic compounds, as well, as sources of energy. Other lithotrophs do not use organic compounds as sources of energy; in fact, they would not transport organic compounds. CO_2 is the sole source of carbon for the methanogens and the nitrifying bacteria and a few other species in other groups. These **lithoautotrophs** are often referred to as "chemoautotrophs", but the term **lithoautotroph** is a more accurate description of their metabolism. The lithotrophs are a very diverse group of procaryotes, united only by their ability to oxidize an inorganic compound as an energy source.

Lithotrophy runs through the **Bacteria** and the **Archaea**. If one considers methanogen oxidation of H_2 a form of lithotrophy, then probably most of the **Archaea** are lithotrophs. Lithotrophs are usually organized into "physiological groups" based on their inorganic substrate for energy production and growth (see the Table below).

Table (38): Physiological groups of lithotrophs

physiological group	**energy source**	**oxidized end product**	**organism**
hydrogen bacteria	H_2	H_2O	*Alcaligenes*, *Pseudomonas*
methanogens	H_2	H_2O	*Methanobacterium*
carboxydobacteria	CO	CO_2	*Rhodospirillum*, *Azotobacter*
nitrifying bacteria*	NH_3	NO_2	*Nitrosomonas*
nitrifying bacteria*	NO_2	NO_3	*Nitrobacter*
sulfur oxidizers	H_2S or S	SO_4	*Thiobacillus*, *Sulfolobus*
iron bacteria	Fe^{++}	Fe^{+++}	*Gallionella*, *Thiobacillus*

*** The overall process of nitrification, conversion of NH_3 to NO_3, requires a consortium of microorganisms.**

The **hydrogen bacteria** oxidize H_2 (hydrogen gas) as an energy source. The hydrogen bacteria are **facultative lithotrophs** as evidenced by the pseudomonads that possess a hydrogenase enzyme that will oxidize H_2 and put the electrons into their respiratory ETS. They will use H_2 if they find it in their environment even though they are typically heterotrophic. Indeed, most hydrogen bacteria are nutritionally versatile in their ability to use a wide range of carbon and energy sources.

The **methanogens** used to be considered a major group of hydrogen bacteria - until it was discovered that they are **Archaea**. The methanogens are able to oxidize H_2 as a sole source of energy while transferring the electrons from H_2 to CO_2 in its reduction to methane. Apparently, H_2 has more energy available than CH_4 and methanogens represent the most prevalent and diverse group of **Archaea**. Methanogens use H_2 and CO_2 to produce cell material and methane.

The **carboxydobacteria** are able to oxidize CO (carbon monoxide) to CO_2, using an enzyme **CODH** (**carbon monoxide dehydrogenase**). The carboxydobacteria are not obligate CO users, i.e., some are also hydrogen bacteria, and some are phototrophic bacteria.

The **nitrifying bacteria** are represented by two genera, *Nitrosomonas* and *Nitrobacter*. Together these bacteria can accomplish the oxidation of NH_3 to NO_3, known as the process of **nitrification**. No single organism can carry out the whole oxidative process. *Nitrosomonas* oxidizes ammonia to NO_2 and *Nitrobacter* oxidizes NO_2 to NO_3. Most of the nitrifying bacteria are **obligate lithoautotrophs**, the exception being a few strains of *Nitrobacter* that will utilize acetate.

Lithotrophic **sulfur oxidizers** include both **Bacteria** (e.g. *Thiobacillus*) and **Archaea** (e.g. *Sulfolobus*). Sulfur oxidizers oxidize H_2S (sulfide) or S (elemental sulfur) as a source of energy. Similarly, the purple and green sulfur bacteria oxidize H_2S or S as an electron donor for photosynthesis, and use the electrons for CO_2 fixation (the dark reaction of photosynthesis). Obligate autotrophy, which is nearly universal among the nitrifiers, is variable among the sulfur oxidizers. Lithoautotrophic sulfur oxidizers are found in environments rich in H_2S, such as volcanic hot springs and fumaroles, and deep-sea thermal vents. Some are found as symbionts and endosymbionts of higher organisms. Since they can generate energy from an inorganic compound and fix CO_2 as autotrophs, they may play a fundamental role in **primary production** in environments that lack sunlight. As a result of their lithotrophic oxidations, these organisms produce sulfuric acid (SO_4), and therefore tend to acidify their own environments. Some of the sulfur oxidizers are **acidophiles** that will grow at a pH of 1 or less. Some are **hyperthermophiles** that grow at temperatures of 115 degrees C.

Iron bacteria oxidize Fe^{++} (ferrous iron) to Fe^{+++} (ferric iron). At least two bacteria probably oxidize Fe^{++} as a source of energy and/or electrons and are capable of lithoautotrophic growth: the stalked bacterium *Gallionella*, which forms flocculant rust-colored colonies attached to objects in nature, and *Thiobacillus ferrooxidans*, which is also a sulfur-oxidizing lithotroph.

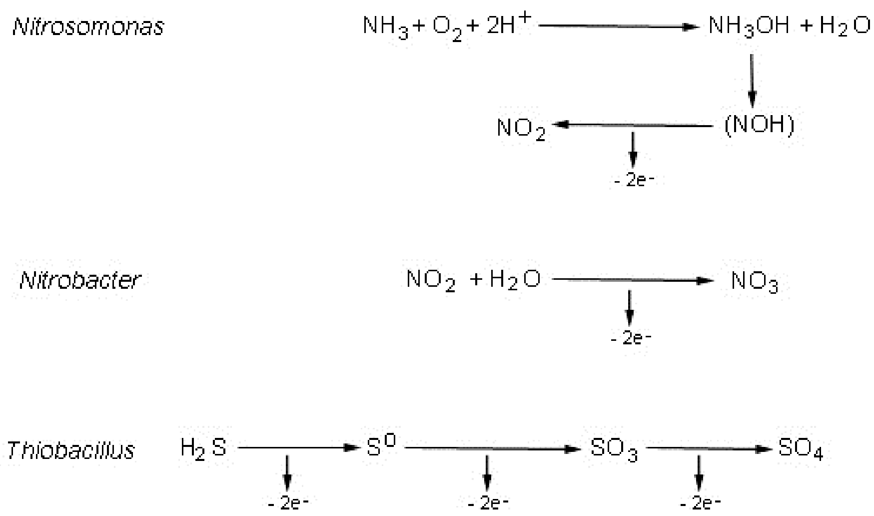


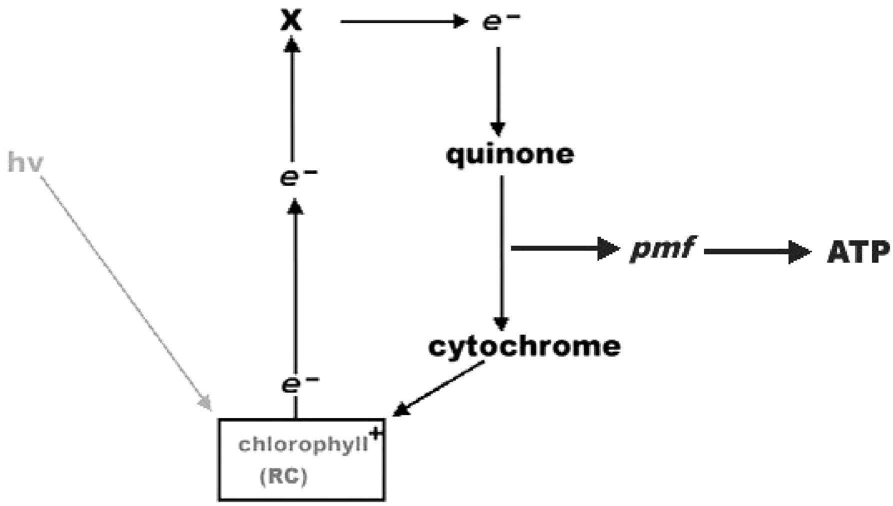
Figure (56): Lithotrophic oxidations

PHOTOTROPHIC METABOLISM

The cyanobacteria conduct plant photosynthesis, called **oxygenic photosynthesis**; the purple and green bacteria conduct bacterial photosynthesis or **anoxygenic photosynthesis**; the extreme halophilic archaea use a type of **nonphotosynthetic photophosphorylation** mediated by bacteriorhodopsin to transform light energy into ATP.

Photosynthesis is the conversion of light energy into chemical energy that can be used in the formation of cellular material from CO_2. Photosynthesis is a type of metabolism involving both catabolic and anabolic component. The catabolic component is the **light reaction,** where light energy is transformed into electrical energy, then chemical energy**.** The anabolic component involves the fixation of CO_2 and its use as a carbon source for growth, usually called the **dark reaction**. In photosynthetic procaryotes there are two types of photosynthesis and two types of CO_2 fixation.

The Light Reactions depend upon the presence of chlorophyll, the **primary light-harvesting pigment** in the membrane of photosynthetic organisms. Absorption of a quantum of light by a chlorophyll molecule causes the displacement of an electron at the reaction center. The displaced electron is an energy source that is moved through a membrane photosynthetic electron transport system, being successively passed from an iron-sulfur protein (X) to a quinone to a cytochrome and back to chlorophyll (the Figure below). As the electron is transported, a proton motive force (pmf) is established on the membrane, and ATP is synthesized by an ATPase enzyme. This manner of converting light energy into chemical energy is called **cyclic photophosphorylation**.



Photosystem I: cyclic electron flow coupled with photophosphorylation

The functional components of the photochemical system are **light harvesting pigments**, a membrane **electron transport system**, and an **ATPase** enzyme.

There are several types of pigments distributed among various phototrophic organisms. **Chlorophyll** is the primary light-harvesting pigment in all photosynthetic organisms. Cyanobacteria have **chlorophyll a**, the same as plants and algae. The chlorophylls of the purple and green bacteria, called **bacteriochlorophylls** are chemically different than chlorophyll a in their substituent side chains. This is reflected in their light absorption spectra. Chlorophyll a absorbs light in two regions of the spectrum, one around 450nm and the other between 650 -750nm; bacteriochlorophylls absorb from 800-1000nm in the far red region of the spectrum.

Carotenoids are always associated with the photosynthetic apparatus. They function as **secondary light- harvesting pigments**, absorbing light in the blue- green spectral region between 400-550 nm. Carotenoids transfer energy to chlorophyll, at near 100 percent efficiency, from wavelengths of light that are missed by chlorophyll. In addition, carotenoids have an indispensable function to protect the photosynthetic apparatus from photooxidative damage. Carotenoids reduce the powerful oxygen radical, singlet oxygen, which is invariably produced in reactions between chlorophyll and O_2 (molecular oxygen). Some nonphotosynthetic bacterial pathogens, i.e., *Staphylococcus aureus*, produce carotenoids that protect the cells from lethal oxidations by singlet oxygen in phagocytes.

Phycobiliproteins are the major light harvesting pigments of the cyanobacteria. They also occur in some groups of algae. They may be red or blue, absorbing light in the middle of the spectrum between 550 and 650nm. Phycobiliproteins consist of proteins that contain covalently-bound linear tetrapyrroles (**phycobilins**). They are contained in granules called **phycobilisomes** that are closely associated with the photosynthetic apparatus. Being closely linked to chlorophyll they can efficiently transfer light energy to chlorophyll at the reaction center.

All phototrophic bacteria are capable of performing cyclic photophosphorylation as described above This universal mechanism of cyclic photophosphorylation is referred to as **Photosystem I**. Bacterial photosynthesis uses only Photosystem I (PSI), but the more evolved cyanobacteria, as well as algae and plants, have an additional light-harvesting system called Photosystem II (PSII).

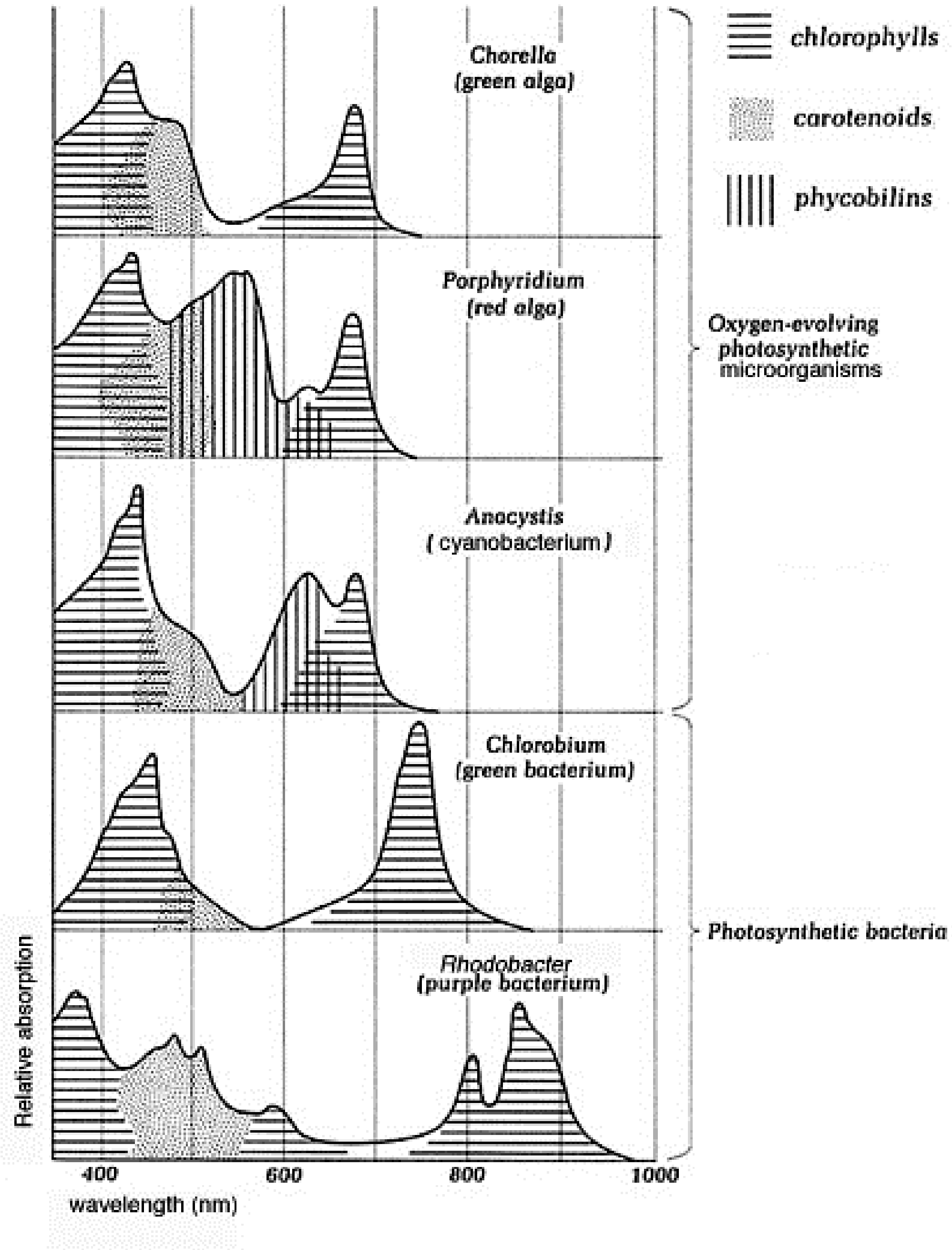


Figure (58): Distribution of photosynthetic pigments among photosynthetic microorganisms

The differences between plant and bacterial photosynthesis are summarized in Table (39) below.

Table (39): Differences between plant and bacterial photosynthesis

Process	plant photosynthesis	bacterial photosynthesis
organisms	plants, algae, cyanobacteria	purple and green bacteria
type of chlorophyll	chlorophyll a absorbs 650-750nm	bacteriochlorophyll absorbs 800-1000nm
Photosystem I **(cyclic photophosphorylation)**	present	present
Photosystem II **(noncyclic photophosphorylation)**	present	absent
Produces O_2	yes	no
Photosynthetic electron donor	H_2O	H_2S, other sulfur compounds or certain organic compounds

AUTOTROPHIC CO_2 FIXATION

The use of RUBP (ribulose biphosphate) carboxylase and the Calvin cycle is the most common mechanism for CO_2 fixation among autotrophs. Indeed, RUBP carboxylase is said to be the most abundant enzyme on the planet (nitrogenase, which fixes N_2 is second most abundant). This is the only mechanism of autotrophic CO_2 fixation among eukaryotes, and it is used, as well, by all cyanobacteria and purple bacteria. Lithoautotrophic bacteria also use this pathway. But the green bacteria and the methanogens, as well as a few isolated groups of procaryotes, have alternative mechanisms of autotrophic CO_2 fixation and do not possess RUBP carboxylase. In a complicated reaction the CO_2 is "fixed" by addition to the RUBP, which is immediately cleaved into two molecules of 3-phosphoglyceric acid (PGA). The fixed CO_2 ends up in the -COO group of one of the PGA molecules. Actually, this is the reaction that initiates the Calvin cycle.

The Calvin cycle is concerned with the conversion of PGA to intermediates in glycolysis that can be used for biosynthesis, and with the regeneration of RUBP, the substrate that drives the cycle.

After the initial fixation of CO_2, 2 PGA are reduced and combined to form hexose-phosphate by reactions which are essentially the reverse of the oxidative Embden-Meyerhof pathway. The hexose phosphate is converted to pentose-phosphate, which is phosphorylated to regenerate RUBP. An important function of the Calvin cycle is to provide the organic precursors for the biosynthesis of cell material. The fixation of CO_2 to the level of glucose ($C_6H_{12}O_6$) requires 18 ATP and 12 $NADPH_2$.

The methanogens, on the other hand, fix CO_2 by means of the enzyme **CODH** (**carbon monoxide dehydrogenase**) and the **Acetyl CoA pathway**.

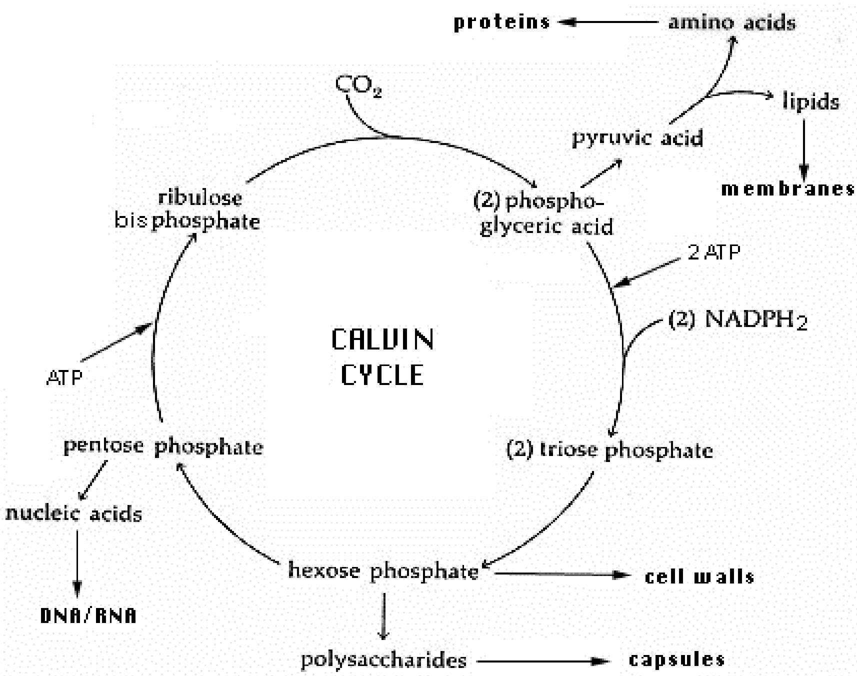


Figure (59): The Calvin cycle and its relationship to the synthesis of cell materials

BIOSYNTHESIS OF CELL MOLECULES

As previously discussed in the above parts, we can now summarize the outlines for the biosynthesis of cell molecules in the following steps:

1- Biosynthesis of low molecular weight organic molecules.

2- Biosynthesis of the macromolecules.

3- Supplying the appropriate energy form to achieve biosynthesis.

The following are some general points for biosynthesis of macromolecules:

- In these processes one of the reactants must provide the necessary energy for the polymerization step.
- The low molecular weight component of the reaction is the molecule activated to provide the energy.

- The complexity of the synthesis of macromolecules is related to the complexity of the macromolecule itself. For example in the synthesis of nucleic acids a sequential action of various enzymes are essential for the process. It is also known that these enzymes have to act under the specification of a second molecule, called a template that carries information to ensure the enzymes are acting in the correct place and order.

In the following paragraphs we will discuss in brief an example of macromolecules biosynthesis.

<u>Polysaccharides and related molecules</u>

Polysaccharides are synthesized by the sequential addition of one monosaccharide unit (X) at a time to a pre- existing polysaccharide chain. The added monosaccharide enters the reaction in an activated form usually the uridine diphosphate derivative (UDP. X) but sometimes with other nucleotides such as pyrimidine. The generalized reaction appears as follows:

…X.X.X.X + UDP-X ⟶ X.X.X.X.X + UDP

n residue   n +1 residue

In case of two monosaccharides involved in the process, the generalized reaction will be in two steps as follows:

(1) X.Y.X.Y.X.Y + UDP-X ⟶ X.Y.X.Y.X.Y.X + UDP

(2) X.Y.X.Y.X.Y.X + UD-Y ⟶ X.Y.X.Y.X.Y.X.Y + UDP

There are also other branched molecules of polysaccharides and the biosynthesis of those molecules start with the same repeated reactions but there are unclear points about where the branching will be added to the growing macromolecule.

When a pathway, such as the Embden-Meyerhof pathway or the TCA cycle, functions to provide energy in addition to chemical intermediates for the synthesis of cell material, the pathway is referred to as an **amphibolic pathway**. Pathways of glycolysis and the TCA cycle are amphibolic pathways because they provide ATP and chemical intermediates to build new cell material. The main metabolic pathways, and their relationship to biosynthesis of cell material, are shown in the Figure below.

The fundamental metabolic pathways of biosynthesis are similar in all organisms, in the same way that protein synthesis or DNA structure are similar in all organisms. Some of the main precursors for synthesis of procaryotic cell structures and components are as follows:

- **Polysaccharide capsules or inclusions** are polymers of **glucose** and **other sugars**.
- **Cell wall** peptidoglycan (NAG and NAM) is derived from **glucose phosphate**.
- **Amino acids** for the manufacture of **proteins** have various sources, the most important of which are **pyruvic acid**, **alpha ketoglutaric acid** and **oxalacetic acid**.

- **Nucleotides** (**DNA** and **RNA**) are synthesized from **ribose phosphate**. **ATP** and **NAD** are part of purine (nucleotide) metabolism.
- **Triose-phosphates** are precursors of **glycerol**, and **acetyl CoA** is a main precursor of **lipids** for **membranes.**
- **Vitamins** and **coenzymes** are synthesized in various pathways that leave central metabolism. In the example given in the Figure, **heme** synthesis proceeds from the serine pathway, as well as from succinate in the TCA cycle.

The main pathways of biosynthesis are illustrated in Figure (60).

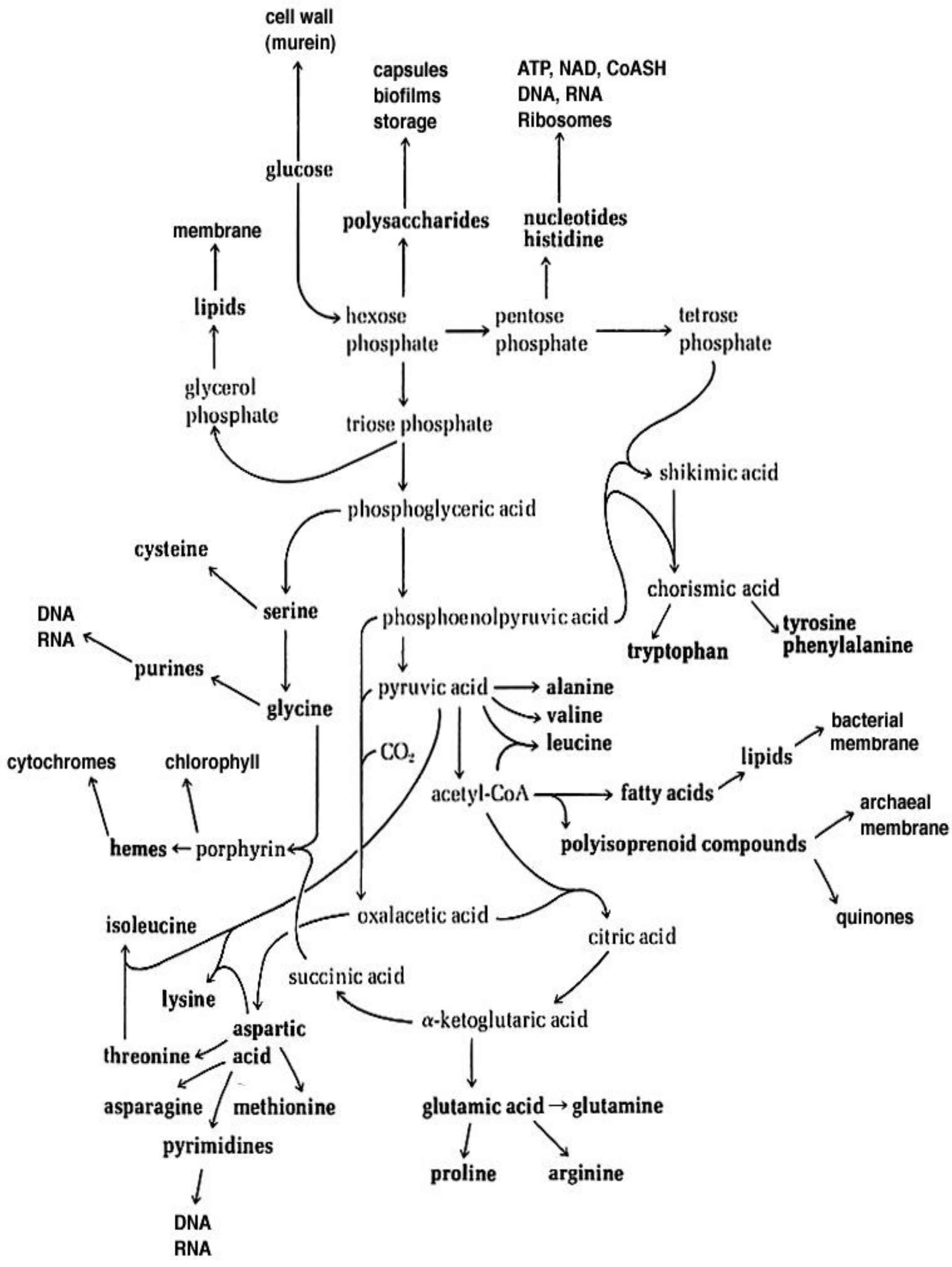


Figure (60): The main pathways of biosynthesis in procaryotic cells

____ ***End of Lecture (7)***

BACTERIAL GENETICS

Most bacteria reproduce by asexual process called **binary fission**: each cell increases in size and divides into two cells. During this process there is an increase in cellular structures and components, replication and segregation of the bacterial DNA, and formation of a **septum** or cross wall which divides the cell into two. The process is evidently coordinated by activities associated with the cell membrane. The DNA molecule is believed to be attached to a point on the membrane where it is replicated. The two DNA molecules remain attached at two points on the membrane while new membrane material is synthesized between the two points. This draws the DNA molecules in opposite directions while new cell wall and membrane are laid down as a septum between the two chromosomal compartments. When septum formation is complete the cell splits into two progeny cells. The interval time required for a bacterial cell to divide or for a population of cells to double is the **generation time**.

GENETIC EXCHANGE IN BACTERIA

Although procaryotes do not undergo sexual reproduction, they are not without the ability to exchange genes and undergo **genetic recombination**. Bacteria are known to exchange genes in nature by three fundamental processes: **conjugation**, **transduction** and **transformation**. Conjugation involves the contact between two cells and DNA crosses a sex pilus from the donor to the recipient cell. During transduction, a virus transfers the genes between mating bacteria. In transformation, DNA is acquired directly from the environment, having been released from another cell.

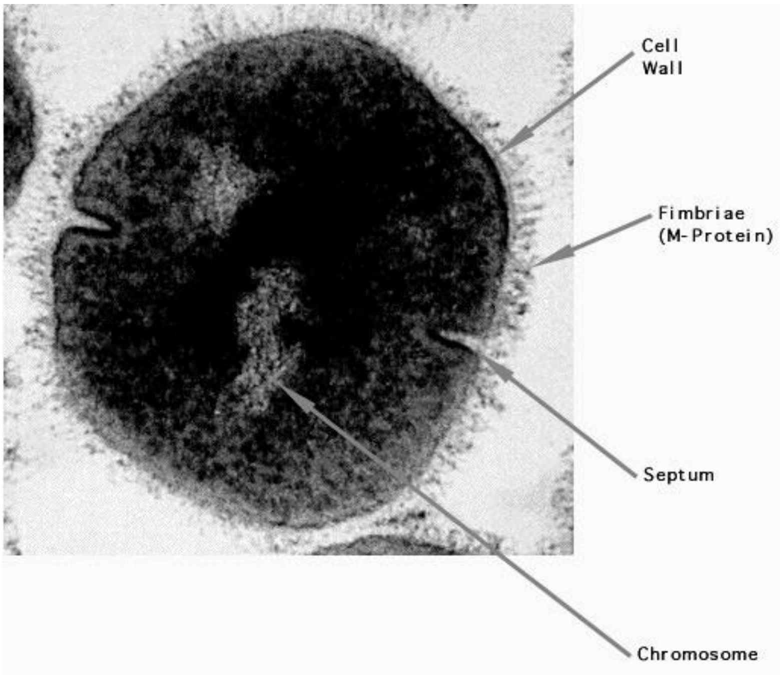


Figure (61): A pair of dividing streptococci: The chromosome has been replicated and is partially segregated as septum formation is beginning. Electron micrograph of *Streptococcus pyogenes*

Genetic recombination can follow the transfer of DNA from one cell to another leading to the emergence of a new genotype. It is common for DNA to be transferred as plasmids between mating bacteria. Since bacteria usually develop their genes for drug resistance on plasmids (called **resistance transfer factors**, or **RTFs**), they are able to spread drug resistance to other strains and species during genetic exchange processes. The genetic engineering of bacterial cells in the research or biotechnology laboratory is often based on the use of plasmids as vectors. The genetic systems of the Archaea are poorly characterized at this point, although the entire genome of *Methanosarcina* has been sequenced which opens up the possibilities for genetic analysis of the group. The following are some definitions used in genetics that you should know.

Some definitions will be discussed in the following paragraphs.

DEFINITIONS

1-Transformation

Gene transfers by soluble DNA, which has been extracted, or liberated from a donor bacterium, to a recipient bacterium.

2- Transduction

The transfer of DNA from a donor to a recipient cell by bacteriophages.

3- Conjugation

The transfer of genetic material from cell to cell by direct contact.

4- Cloning

A method for obtaining many copies of a given gene or other piece (s) of DNA.

5- The polymerase chain reaction (PCR)

A method of copying DNA in which a repeated replication of a given sequence (usually > 2kb long, or 2000 base) forms millions of copies within hours by using a PCR equipment. Nucleotide sequence is the way in which they occur in the DNA and it is the main source of information for the DNA molecule. The technique is based on the use of a DNA polymerase enzyme derived from a thermophilic bacterium, *Thermus aquaticus*.

6- Chromosomes and plasmids

The chromosome consists of the nucleic acids DNA and RNA molecules that can carry information in their sequences. Chromosomal DNA carries all the required information for both structure and behaviour of the bacterial cell (genotype and phenotype characteristics).

DNA encodes all the enzymes (for controlling structure and metabolism) and encodes various RNA molecules that are involved in the synthesis of protein and other functions. DNA also controls growth and differentiation and its own replication. This includes self- control for monitoring and repairing. All of these information is passed to the daughter cells when chromosome replicates and parent cell divides.

Many bacteria contain one or more plasmids that is an extra piece of DNA usually smaller than the chromosome and can replicate independently. The common shape of the plasmid is the circular shape but some plasmids are linear- shaped also. Plasmids encode different functions such as enzymes to inactivate specific antibiotics (R plasmids or resistance plasmids). Such plasmids usually make the host cell resistant to the relevant antibiotic. Some other plasmids encode structural elements (e.g. gas vacuoles in certain strains of *Halobacterium*). The “Cit” plasmid encodes a transport system (the uptake of citrate) in strains of *E. coli* and many others. Some plasmids encode to transfer themselves from a cell to another during bacterial conjugation as discussed above. Plasmids are widely used in the recombinant DNA technology. The recombinant

DNA is used for many applications in biotechnology such as food industries to change or alter some characteristics or the product.

7- DNA monitoring and repair

Abnormal DNA can result in some cases such as the insertion of abnormal nucleotide during replication. This abnormality may be recognized and repaired immediately through "proof reading": The enzyme DNA polymerase can cleave the wrong nucleotide from the growing strand, allowing replacement with a normal one.

8- Mutation

The bacterial mutation is a stable change in the sequence of DNA nucleotide. Mutations are mainly errors in replication and repair. The causative agents are called **mutagens**. Mutagens are mainly physical and chemical agents (such as ultraviolet, X- ray, nitrous oxide, bisulphite, etc.). In a bacterial population, mutation occurs randomly, affecting different genes in different individuals. A cell in which a mutation has occurred is called a **mutant**.

Mutation is usually harmful or may be lethal if the affected sequence of nucleotides encodes a vital product or function, but, there are also beneficial mutations such as that increasing cell resistance to antibiotics. For example, a mutation may result in an altered ribosome, thus the antibiotic (that usually binds to the ribosome) does not bind to this altered form and the result is a mutant that is resistant to the antibiotic.

End of Lecture (8)

APPLIED BACTERIOLOGY

EXPLOITATION OF BACTERIA BY HUMANS

In addition to other ecological roles, prokaryotes, especially bacteria, are used industrially in the manufacture of foods, antibiotics, drugs, vaccines, insecticides, enzymes, hormones and other useful biological products. The genetic systems of bacteria are the foundation of the biotechnology industry.

In the foods industry, lactic acid bacteria such as *Lactobacillus* and *Streptococcus* are used for the manufacture of dairy products such as yogurt, cheese, buttermilk, sour cream, and butter. Lactic acid fermentations are also used in pickling processes. Bacterial fermentations can be used to produce lactic acid, acetic acid, ethanol or acetone. In many parts of the world, various human cultures ferment indigenous plant material using *Zymomonas* bacteria to produce the regional alcoholic beverage.

In the pharmaceutical industry, bacteria are used to produce antibiotics, vaccines, and medically- useful enzymes. Most antibiotics are made by bacteria that live in soil. Actinomycetes such as *Streptomyces* produce tetracyclines, erythromycin, streptomycin, rifamycin and ivermectin. *Bacillus* species produce bacitracin and polymyxin. Bacterial products are used in the manufacture of vaccines for immunization against infectious disease. Vaccines against diphtheria, whooping cough, tetanus, typhoid fever and cholera are made from components of the bacteria that cause the respective diseases. Note that the use of antibiotics and the practice of vaccination (immunization) against infectious diseases developments that have drastically increased the quality of life and the average life expectancy of individuals in developed countries.

BIOTECHNOLOGY

The biotechnology industry uses bacterial cells for the production of human hormones such as insulin and human growth factor (protropin), and human proteins such as interferon, interleukin-2, and tumor necrosis factor. These products are used for the treatment of a variety of diseases ranging from diabetes to tuberculosis and AIDS. Other biotechnological applications of bacteria involve the genetic construction of “super strains” of organisms to perform a particular metabolic task in the environment. For example, bacteria which have been engineered genetically to degrade petroleum products can be used in cleanup of oil spills in seas and oceans. One area of biotechnology involves improvement of the qualities of plants through genetic engineering. Genes can be introduced into plants by a bacterium *Agrobacterium tumefaciens*. Using *A. tumefaciens*, plants have been genetically engineered so that they are resistant to certain pests, herbicides, and diseases. Finally, the polymerase chain reaction (PCR), is now representing a core of the biotechnology industry because it allows scientists to duplicate genes starting with a single molecule of DNA. The following are examples on some industrial and biotechnological processes of bacteria.

I. FOOD INDUSTRY

Bacteria are used for fermentations in dairy industry, processing of raw material for the manufacture of coffee and cocoa, manufacture of food additives, vinegar production and the production of food for farm animals.

Dairy products

Products from fermented milk are well known very long time ago. Such fermentations are related with the area where there are large number of lactating animals, cows, goats and sheep, and Europe is the major area of production.

Fermented dairy products account for about 10% of all fermented food production. The main organisms responsible for these fermentations are lactic acid bacteria. In the past days, these fermentations were accidentally occurring by the natural presence of lactic acid bacteria. In the present time, an inoculum (of pure cultured bacteria) is added to the milk for obtaining the best final product.

The main benefits gained from the use of lactic acid bacteria are:

1- They inhibit many undesirable bacteria while they are harmless bacteria. Therefore, they preserve milk in this way.

2- They create the required texture and flavour in the fermented milk.

3- They have beneficial health effects on intestinal microflora.

Lactic acid bacteria inoculated in milk will break down milk sugar (lactose) to lactic acid. Different end products may occur according to the composition of the substrate, types of additives and mode of fermentation. Therefore, different products can be obtained from fermented milk such as buttermilk, yoghurt and many different kinds of cheeses.

The most important industry from milk is the production of **cheeses**. There are over 900 types of cheeses produced by various manufacturers. The main idea in producing cheese is to separate the milk protein (casein) from the liquid (whey). The kind of cheese depends on the fermentation mode and the starter or the microorganism used for fermentation.

The early cheese production processes arisen from the use of animal stomachs (sheep) in which the milk is heated and soured by naturally occurring bacteria and contaminated with enzymes "*rennet*" from the stomach lining. This results in the transformation of milk into solid curds and liquid whey. Current production of cheese is essentially a dehydration process in which the milk protein (casein) and fats are concentrated 6- 12- fold. The basic process steps for cheese production are:

1. Acidification of the milk by the conversion of sugar lactose into lactic acid by the lactic acid bacteria.
2. Coagulation of the casein by a combination of proteolysis and acidification.

Proteolysis is started by the rennet (chymosin enzyme from animal or fungal origin) and the coagulated caseins form a gel that entraps any fat present. The separated curd is cut into blocks, drained and pressed into shapes, matured and made into cheeses. The details of cheese production are very complicated and involve many strains of bacteria and sometimes filamentous fungi (e. g. Camembert, blue-cheese), special milks and related additives and differing process techniques.

II. OTHER APPLICATIONS

(1) Biological control

Biological control is "the use of one species of organism to control the numbers or activities of another". This is used on a commercial scale in agriculture and forestry for example. It mainly involves the use of certain microorganisms and/ or their toxins to kill or disable insects that affect certain plants. These microorganisms are called bioinsecticides and biopesticides. For example, certain strains of *Bacillus thuringiensis*, *B. sphaericus* and *Clostridium bifermentans* form toxins that kill mosquitoes and efforts are made to develop a product to control mosquito- borne diseases such as malaria and yellow fever.

(2) Biomining

The use of certain bacterial species to extract metals from low-grade ores is called biomining. For example, this process is used commercially to recover copper from ores containing chalcopyrite ($CuFeS_2$) and iron pyrites (FeS_2). These processes involve the chemolithotrophic bacteria such as species of *Thiobacillus* and *Sulfolobus*. A mixture of the bacteria and sulphuric acid is allowed to pass through the crushed ore in a recycling manner. The iron and sulphur ions leached from the ore are oxidized by bacteria and thus the recycling of this process allows approximately complete solubilization of the formed compounds from the ore. The copper is removed by electrolysis.

(4) <u>Production of bioplastics</u> (BIOPOL)

The production of biodegradable plastics, that are environmentally harmless, from bacteria depends mainly on the polymer poly-β-hydroxybutyrate (PHB). The process involves growing *Alcaligenes eutrophus* in the appropriate medium, that is controlling the proportions, to produce the PHB's along with a co- polymer (hydroxyvalerate) in the required amounts. The formed intracellular granules are collected and purified to form a fine powder that is used to produce fibers, films, coatings and containers. The most important benefit of these plastics is that they completely biodegradeable after disposal (e. g. in soil) without leaving any toxic or harmful substances in the environment.

____***End of Lecture (9)***

SOME BACTERIAL DISEASES

(1) Diseases caused by *Streptococcus pyogenes*

Streptococcus pyogenes (Group A *streptococcus*) is a Gram-positive, nonmotile, nonsporeforming coccus that occurs in chains or in pairs of cells. Individual cells are round to ovoid cocci, 0.6-1.0 µm in diameter. Streptococci divide in one plane and thus occur in pairs (especially in liquid media or clinical material) or in chains of varying lengths. The metabolism of *S. pyogenes* is fermentative; the organism is a catalase-negative aerotolerant anaerobe (facultative anaerobe), and requires enriched medium containing blood in order to grow. Group A streptococci typically have a capsule composed of hyaluronic acid and exhibit beta (clear) hemolysis on blood agar.

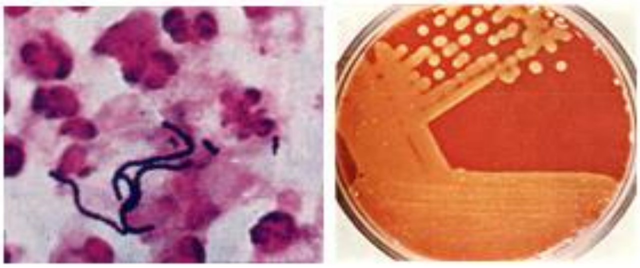


Figure (62): ***Streptococcus pyogenes:*** **Left. Gram stain of** ***Streptococcus pyogenes*** **in a clinical specimen. Right. Colonies of** ***Streptococcus pyogenes*** **on blood agar exhibiting beta hemolysis.**

Streptococcus pyogenes is one of the most frequent pathogens of humans. It is estimated that between 5- 15% of normal individuals harbor the bacterium, usually in the respiratory tract, without signs of disease. As normal flora, *S. pyogenes* can infect the host when the bacteria are introduced or transmitted to vulnerable tissues and a variety of types of **infections** can occur.

Acute *Streptococcus pyogenes* infections may present as illustrated in the following Figure (Fig. 63).

Pathogenesis

Streptococcus pyogenes owes its major success as a pathogen to its ability to colonize and rapidly multiply and spread in its host while escaping phagocytosis and confusing the immune system.

Acute diseases associated with *Streptococcus pyogenes* occur chiefly in the **respiratory tract**, **bloodstream**, or the **skin**. Streptococcal disease is most often a respiratory infection (pharyngitis or tonsillitis) or a skin infection (pyoderma). Generally, streptococcal isolates from the pharynx and respiratory tract do not cause skin infections.

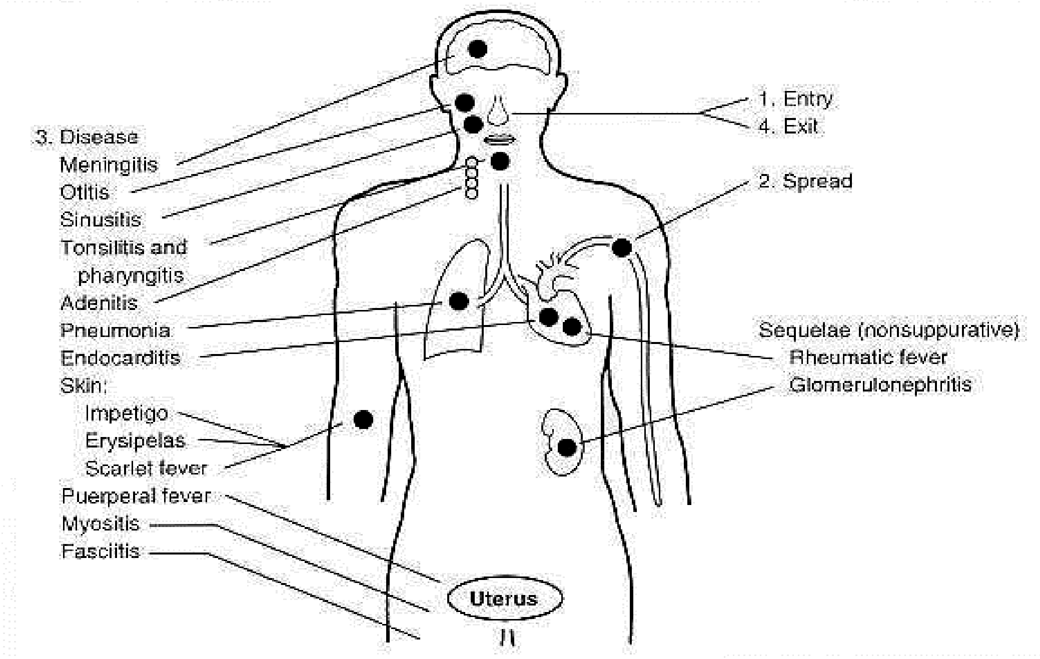


Figure (63): ***Streptococcus pyogenes*** **infections**

The **cell surface** of *Streptococcus pyogenes* accounts for many of the bacterium determinants of virulence, including colonization and avoidance of phagocytosis and host immune responses. The surface of *Streptococcus pyogenes* is incredibly complex and chemically-diverse. Antigenic components include **capsular polysaccharide (C-substance)**, cell wall **peptidoglycan** and **lipoteichoic acid (LTA)**, and a variety of surface proteins, including **M protein**, **fimbrial proteins**, **fibronectin- binding proteins**, (e.g. **Protein F**) and cell- bound **streptokinase**.

The cell envelope of a Group A *Streptococcus* is illustrated in the following Figure. The complexity of the surface can be seen in several of the electron micrographs of the bacterium.

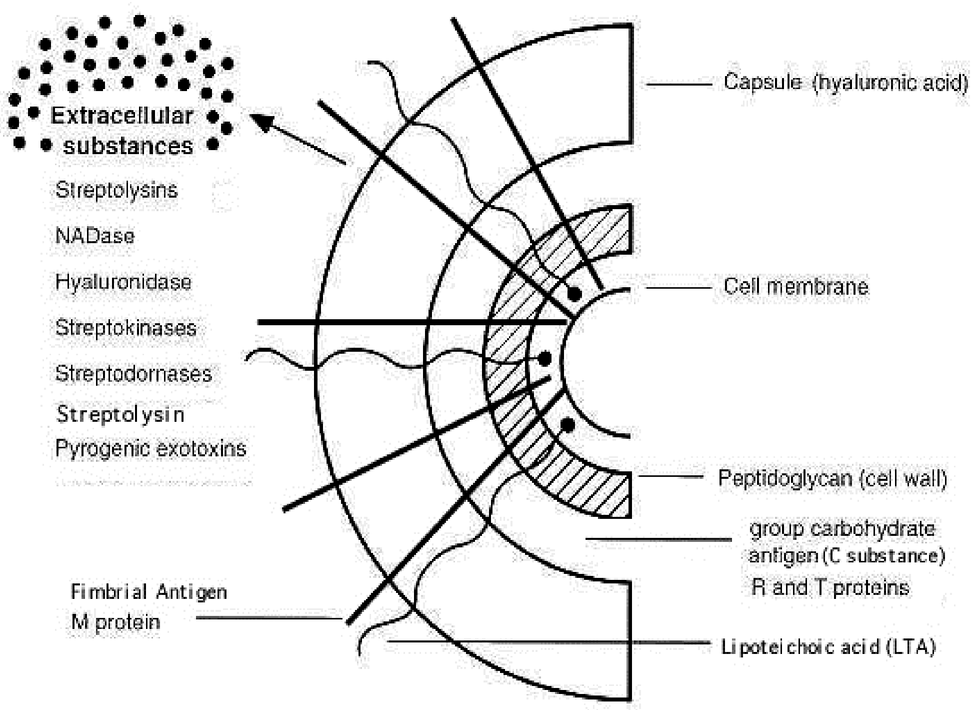


Figure (64): Surface structure of *S. pyogenes* cells and secretions involved in virulence

In group A streptococci, the **R** and **T** proteins are used as epidemiologic markers and have no known role in virulence. The group carbohydrate antigen (composed of N- acetylglucosamine and rhamnose) has been thought to have no role in virulence, but emerging strains with increased invasive capacity produce a very mucoid colony, suggesting a role of the capsule in virulence.

The **M proteins** are clearly virulence factors associated with both colonization and resistance to phagocytosis. The M proteins of certain M- types are considered **rheumatogenic** since they contain antigenic epitopes related to heart muscle, and they therefore may lead to autoimmune rheumatic carditis (rheumatic fever) following an acute infection.

The **capsule** of *S. pyogenes* is non antigenic since it is composed of **hyaluronic acid**, which is chemically similar to that of host connective tissue. This allows the bacterium to hide its own antigens and to be unrecognized as antigenic by its host. The Hyaluronic acid capsule also prevents phagocytosis by neutrophils or mancrophages.

Adhesins

There is evidence that *Streptococcus pyogenes* utilizes **lipoteichoic acids (LTA)**, **M protein**, and multiple **fibronectin- binding proteins** in its adhesins. LTA is anchored to proteins on the bacterial surface, including the M protein. The fibronectin- binding protein, **Protein F**, has also been shown to mediate streptococcal adherence to the amino terminals of fibronectin on mucosal surfaces.

Extracellular products: invasins and exotoxins

Colonization of the upper respiratory tract and acute pharyngitis may spread to other portions of the upper or lower respiratory tracts resulting in infections of the middle ear (otitis media), sinuses (sinusitis), or lungs (pneumonia). In addition, meningitis can occur by direct extension of infection from the middle ear or sinuses to the meninges or by way of bloodstream invasion from the pulmonary focus. Bacteremia can also result in infection of bones (osteomyelitis) or joints (arthritis). During these aspects of acute disease the streptococci bring into play a variety of secretory proteins that mediate their invasion.

For the most part, streptococcal invasins and protein toxins interact with mammalian blood and tissue components in ways that kill host cells and exhibit a damaging inflammatory response (see the extracellular substances in Figure 64). This large range of products is important in the pathogenesis of *S. pyogenes* infections. Even so, antibodies to these products are relatively insignificant in protection of the host.

The streptococcal invasins act in a variety of ways. Streptococcal invasins lyse eukaryotic cells, including red blood cells and phagocytes; they lyse other host macromolecules, including enzymes and informational molecules; they allow the bacteria to spread among tissues by dissolving host fibrin and intercellular ground substances.

Pyrogenic Exotoxins (antigens)

Three **streptococcal pyrogenic exotoxins** (SPE), formerly known as **Erythrogenic toxin**, are recognized: types A, B, C. The erythrogenic toxin is so- called for its association with scarlet fever which occurs when the toxin is disseminated in the blood. Re-emergence of exotoxin- producing strains of *S. pyogenes* has been associated with a **toxic shock-like syndrome** similar in pathogenesis and manifestation to staphylococcal toxic shock syndrome, and with other forms of invasive disease associated with severe tissue destruction. The latter condition is termed **necrotizing fasciitis**. Outbreaks of sepsis, toxic shock and necrotizing fasciitis have been reported at increasing frequency.

Host defenses

S. pyogenes is usually an **exogenous secondary invader**, following viral disease or disturbances in the normal bacterial flora. In the normal human the skin is an effective barrier against invasive streptococci, and nonspecific defense mechanisms prevent the bacteria from penetrating beyond the superficial epithelium of the upper respiratory tract. These mechanisms include mucociliary movement, coughing, sneezing and epiglottal reflexes.

The **host phagocytic system** is a second line of defense against streptococcal invasion. *S. pyogenes* is rapidly killed following phagocytosis enhanced by specific antibody. The bacteria do not produce catalase or significant amounts of superoxide dismutase to inactivate the oxygen metabolites (hydrogen peroxide, superoxide) produced by the oxygen-dependent mechanisms of the phagocyte. Therefore, they are quickly killed after engulfment by phagocytes.

In immune individuals, antibodies reactive with M protein promote phagocytosis which results in killing of the organism. This is the major mechanism by which Group A streptococcal infections are terminated. **M protein vaccines** are a major candidate for use against rheumatic fever, but certain M protein types cross- react antigenically with the heart and , then, may be responsible for rheumatic carditis. This risk of autoimmunity has prevented the use of Group A streptococcal vaccines.

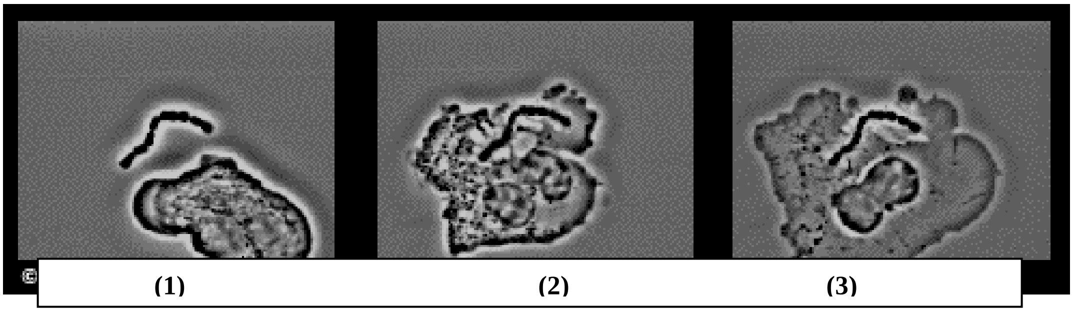
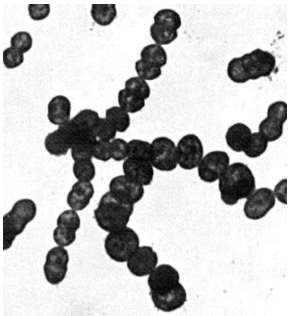


Figure (65): Phagocytosis of *S. pyogenes* by a macrophage (white blood cell)

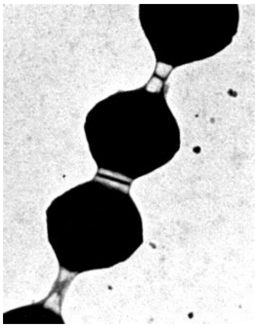
Treatment and prevention

Penicillin is still effective in treatment of Group A streptococcal disease. It is important to identify and treat Group A streptococcal infections in order to prevent post infectious diseases. No effective vaccine has been produced, but specific M- protein vaccines are being tested.

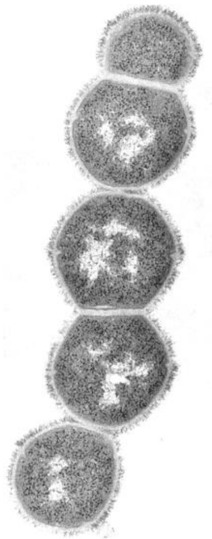
Figure (66): Electron micrographs of some strains of Streptococcus



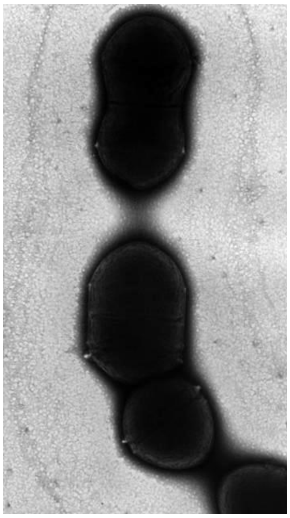
A) Critical point dried whole group A streptococci (*Streptococcus pyogenes*) viewed directly by transmission electron microscopy (TEM 6,500X). Chains of streptococci are clearly evident.



B) Dividing streptococci (12,000X). Electron micrograph of *Streptococcus pyogenes.*



C) Electron micrograph of an ultra-thin section of a chain of group A streptococci (20,000X). The cell surface fibrils, consisting primarily of M protein, are clearly evident. The bacterial cell wall, to which the fibrils are attached, is seen as the light staining region between the fibrils and the dark cell interior. Cell division is indicated by the nascent septum formation. Electron micrograph of *Streptococcus pyogenes* (cell diameter+ approximately 1µm.



D) Negative staining of group A streptococci (TEM, 28,000X). The "halo" around the chain of cells (approximately equal in thickness to the cell diameter) is the capsule surrounding the exterior of certain strains of group A streptococci. The septa between pairs of dividing cells may also be seen.

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(2) Diseases caused by *Streptococcus pneumoniae*

Introduction

Pneumonia is a disease of the lung that is caused by a variety of bacteria including *Streptococcus, Staphylococcus, Pseudomonas, Haemophilus, Chlamydia and Mycoplasma,* several viruses, and certain fungi and protozoans. The disease may be divided into two forms, bronchial (i. e. for air tubes in the lung) and lobar (i. e. for lung lobes) pneumonia. Bronchial pneumonia is most prevalant in infants, young children and aged adults. It is caused by various bacteria, including *Streptococcus pneumoniae.* Lobar pneumonia is more prone to occur in younger adults. A majority (more than 80%) of the cases of lobar pneumonia are caused by *Streptococcus pneumoniae.* Lobar pneumonia involves all of a single lobe of the lungs (although more than one lobe may be involved), where the entire area of involvement tends to become a consolidated mass, in contrast to the spongy texture of normal lung tissue. *Streptococcus pneumoniae* is known in medical microbiology as the **pneumococcus**, referring to its morphology and its consistent involvement in pneumonia.

Streptococcus pneumoniae are Gram +ve, elongated cocci with a slightly pointed outer curvature. Usually they are seen as pairs of cocci (diplococci), but they may also occur singly and in short chains. Individual cells are between 0.5 and 1.25 µm in diameter. They do not form spores, and they are nonmotile. Like other streptococci, they lack catalase and ferment glucose to lactic acid. Unlike other streptococci, they do not display an M protein, they hydrolyze inulin, and their cell wall composition is characteristic both in terms of their peptidoglycan and their teichoic acid.

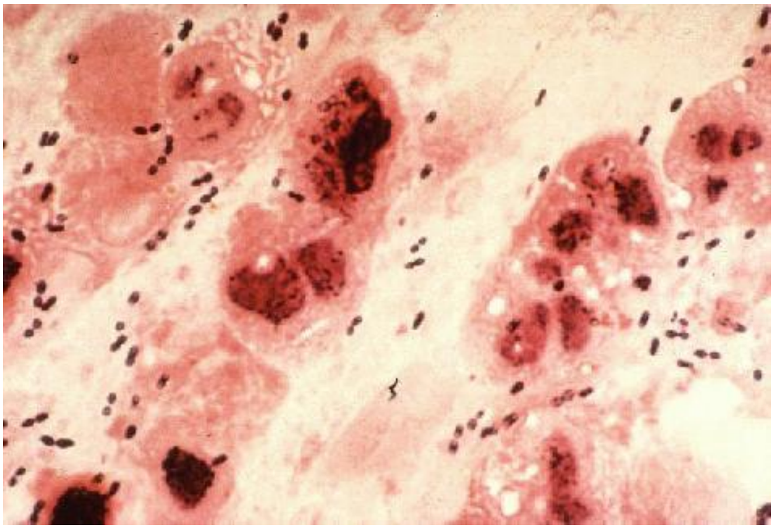

Figure (67): Gram Stain of a film of sputum from a case of lobar pneumonia

Cultivation

Streptococcus pneumoniae grows best in 5% carbon dioxide. Nearly 20% of fresh clinical isolates require fully anaerobic conditions. In all cases, growth requires a source of catalase (e.g. blood) to neutralize the large amount of hydrogen peroxide produced by the bacteria. In complex media containing blood, at 37°C, the bacterium has a doubling time of 20-30 minutes. On agar, pneumococci grow as glistening mucoid colonies, about 1 mm in diameter. A transparent colony type is adapted to colonization of the nasopharynx, whereas an opaque variant occurs in blood. The chemical basis for the difference in colony appearance is not known. *Streptococcus pneumoniae* is a fermentative aerotolerant anaerobe. It is usually cultured in media that contain blood. On blood agar, colonies characteristically produce a zone of alpha (green) hemolysis, which differentiates *S. pneumoniae* from the group A (beta hemolytic) streptococcus. Special tests such as inulin fermentation, bile solubility, and optochin (an antibiotic) sensitivity must be routinely employed to differentiate the pneumococcus from *Streptococcus viridans.*

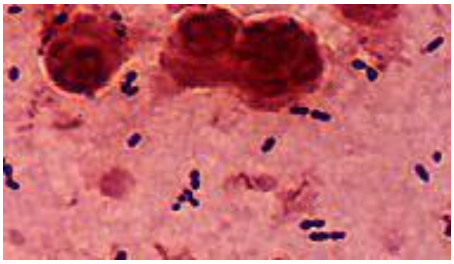

Figure (68): ***S. pneumoniae*****: Gram stain of blood broth culture**

Identification

The minimum criteria for identification and distinction of pneumococci from other streptococci are bile or optochin sensitivity, Gram staining, and hemolytic activity. Pneumococci cause alpha hemolysis on agar containing horse, human, rabbit and sheep erythrocytes. Under anaerobic conditions they switch to beta hemolysis. Typically, pneumococci form a 16- mm zone of inhibition around a 5 mg optochin disc, and undergo lysis by bile salts (e.g. deoxycholate). Addition of a few drops of 10% deoxycholate at 37°C lyses the entire culture in minutes. The ability of deoxycholate to dissolve the cell wall depends upon the presence of an autolytic enzyme, LytA. Virtually all clinical isolates of pneumococci harbor the autolysin and undergo deoxycholate lysis.

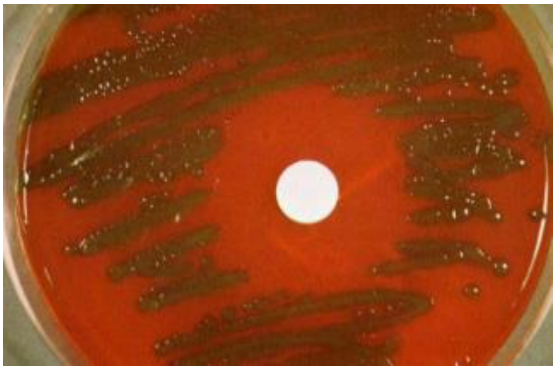

Figure (69): A mucoid strain of *S. pneumoniae* on blood agar showing alpha hemolysis: (green zone surrounding colonies, not shown here). Note the zone of inhibition around a filter paper disc dipped in optochin.

Serotyping

The **quellung reaction** (swelling reaction) forms the basis of serotyping and relies on the swelling of the capsule upon binding of homologous antibody The test consists of mixing a loopful of colony with equal quantity of specific antiserum and then examining microscopically at 1000X for capsular swelling. Although generally highly specific, cross- reactivity has been observed between other capsular types and with *E. coli, Klebsiella, H. influenzae* and certain viridans streptococci.

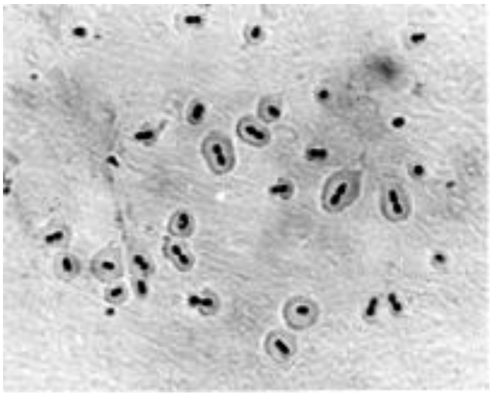

Figure (70): Quellung (swelling) reaction of *S. pneumoniae*

Cell Surface Structure

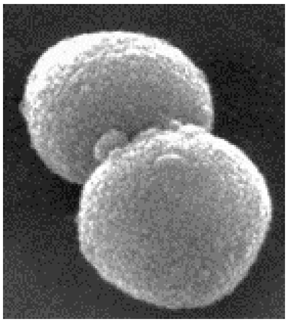

Figure (71): A pair of diplococci of *S. pneumoniae* (SEM micrograph)

Capsule

A capsule composed of polysaccharide completely envelops the pneumococcal cells. During invasion the capsule is an essential determinant of virulence. Anti- pneumococcal vaccines are based on formulations of various capsular (polysaccharide) antigens derived from the highly- prevalent strains.

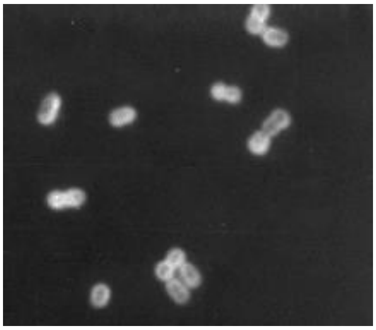

Figure (72): Fluorescent antibody staining of *S. pneumoniae* capsule

Colonization

Pneumococci adhere tightly to the nasopharyngeal epithelium by multiple mechanisms that, for most individuals, appears to result in an immune response that generates type- specific immunity. For some people, however, progression into the lungs or middle ear occurs. Inflammation in the middle ear is caused by pneumococcal cell wall components. Upon reaching the lower respiratory tract by aerosol, pneumococci bypass the ciliated upper respiratory epithelial cells unless there is damage to the epithelium. Instead, they progress to the alveolus (i. e. lung air sacs) and associate with specific alveolar cells. Experimentally, in healthy tissues, it requires approximately 100,000 bacteria/ ml to trigger an inflammatory response. However, if a proinflammatory signal is supplied, inflammation follows with as few as 10 bacteria. The inflammatory response can cause considerable tissue damage.

Invasion

The bacteria invade and grow primarily due to their resistance to the host phagocytic response. The cell wall components directly activate multiple inflammatory cascades. In addition, as pneumococci begin to lyse in response to host defenses and antimicrobial agents, they release cell wall components, pneumolysin and other substances that lead to greater inflammation and cytotoxic effects. Pneumolysin and hydrogen peroxide kill cells and induce production of nitric oxide. If bacteremia occurs, the risk of meningitis increases. Once in the cerebrospinal fluid, a variety of pneumococcal components, particularly cell wall components, incite the inflammatory response.

Vaccines

Given the 90 different capsular types of pneumococci, a comprehensive vaccine based on polysaccharide alone is not feasible. Thus, vaccines based on a subgroup of highly prevalent types have been formulated. The number of serotypes in the vaccine has increased from four in 1945, to 14 in the 1970s, and finally to the current 23- valent formulation. These serotypes represent 85-90% of those that cause invasive disease and the vaccine efficacy is estimated at 60%. However, underutilization of the vaccine is is leading to that the pneumococcus remains the most common infectious agent leading to hospitalization in all age groups.

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(3) Diseases caused by *Listeria monocytogenes*

Figure (73): *Listeria monocytogenes* (TEM micrograph)

Introduction

Listeria monocytogenes is a Gram +ve rod- shaped bacterium. It is the agent of **listeriosis**, a serious infection caused by eating food contaminated with the bacteria. The disease affects primarily pregnant women, newborns, and adults with weakened immune systems. Listeriosis is a serious disease for humans; the **overt form** of the disease has a mortality greater than 25 percent. The two main clinical manifestations are sepsis and meningitis. Meningitis is often complicated by encephalitis, a pathology that is unusual for bacterial infections.

Microscopically *Listeria* species appear as small, Gram +ve rods, which are sometimes arranged in short chains. In direct smears they may be coccoid, so they can be mistaken for streptococci. Longer cells may resemble corynebacteria. (as Gram +ve, nonspore-forming, catalase- positive rods). The genus *Listeria* was classified in the family Corynebacteriaceae through the seventh edition of of Bergey's Manual. Flagella are produced at room temperature but not at 37° C. Hemolytic activity on blood agar have been used as a marker to distinguish *Listeria monocytogenes* among other *Listeria* species, but it is not an absolutely definitive criterion. Further biochemical characterization may be necessary to distinguish between the different *Listeria* species.

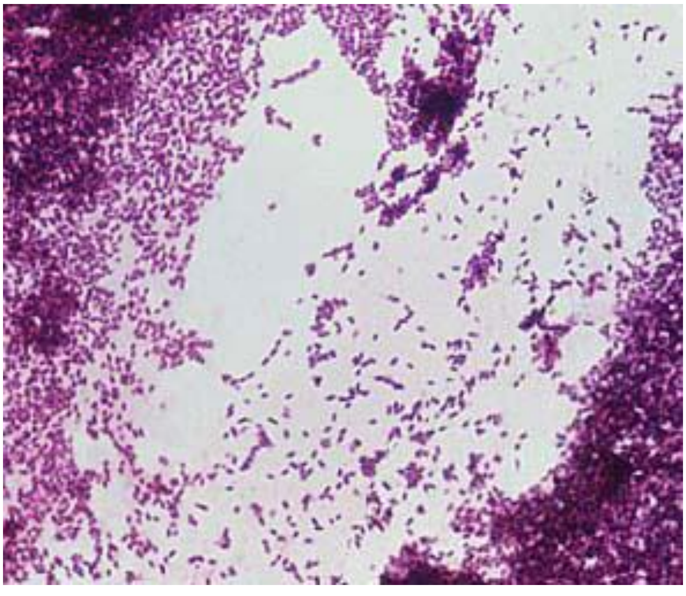

Figure (74): ***Listeria monocytogenes*** **(Gram Stain**ing)

Natural Habitats

Listeriae, including the pathogenic species *L. monocytogenes* and *L. ivanovii* have been isolated from a variety of sources, and they are now widely distributed in nature. In addition to humans, at least 42 species of wild and domestic mammals and 17 avian species, including domestic chickens, can harbor listeriae. *Listeria monocytogenes* is reportedly carried in the intestinal tract of 5- 10% of the human population without any apparent symptoms of disease. Listeriae have also been isolated from crustaceans, fish, oysters, ticks, and flies.

The term **listeriosis** includes a wide variety of disease symptoms that are similar in animals and humans. *Listeria monocytogenes* causes listeriosis in animals and humans; *L. ivanovii* causes the disease in animals only, mainly sheep. Encephalitis is the most common form of the disease in ruminant animals. The true incidence of listeriosis in humans is not known, because in the average healthy adult, infections are usually asymptomatic, or at most produce a mild influenza- like disease. Illness is most likely to occur in pregnant women, newborn children, the elderly, but apparently healthy individuals may also be affected. In the serious form of the disease, meningitis, frequently accompanied by septicemia, is the most commonly encountered disease manifestation. In pregnant women, however, even though the most usual symptom is a mild influenza- like illness without meningitis, infection of the fetus is extremely common and can lead to abortion, stillbirth, or delivery of an acutely ill infant.

Pathogenesis

Listeria monocytogenes is presumably ingested with raw, contaminated food. Listeriae penetrate the host cells of the epithelial lining. The bacterium is widely distributed so this event may occur frequently. Normally, the immune system eliminates the infection before it spreads. Adults with no history of listeriosis have T lymphocytes specific for *Listeria* antigens. However, if the immune system is compromised (some drugs can expose the immune system to danger), systemic disease may develop. *Listeria monocytogenes* multiplies not only extracellularly but also intracellularly, within macrophages after phagocytosis, or within parenchymal cells that were entered by induced phagocytosis.

Motility

Although *Listeria* is actively motile by means of peritrichous flagella at room temperature (20- 25°C), the organisms do not synthesize flagella at body temperatures (37°C). Instead, virulence is associated with another type of motility: the ability of the bacteria to move themselves into, and between host cells by polymerization of host cell actin at one end of the bacterium ("growing actin tails") that can push the bacteria through cytoplasm.

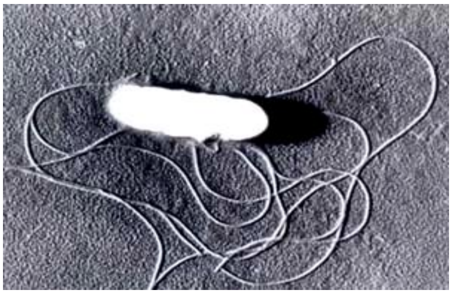

Figure (75): ***Listeria monocytogenes*** **(SEM showing Flagella)**

Adherence and Invasion

Listeria can attach to and enter mammalian cells. The bacterium is thought to attach to epithelial cells by means of D- galactose residues on the bacterial surface which adhere to D- galactose receptors on the host cells. The bacteria are then taken up by **induced phagocytosis**, analogous to the situation in *Shigella.* After engulfment, the bacterium may escape from the phagosome before phagolysosome fusion occurs mediated by a toxin. Within the host cell environment, the bacteria reside and multiply (Figure 76 below).

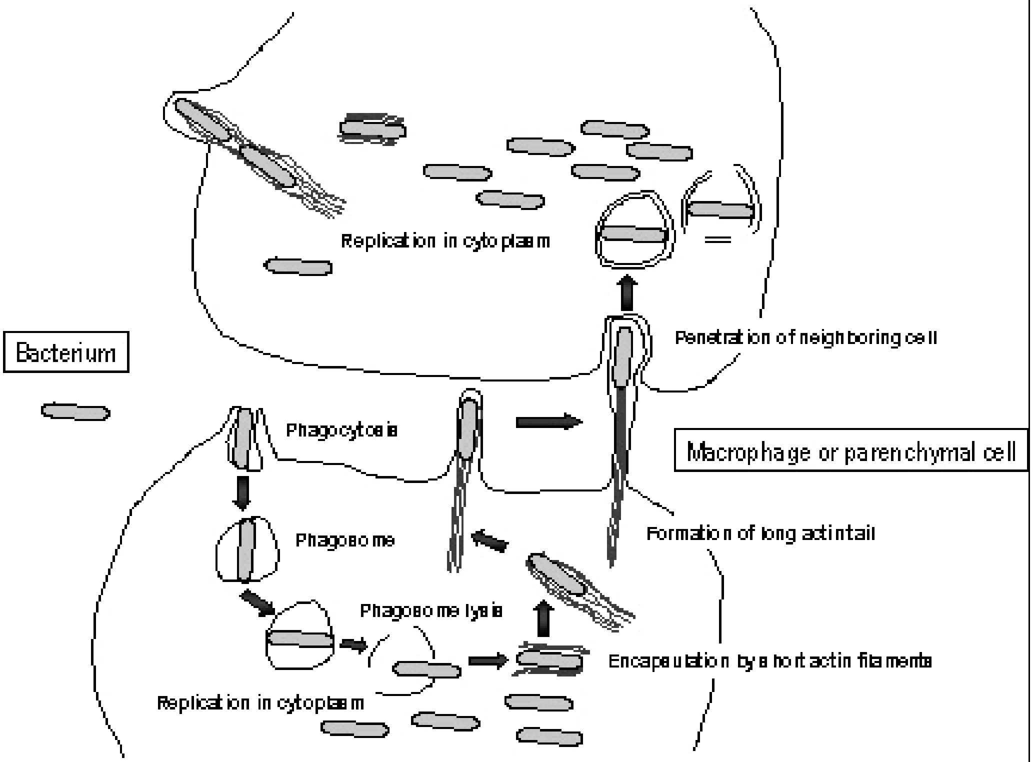

Figure (76): *Listeria* invasion and intracellular spread: The bacterium attaches its D-galactose residues to intestinal mucosa on their receptors then taken up by phagocytosis. Once ingested, the bacterium produces listeriolysin (LLO) to escape from the phagosome then multiplies rapidly in the cytoplasm and moves through the cytoplasm to invade adjacent cells.

Host Defenses

Because *L. monocytogenes* multiplies intracellularly, it is largely protected against immune factors such as antibodies and mediated lysis.

Treatment and Prevention

If diagnosed early enough, antibiotic treatment of pregnant women or immuno- compromised individuals can prevent serious consequences of the disease. However, processed foods known to be the source of *Listeria* that may still be in the market place, restaurant or home should obviously not be used, and should be recalled from the market. It must also be constantly recognized that *L. monocytogenes* is able to grow at low temperatures.

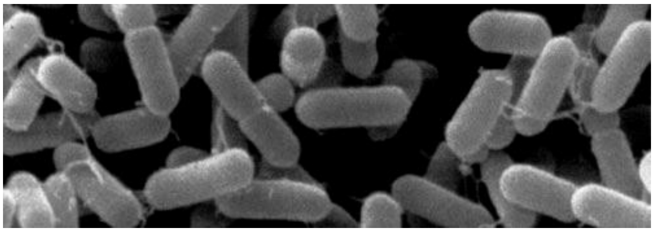

Figure (77): ***Listeria monocytogenes*** **(SEM** photo)

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(4) Diseases caused by *Haemophilus influenzae*

Introduction

Haemophilus influenzae is a small, nonmotile Gram -ve bacterium in the family *Pasteurellaceae*, on the level with the *Vibrionaceae* and the *Enterobacteriaceae*. The family also includes *Pasteurella* and *Actinobacillus*, two other genera of bacteria that are parasites of animals. Encapsulated strains of *Haemophilus influenzae* isolated from cerebrospinal fluid are coccobacilli. Non encapsulated organisms from sputum are pleomorphic and often exhibit long threads and filaments. The organism may appear Gram +ve unless the Gram stain procedure is very carefully carried out. Furthermore, elongated forms from sputum may exhibit bipolar staining, leading to confusion in diagnosis with *Streptococcus pneumoniae*.

Figure (78): Gram stain of *Haemophilus influenzae* from sputum

H. influenzae is highly adapted to its human host. It is present in the nasopharynx of approximately 75 percent of healthy children and adults. It is usually the non encapsulated strains that are harboured as normal flora, but a minority of healthy individuals (3- 7 %) intermittently harbor *H. influenzae* type b (Hib) encapsulated strains in the upper respiratory tract. Pharyngeal carriage of Hib is important in the transmission of the bacterium.

Haemophilus influenzae is widespread among the human population. It was first isolated by Pfeiffer during the influenza pandemic of 1890. It was mistakenly thought to be the cause of the disease influenza, and it was named accordingly. Probably, *H. influenzae* was an important secondary invader to the influenza virus in the 1890 pandemic, as it has been during many subsequent influenza epidemics. In pigs, a synergistic association between swine influenza virus and *Haemophilus suis* is necessary for swine influenza. Similar situations between human influenza virus and *H. influenzae* have been observed in chick embryos and infant rats.

Haemophilus "loves heme", more specifically it requires a precursor of heme in order to grow. Nutritionally, *H. influenzae* prefers a complex medium and requires preformed growth factors that are present in blood. The bacterium grows best at 35- 37°C and has an optimal pH of 7.6. It is generally grown in the laboratory under aerobic conditions or under slight CO_2 tension (5% CO_2), although it is capable of glycolytic growth and of respiratory growth using nitrate as a final electron acceptor.

Pathogenesis

The pathogenesis of *H. influenzae* infections is not completely understood, although the presence of the **type b polysaccharide capsule** is known to be the major factor in virulence. Encapsulated organisms can penetrate the epithelium of the nasopharynx and invade the blood capillaries directly. Their capsule allows them to resist phagocytosis and complement- mediated lysis in the the nonimmune host. Non encapsulated strains are less invasive, but they are apparently able to induce an inflammatory response that causes disease. Outbreaks of *H. influenzae* type b infection may occur in nurseries and child care centers, and prophylactic administration of antibiotics is warranted. Vaccination with type b polysaccharide (in the form of **Hib conjugate vaccines**) is effective in preventing infection, and several vaccines are now available for routine use. Naturally- acquired disease caused by *H. influenzae* seems to occur in humans only. In infants and young children, under 5 years of age, *H. influenzae* type b causes **bacteremia** and acute bacterial **meningitis**. Other diseases are illustrated in the Figure below.

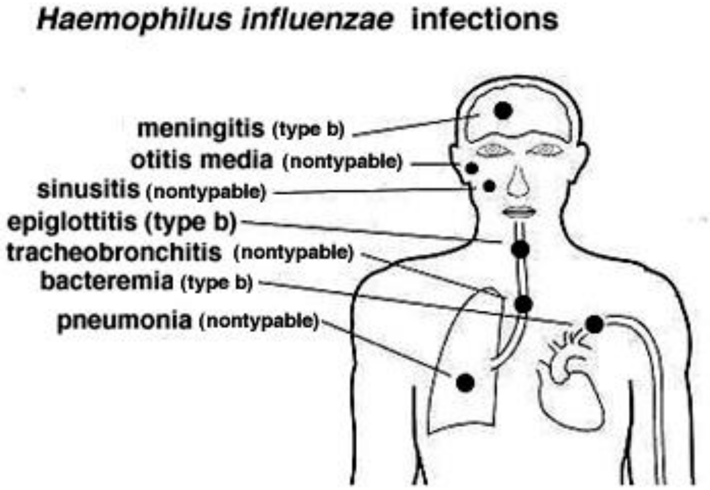

Figure (79): Tissues infected by type b and nontypable strains of *H. influenzae*

Disease caused by *H. influenzae* usually begins in the upper respiratory tract as nasopharyngitis and may be followed by sinusitis and otitis, possibly leading to pneumonia. In severe cases, bacteremia may occur which frequently results in joint infections or meningitis.

Treatment and Prevention

Virtually all patients treated early in the course of *H. influenzae* meningitis are cured. The mortality rate of treated infections is less than 10 percent, but nearly 30 percent of the children who recover have residual neuro effects. Ampicillin has been effective treatment but over 20 percent of *H. influenzae* strains are resistant to ampicillin because of plasmid- mediated ß- lactamase production. The recommended treatment for *H. influenzae* meningitis is ampicillin for strains of the bacterium that do not make ß-lactamase, and a third- generation cephalosporin or chloramphenicol for strains that do. Amoxicillin, together with a substance such as clavulanic acid, that blocks the activity of ß-lactamase, has been unreliable in treatment of meningitis, although it is effective in treatment of sinusitis, otitis media and respiratory infections. Chloramphenicol was long considered the drug of choice for meningitis caused by penicillin- resistant *H. influenzae,* and it is still highly effective, but not without potential toxic side effects. Tetracyclines and sulfa drugs remain effective in treating sinusitis or respiratory infection caused by nontypable *H. influenzae*. Amoxicillin plus clavulanic acid (Augmentin) is effective against ß-lactamase producing strains. Erythromycin is ineffective in treatment of H. influenzae infections.

There are **several types of Hib conjugate vaccines** available for use. All of the vaccines are approved for use in children 15 months of age and older and some are approved for use in children beginning at 2 months of age. All of the vaccines are considered effective. The vaccines are given by injections. More than 90% of infants obtain long term immunity with 2- 3 doses of the vaccine.

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(5) Diseases caused by *Pseudomonas aeruginosa*

Figure (80): Gram staining of *P. aeruginosa*

Pseudomonas aeruginosa is a Gram -ve, aerobic rod belonging to the bacterial family ***Pseudomonadaceae.*** The family includes other genera, which, together with certain other organisms, constitute the bacteria known as **pseudomonads**. These bacteria are common inhabitants of soil and water. They occur regularly on the surfaces of plants and occassionally on the surfaces of animals. They are well known to plant microbiologists because they are one of the few true pathogens of plants. But *Pseudomonas aeruginosa* and two former *Pseudomonas* species (now reclassified as *Burkholderia*) are pathogens of humans.

Pseudomonas aeruginosa is a Gram-negative rod measuring 0.5 to 0.8 µm by 1.5 to 3.0 µm. Almost all strains are motile by means of a single polar flagellum. The bacterium is everywhere in soil, water, and on surfaces in contact with soil or water. Its metabolism is respiratory and never fermentative, but it will grow in the absence of O_2 if NO_3 is available as a respiratory electron acceptor. The typical *Pseudomonas* bacterium in nature might be found in a **biofilm**, attached to some surface or substrate, or in a **planktonic form**, as a unicellular organism, actively swimming by means of its flagellum. *Pseudomonas* is one of the most vigorous, fast-swimming bacteria seen in hay infusions and pond water samples. It has a combination of physiological traits that may relate to its pathogenesis. These are:

--*P. aeruginosa* has very simple nutritional requirements. It is often observed "growing in distilled water".

--*P. aeruginosa* possesses the metabolic versatility for which pseudomonads are so renowned. Organic growth factors are not required, and it can use more than seventy five organic compounds for growth.

--Its optimum temperature for growth is 37°C, and it is able to grow at temperatures as high as 42°C.

--It is tolerant to a wide variety of physical conditions, including temperature. It is resistant to high concentrations of salts and dyes, weak antiseptics, and many commonly used antibiotics.

--It has a tendency for growth in moist environments, which is probably a reflection of its natural existence in soil and water.

P. aeruginosa isolates may produce three **colony types**. Natural isolates from soil or water typically produce a small, **rough** colony. Clinical samples, in general, yield one or another of two smooth colony types. One type has a fried- egg appearance which is large, **smooth**, with flat edges and an elevated appearance. Another type, frequently obtained from respiratory and urinary tract secretions, has a **mucoid** appearance, which is attributed to the production of **alginate slime**. The smooth and mucoid colonies are presumed to play a role in colonization and virulence.

Figure (81): Colonies of *P. aeruginosa* on agar

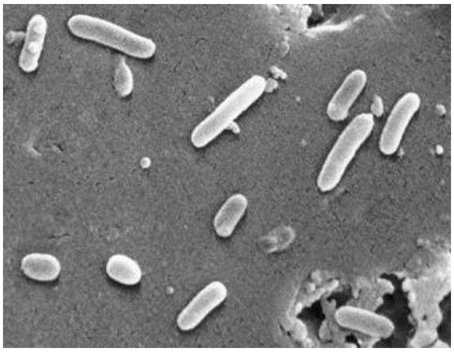

Figure (82): *P. aeruginosa* (SEM micrograph)

P. aeruginosa strains produce two types of soluble pigments, the fluorescent pigment **pyoverdin** and the blue pigment **pyocyanin**. The latter is produced abundantly in media of low iron content and functions in iron metabolism in the bacterium. Pyocyanin refers to "blue pus" which is a characteristic of infections caused by *P. aeruginosa.*

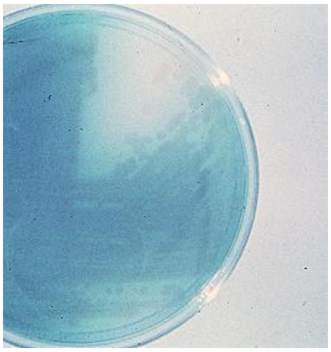

Figure (83): The soluble blue pigment pyocyanin (colour not shown here) produced by many strains of *P. aeruginosa*

Only a few antibiotics are effective against *Pseudomonas*, including fluoroquinolones, gentamicin and imipenem, but not effective against all strains.

Diagnosis

Diagnosis of *P. aeruginosa* infection depends upon isolation and laboratory identification of the bacterium. It grows well on most laboratory media and commonly is isolated on blood agar or eosin-methylthionine blue agar. It is identified on the basis of its Gram morphology, inability to ferment lactose, a positive oxidase reaction, its fruity odor, and its ability to grow at 42°C. Fluorescence under ultraviolet light is helpful in early identification of *P. aeruginosa* colonies. Fluorescence is also used to suggest the presence of *P. aeruginosa* in wounds.

Pathogenesis

P. aeruginosa causes a variety of disease as illustrated in Table (40) below.

Table (40): Diseases caused by *Pseudomonas aeruginosa*

Endocarditis: *P. aeruginosa* infects heart valves. The organism establishes itself on the endocardium by direct invasion from the blood stream.

Respiratory infections: Respiratory infections caused by *P. aeruginosa* occur almost exclusively in individuals with a compromised lower respiratory tract or a compromised systemic defense mechanism. Primary pneumonia occurs in patients with chronic lung disease and congestive heart failure. Bacteremic pneumonia commonly occurs in some cancer patients undergoing chemotherapy. Lower respiratory tract colonization of cystic fibrosis patients by mucoid strains of *Pseudomonas aeruginosa* is common and difficult, if not impossible, to treat.

Bacteremia and Septicemia: *P. aeruginosa* causes bacteremia primarily in immuno- compromised patients. Most *Pseudomonas* bacteremia is acquired in hospitals. *Pseudomonas* accounts for about 25 percent of all hospital acquired Gram-negative bacteremias.

Central Nervous System infections: *P. aeruginosa* causes meningitis and brain abscesses and invades through the inner ear or paranasal sinus. It may be inoculated directly by means of head trauma, surgery or invasive diagnostic procedures, or indirectly from a distant site of infection such as the urinary tract.

Ear infections including external otitis: *P. aeruginosa* is the predominant bacterial pathogen in some cases of external otitis including "swimmer's ear". The bacterium is infrequently found in the normal ear, but often inhabits the external auditory canal in association with injury, maceration, inflammation, or simply wet and humid conditions.

Eye infections: *P. aeruginosa* can cause devastating infections in the human eye. It is one of the most common causes of bacterial keratitis. *Pseudomonas* can colonize the ocular epithelium. The bacterium can proliferate rapidly and, through the production of enzymes, cause a rapidly destructive infection that can lead to loss of the entire eye.

Bone and joint infections: *Pseudomonas* infections of bones and joints result from direct inoculation of the bacteria or from other primary sites of infection. Blood- borne infections are most often seen in drug users, and in conjunction with urinary tract or pelvic infections. *P. aeruginosa* has a particular tropism for some joints. *P. aeruginosa* causes chronic osteomyelitis, usually resulting from direct inoculation of bone.

Urinary tract infections: Urinary tract infections (UTI) caused by *P.aeruginosa* are usually hospital- acquired and related to urinary tract instrumentation or surgery. *Pseudomonas aeruginosa* is the third leading cause of hospital-acquired UTIs, accounting for about 12 percent of all infections of this type.

Gastrointestinal infections: *P. aeruginosa* can produce disease in any part of the gastrointestinal tract from the oropharynx to the rectum. The organism has been concerned in perirectal infections, pediatric diarrhea, typical gastroenteritis, and necrotizing enterocolitis.

Skin and soft tissue infections: *P. aeruginosa* can cause a variety of skin infections, both localized and diffuse. The common factors are breakdown of the outer skin layer which may result from burns, trauma or dermatitis; high moisture conditions such as those found in the ear of swimmers and the toe webs of athletes and combat troops, and under diapers of infants. Individuals with AIDS are easily infected.

Epidemiology and Control of *P. aeruginosa* Infections

P. aeruginosa is a common inhabitant of soil, water, and vegetation. It is found on the skin of some healthy persons and has been isolated from the throat and stool. Within the hospital, *P. aeruginosa* finds numerous reservoirs: disinfectants, respiratory equipment, food, sinks, taps, and mops. Furthermore, it is constantly reintroduced into the hospital environment on fruits, plants, vegetables, as well by visitors and patients transferred from other facilities. Spread occurs from patient to patient on the hands of hospital personnel, by direct patient contact with contaminated reservoirs, and by the ingestion of contaminated foods and water.

P. aeruginosa is frequently resistant to many commonly used antibiotics. Although many strains are susceptible to gentamicin, tobramycin, colistin, and amikacin, resistant forms have developed. The combination of gentamicin and carbenicillin is frequently used to treat severe *Pseudomonas* infections. Several types of vaccines are being tested, but none is currently available for general use.

____***End of Lecture (10)***

(6) Diseases caused by *Escherichia coli*

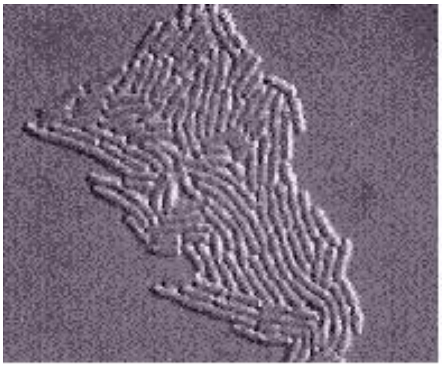

Figure (84): *Escherichia coli* cells and colonies: Left: *E. coli* cells. Right: *E.coli* colonies on EMB Agar

The GI tract (gastrointestinal) of most warm- blooded animals is colonized by *E. coli* within hours or a few days after birth. The bacterium is ingested in foods or water or obtained directly from other individuals handling the infant. The human bowel is usually colonized within 40 hours of birth. *E. coli* can adhere to the mucus overlying the large intestine. Once established, an *E. coli* strain may persist for months or years. The bases for the ecology of Escherichia coli, in the intestine of humans, are poorly understood.

E. coli is the head of the large bacterial family, ***Enterobacteriaceae***, the **enteric bacteria**, which are faculatively anaerobic Gram -ve rods that live in the intestinal tracts of animals in health and disease situations. The *Enterobacteriaceae* are among the most important bacteria medically. A number of genera within the family are human intestinal pathogens (e.g. *Salmonella*, *Shigella*, *Yersinia*). Several others are normal colonists of the human gastrointestinal tract (e.g. *Escherichia*, *Enterobacter*, *Klebsiella*), but these bacteria, may occasionally be associated with diseases of humans.

Physiologically, *E. coli* is versatile and well adapted to its characteristic habitats. It can grow in media with glucose as the sole organic constituent. Wild- type *E. coli* has no growth factor requirements, and metabolically it can transform glucose into all of the macromolecular components that make up the cell. The bacterium can grow in the presence or absence of O_2.

Under anaerobic conditions it will grow by means of fermentation, producing characteristic "mixed acids and gas" as end products. However, it can also grow by means of anaerobic respiration, since it is able to utilize NO_3, NO_2 or fumarate as final electron acceptors. This adapts *E. coli* to its intestinal (anaerobic) and its extra intestinal (aerobic or anaerobic) habitats.

E. coli can respond to environmental signals such as chemicals, pH, temperature, osmolarity, etc., in a number of ways considering it is a single- celled organism. For example, it can sense the presence or absence of chemicals and gases in its environment and swim towards or away from them. Or it can stop swimming and grow fimbriae that will specifically attach it to a cell or surface receptor. With its complex mechanisms for regulation of metabolism the bacterium can survey the chemical contents in its environment before synthesizing any enzymes for these compounds. It does not produce enzymes for degradation of carbon sources unless they are available, and it does not produce enzymes for synthesis of metabolites if they are available as nutrients in the environment.

E. coli is a consistent inhabitant of the human intestinal tract, and it is the predominant facultative organism in the human GI tract. However, it makes up a very small proportion of the total bacterial content. The anaerobic *Bacteroides* species in the bowel outnumber *E. coli* by at least 20:1. Moreover, the regular presence of *E. coli* in the human intestine and feces has led to tracking the bacterium in nature as an indicator of fecal pollution and water contamination. Therefore, wherever *E. coli* is found, there may be fecal contamination by intestinal parasites of humans.

Pathogenesis of *E. coli*

E. coli is responsible for three types of infections in humans: **urinary tract infections (UTI)**, **neonatal meningitis**, and **intestinal diseases (gastroenteritis)**. These three diseases depend on a specific array of pathogenic (virulence) determinants. The virulence determinants of various strains of pathogenic *E. coli* are summarized in Table (41) below.

Table (41): Summary of the virulence determinants of pathogenic *E. coli*

Adhesins: mainly fimbriae

Invasins: e. g.:intracellular invasion

Motility/chemotaxis: flagella

Toxins: e. g.: cytotoxins and endotoxin (LPS)

Antiphagocytic surface properties: e. g.: capsulesand LPS

Defense against serum bactericidal reactions: e. g.: LPS and other antigens

Defense against immune responses: e. g.: LPS, capsules and other antigens

Genetic attributes: e. g.: genetic exchange by transduction and conjugation and transmissible plasmids

Urinary tract pathogenic *E. coli* cause 90% of the urinary tract infections (UTI) in anatomically normal urinary tracts. The bacteria colonize from the feces or perineal region and ascend the urinary tract to the bladder. Bladder infections are 14-times more common in females than males.

Neonatal Meningitis affects1/ 2000- 4000 infants. Eighty percent of *E. coli* strains involved synthesize K-1 capsular antigens. Neonatal meningitis requires antibiotic therapy that usually includes ampicillin and a third-generation cephalosporin.

Intestinal Diseases: As a pathogen, *E. coli*, of course, is best known for its ability to cause intestinal diseases. Five classes of *E. coli* that cause diarrheal diseases are now recognized.

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(7) Diseases caused by *Salmonella*

Figure (85): ***Salmonella enterica***

Salmonella is a Gram -ve facultative rod- shaped bacterium in the same family as *Escherichia coli*, ***Enterobacteriaceae,*** known as "enteric" bacteria. In humans, *Salmonella* are the cause of two diseases called **salmonellosis**: **enteric fever** (**typhoid**), resulting from bacterial invasion of the bloodstream, and **acute gastroenteritis**, resulting from a foodborne infection and intoxication.

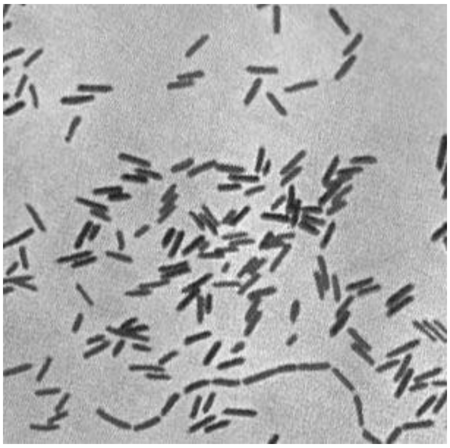

Figure (86): *Salmonella typhi,* the agent of typhoid (Gram stain)

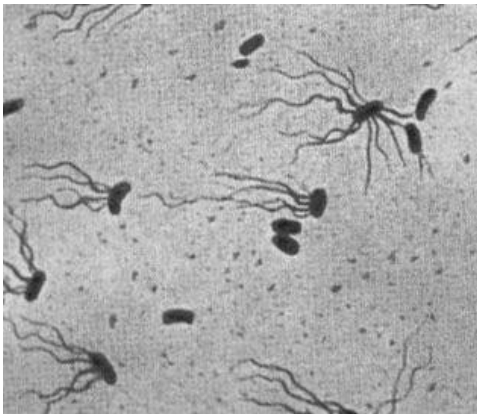

Figure (87): Flagellar stain of *S. typhi*: Like *E. coli, Salmonella* are motile by means of peritrichous flagella. A close relative that causes enteric infections is the bacterium *Shigella* that is nonmotile.

Habitats

The principal habitat of the salmonellae is the intestinal tract of humans and animals. *Salmonella* serovars can be found predominantly in one particular host, can be everywhere, or can have an unknown habitat. Typhi and Paratyphi A are strictly human serovars that may cause serious diseases often associated with invasion of the bloodstream. Salmonellosis in these cases is transmitted through fecal contamination of water or food.

Ubiquitous (non- host- adapted) *Salmonella* serovars (e.g., *S. typhimurium*) cause diverse clinical symptoms, from asymptomatic infection to serious typhoid- like syndromes in infants or certain highly susceptible animals (mice). In human adults, ubiquitous *Salmonella* organisms are mostly responsible for foodborne toxic infections.

Salmonella in the Natural Environment

Salmonellae are disseminated in the natural environment (water, soil and sometimes plants used as food) through human or animal excretion. Humans and animals (either wild or domesticated) can excrete *Salmonella* either when clinically diseased or after having had salmonellosis, if they remain carriers. *Salmonella* organisms do not seem to multiply significantly in the natural environment (out of digestive tracts), but they can survive several weeks in water and several years in soil if conditions of temperature, humidity, and pH are favourable.

Isolation and Identification of _Salmonella_

The most commonly used media selective for *Salmonella* are SS agar, bismuth sulfite agar, Hektoen enteric (HE) medium, brilliant green agar and xylose- lisine- deoxycholate (XLD) agar. All these media contain both selective and differential ingredients and they are commercially available.

Media used for *Salmonella* identification are those used for identification of all *Enterobacteriaceae*. Most *Salmonella* strains are motile with peritrichous flagella. However, nonmotile variants may occur occasionally. Most strains grow on nutrient agar as smooth colonies, 2- 4 mm in diameter. Most strains do not require any growth factors.

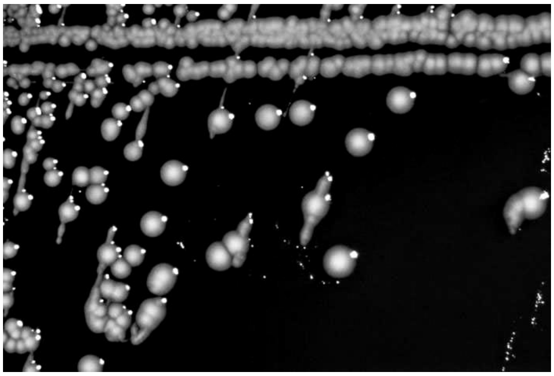

Figure (88): Growth of *S. choleraesuis* subsp. Arizonae: colonies on a blood agar culture plate. The bacterium can infect humans, birds, reptiles, and other animals.

Table (42): Characteristics shared by most *Salmonella* strains

Motile, Gram-negative bacteria
Lactose negative; acid and gas from glucose, mannitol, maltose, and sorbitol; no Acid from adonitol, sucrose, salicin, lactose
Indole test negative
Methyl red test positive
Voges-Proskauer test negative
Citrate positive (growth on Simmon's citrate agar)
Lysine decarboxylase positive
Urease negative
Ornithine decarboxylase positive
H_2S produced from thiosulfate
Phenylalanine and tryptophan deaminase negative
Gelatin hydrolysis negative

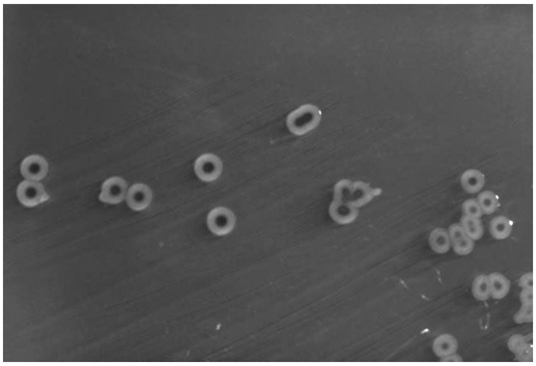

Figure (89): Growth pattern of *S. typhimurium* colonies on Hektoen enteric (HE) agar. *S. typhimurium* colonies grown on HE agar are blue- green in color (not shown here) indcating that the bacterium does not ferment lactose. However it produces hydrogen sulfide (H_2S), as indicated by black deposits in the centers of the colonies. HE agar is the medium for isolating fecal bacteria belonging to the family Enterbacteriaceae.

Pathogenesis

Salmonella infections in humans vary with the serovar, the strain, the infectious dose, the nature of the contaminated food, and the host status.

In the **pathogenesis of typhoid** the bacteria enter the human digestive tract, penetrate the intestinal mucosa (causing no lesion), and are stopped in the mesenteric lymph nodes. There, bacterial multiplication occurs, and part of the bacterial population lyses. From the mesenteric lymph nodes, viable bacteria and LPS (endotoxin) may be released into the bloodstream resulting in septicemia Release of endotoxin is responsible for cardiovascular "collapsus and tuphos" (a stuporous state—origin of the name typhoid).

Salmonella excretion by human patients may continue long after clinical cure. Asymptomatic carriers are potentially dangerous when unnoticed. About 5% of patients clinically cured from typhoid remain carriers for months or even years. Antibiotics are usually ineffective on *Salmonella* carriage (even if salmonellae are susceptible to them) because the site of carriage may not allow penetration by the antibiotic.

Salmonellae survive sewage treatments if suitable germicides are not used in sewage processing. In a typical cycle of typhoid, sewage from a community is directed to a sewage plant. Effluent from the sewage plant passes into a coastal river where edible shellfish (mussels, oysters) live. Shellfish concentrate bacteria as they filter several liters of water per hour. Ingestion by humans of these seafoods (uncooked or superficially cooked) may cause typhoid or other salmonellosis.

Typhoid is strictly a human disease. The incidence of human disease decreases when the level of development of a country increases (i.e., controlled water sewage systems, pasteurization of milk and dairy products). Where these hygienic conditions are missing, the probability of fecal contamination of water and food remains high and so is the incidence of typhoid.

Foodborne *Salmonella* toxic infections are caused by ubiquitous *Salmonella* serovars (e.g., *S. typhimurium*). About 12- 24 hours following ingestion of contaminated food (containing a sufficient number of *Salmonella*), symptoms appear (diarrhea, vomiting, fever) and last 2- 5 days. Spontaneous cure usually occurs. *Salmonella* may be associated with all kinds of food. Contamination of meat (cattle, pigs, goats, chicken, etc.) may originate from animal salmonellosis, but most often it results from contamination of muscles with the intestinal contents during washing, and transportation. Surface contamination of meat is usually of little consequence, as proper cooking will sterilize it (although handling of contaminated meat may result in contamination of hands, tables, kitchenware, towels, other foods, etc.). However, when contaminated meat is ground, multiplication of *Salmonella* may occur within the ground meat and if cooking is superficial, ingestion of this highly contaminated food may produce a *Salmonella* infection. Infection may follow ingestion of any food that supports multiplication of *Salmonella* such as eggs, cream, mayonnaise, creamed foods, etc.), as a large number of ingested salmonellae are needed to give symptoms. Prevention of *Salmonella* toxic infection relies on avoiding contamination (improvement of hygiene), preventing multiplication of *Salmonella* in food (constant storage of food at 4°C), and use of pasteurized and sterilized milk and milk products. Vegetables and fruits may carry *Salmonella* when contaminated with fertilizers of fecal origin, or when washed with polluted water.

Antibiotic Susceptibility

Antibiotic resistance and multiresistance of *Salmonella* spp. have increased a great deal. Resistance to ampicillin, streptomycin, kanamycin, chloramphenicol, tetracycline, and sulfonamides is commonly observed. Colistin resistance has not yet been observed.

Until 1972, Typhi strains had remained susceptible to antibiotics, including chloramphenicol (the antibiotic most commonly used against typhoid) but in 1972, a widespread epidemic in Mexico was caused by a chloramphenicol- resistant strain of *S. typhi*. Other chloramphenicol- resistant strains have since been isolated in India, Thailand, and Vietnam. *Salmonella* strains should be systematically checked for antibiotic resistance to aid in the choice of an efficient drug when needed and to detect any change in antibiotic susceptibility of strains (either from animal or human source). Random distribution and use of antibiotics should be discouraged.

Vaccination against typhoid fever

Three types of typhoid vaccines are currently available for use: (1) an oral live- attenuated vaccine; (2) a heat- phenol- inactivated vaccine; (3) a newly licensed capsular polysaccharide vaccine. A fourth vaccine, an acetone- inactivated vaccine, is currently available only to some armed forces.

1. **Live oral vaccines**. Although oral killed vaccines are without efficiency, vaccines using living avirulent bacteria have shown promise. The Live Vaccine should not be given to children younger than 6 years of age.

2. The **heat- phenol- inactivated vaccine** has been widely used for many years. The inactivated Typhoid Vaccine should not be given to children younger than 2 years of age. One dose provides protection. It should be given at least 2 weeks before travel to allow the vaccine time to work. A booster dose is needed every 2 years for people who remain at risk.

3. The newly **capsular polysaccharide** vaccine is composed of purified virulent antigen, the capsular polysaccharide elaborated by *S.typhi* isolated from blood cultures. It has not been tested among

children less than 1 year of age. No typhoid vaccine is 100% effective and is not a substitute for being careful about food and drinks.

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(8) Diseases caused by *Clostridium* spp.

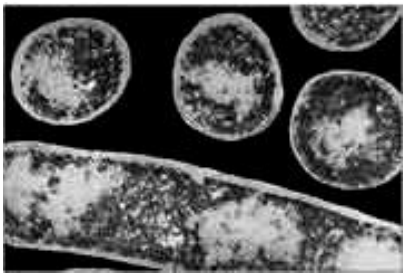

Figure (90): ***Clostridium botulinum***

The Genus *Clostridium*

The **clostridia** are relatively large, Gram +ve, rod- shaped bacteria. All species form endospores and have a strictly fermentative mode of metabolism. Most clostridia will not grow under aerobic conditions and vegetative cells are killed by exposure to O_2, but their spores are able to survive long periods of exposure to air. The clostridia live in almost all of the anaerobic habitats of nature where organic compounds are present, including soils, aquatic sediments and the intestinal tracts of animals.

Clostridia are able to ferment a wide variety of organic compounds. They produce end products such as butyric acid, acetic acid, butanol and acetone, and large amounts of gas (CO_2 and H_2) during fermentation of sugars. A variety of foul smelling compounds are formed during the fermentation of amino acids and fatty acids. The clostridia also produce a wide variety of extracellular enzymes to degrade large biological molecules in the environment into fermentable components. Hence, the clostridia play an important role in nature in biodegradation and the carbon cycle. In anaerobic clostridial infections, these enzymes play a role in invasion and pathology.

Most of the clostridia are saprophytes but a few are pathogenic for humans. *Clostridium tetani* and *Clostridium botulinum* produce the most potent biological toxins known to affect humans. As pathogens of tetanus and food- borne botulism, their virulence is due entirely to their toxigenicity.

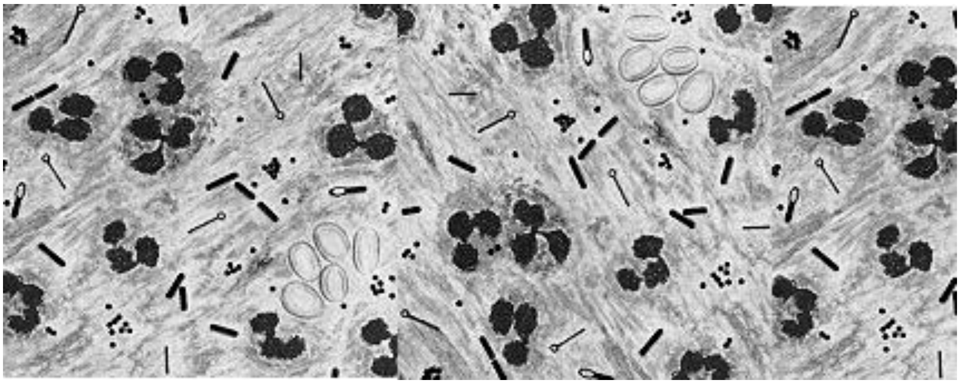

Figure (91): Stained pus from anaerobic infection containing at least three different clostridia

Clostridium perfringens

Figure (92): ***C. perfringens*** **cells**

Clostridium perfringens, which produces a huge array of invasins and exotoxins, causes wound and **surgical infections** that lead to **gas gangrene**, in addition to severe **uterine** infections. Clostridial hemolysins and extracellular enzymes such as proteases, lipases, collagenase and hyaluronidase, contribute to the invasive process.

Clostridium perfringens also produces an enterotoxin and is an important cause of **food poisoning**. Usually the organism is encountered in improperly sterilized (canned) foods in which endospores have germinated.

Figure (93): *Clostridium perfringens* (Gram Stain): most clostridia are Gram-variable

Clostridium tetani

Figure (94): *C. tetani* cells

Clostridium tetani is the causative agent of **tetanus**. The organism is found in soil, especially heavily- manured soils, and in the intestinal tracts and feces of various animals. Carrier rates in humans vary from 0 to 25%, and the organism is thought to be a transient member of the flora that depends upon ingestion. The organism produces terminal spores within a swollen sporangium giving it a distinctive drumstick appearance. Although the bacterium has a typical Gram +ve cell wall, it may be Gram -ve or Gram variable, especially in older cells.

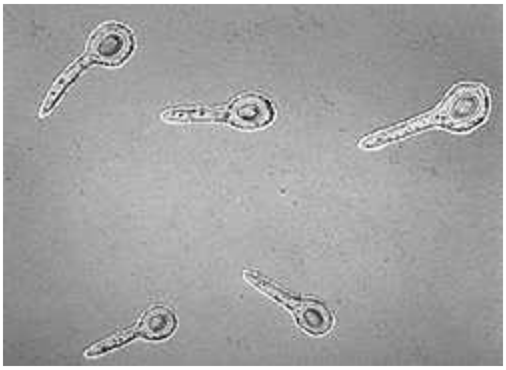

Figure (95): Characteristic terminal "drum stick" endospores of *C. tetani*

Tetanus is a highly fatal disease of humans. Mortality rates reported vary from 40% to 78%. The disease stems from a potent neurotoxin (**tetanus toxin** or **tetanospasmin**) produced when spores germinate and vegetative cells grow after gaining access to wounds. The organism multiplies locally and symptoms appear remote from the infection site. The disease is a significant problem world- wide (more than 300,000 cases annually).

Pathogenesis

Most cases of tetanus result from small puncture wounds that become contaminated with *C. tetani* spores that germinate and produce toxin. The infection remains localized often with only minimal inflammatory damage. The toxin is produced during cell growth, sporulation and lysis. It migrates along neural paths from a local wound to sites of action in the **central nervous system**. The clinical pattern of generalized tetanus consists of severe painful **spasms and rigidity of the voluntary muscles** with the characteristic symptom of "lockjaw". It is an early symptom which is followed by progressive rigidity and violent spasms of the trunk and limb muscles. Spasms of the pharyngeal muscles cause difficulty in swallowing. Death usually results from interference with the mechanics of respiration.

Neonatal tetanus accounts for about half of the tetanus deaths in developing countries. In a study of neonatal mortality in Bangladesh, 112 of 330 infant deaths were due to tetanus.

Tetanus Toxin

There have been 11 strains of *C. tetani* distinguished primarily on the basis of flagellar antigens. They differ in their ability to produce tetanus toxin (tetanospasmin), but all strains produce a toxin which is identical in its immunological and pharmacological properties. Tetanospasmin is **encoded on a plasmid** which is present in all toxigenic strains.

Tetanus toxin is one of the three most poisonous substances known, the other two being the toxins of botulism and diphtheria. The toxin is produced by growing cells and released only on cell lysis. Cells lyse naturally during germination the outgrowth of spores, as well as during vegetative growth. After inoculation of a wound with *C. tetani* spores, only a minimal amount of spore germination and vegetative cell growth are required until the toxin is produced.

Immunity

Prophylactic immunization is accomplished with tetanus toxoid. Three injections are given in the first year of life, and a booster is given about a year later, and again on the entrance into elementary school. Whenever a previously- immunized individual sustains a potentially dangerous wound, a booster of toxoid should be injected. Wherever employed, intensive programs of immunization with toxoid have led to a remarkable reduction in the incidence of the disease.

Clostridium botulinum

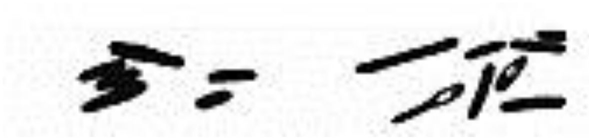

Figure (96): ***C. botulinum*** **cells**

C. botulinum is a large anaerobic bacillus that forms subterminal endospores. It is widely distributed in soil, sediments of lakes and ponds, and decaying vegetation. Hence, the intestinal tracts of birds, mammals and fish may occasionally contain the organism as a transient. Seven toxigenic types of the organism exist, each producing an immunologically distinct form of botulinum toxin. Not all strains of *C. botulinum* produce the botulinum toxin.

Food-borne Botulism

Food- borne botulism is not an infection but an **intoxication** since it results from the ingestion of foods that contain the preformed clostridial toxin. In this respect it resembles staphylococcal food poisoning. Botulism results from eating uncooked foods in which contaminating spores have germinated and produced the toxin. *C. botulinum* spores are relatively heat resistant and may survive the sterilizing process of improper canning procedures. The anaerobic environment produced by the canning process may further encourage the outgrowth of spores. The organisms grow best in neutral or "low acid" vegetables (> pH 4.5).

Clinical symptoms of botulism begin 18- 36 hours after toxin ingestion with weakness, dizziness and dryness of the mouth. Nausea and vomiting may occur. Neurologic features soon develop: blurred vision, inability to swallow, difficulty in speech, descending weakness of skeletal muscles and respiratory paralysis.

Botulinum toxin may be transported within nerves in a manner analogous to tetanospasmin, and can thereby gain access to the CNS (central nervous system). However, symptomatic CNS involvement is rare.

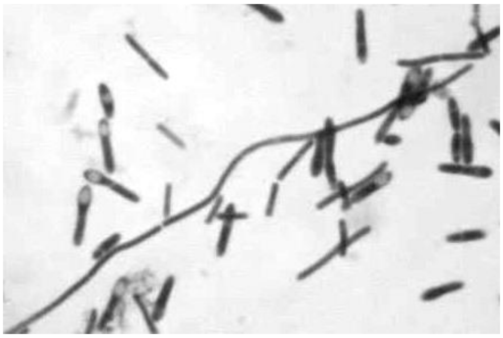

Figure (97): ***C. botulinum***

Immunity and prevention

As with tetanus, immunity to botulism does not develop, even with severe disease, because the amount of toxin necessary to induce an immune response is toxic. Repeated occurrence of botulism has been reported. Once the botulinum toxin has bound to nerve endings, its activity is unaffected by antitoxin. Any unfixed toxin can be neutralized by intravenous injection of antitoxin. Individuals known to have ingested food with botulism should be treated immediately with antiserum.

The most important aspect of botulism prevention is proper food handling and preparation. The spores of *C. botulinum* can survive boiling (100 degrees at 1 atm) for more than one hour although they are killed by autoclaving. Because the toxin is heat- labile, boiling or intense heating (cooking) of contaminated food will inactivate the toxin. Food containers that swell may contain gas produced by *C. botulinum* and should not be opened or tasted. Other foods that appear to be spoiled should not be tasted.

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(9) Diseases caused by *Bacillus cereus*

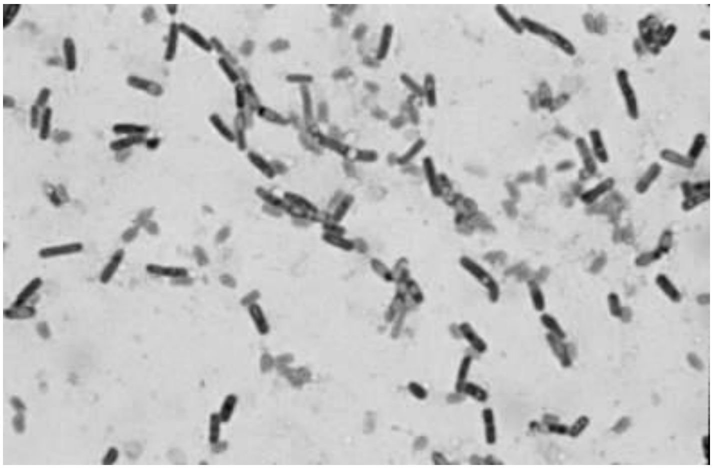

Figure (98): Spore staining of *Bacillus cereus*

Bacillus cereus has been recognized as an agent of food poisoning since 1955. *B. cereus* causes two types of **food- borne intoxications.** One type is characterized by nausea and vomiting and abdominal cramps and has an incubation period of 1 to 6 hours. It resembles *Staphylococcus aureus* food poisoning in its symptoms and incubation period. This is the "short- incubation" form of the disease. The second type is manifested primarily by abdominal cramps and diarrhea with an incubation period of 8 to 16 hours. This type is referred to as the "long- incubation" or **diarrheal form** of the disease, and it resembles more food poisoning caused by *Clostridium perfringens.* In either type, the illness usually lasts less than 24 hours after onset. In a few patients symptoms may last longer.

B. cereus food poisoning occurs year- round and is without any particular geographic distribution. The short- incubation form is most often associated with fried rice that has been cooked and then held at warm temperatures for several hours. The disease is often associated with Chinese restaurants. In one reported outbreak, macaroni and cheese made from powdered milk turned out to be the source of the bacterium.

Long- incubation *B. cereus* food poisoning is frequently associated with meat or vegetable- containing foods after cooking. The bacterium has been isolated from 50% of dried beans and cereals and from 25% of dried foods such as spices, seasoning mixes and potatoes.

Since bacteria grow best at temperatures ranging from 40 to 140°F, infection may be prevented if cold food is refrigerated and if hot food is held at greater than 140°F before serving. Nonanthrax *Bacillus* species, especially *B. cereus,* are occasionally implicated in local infections especially involving the eye such as conjunctivitis. An intra- ocular foreign body such as a metal projectile is often present, or the injury occurs in a rural or farm location where there is a greater risk of eye contamination with dust or soil. *B. cereus* is one of the most destructive organisms to infect the eye. *Bacillus thuringiensis* has also been known to infect the eye.

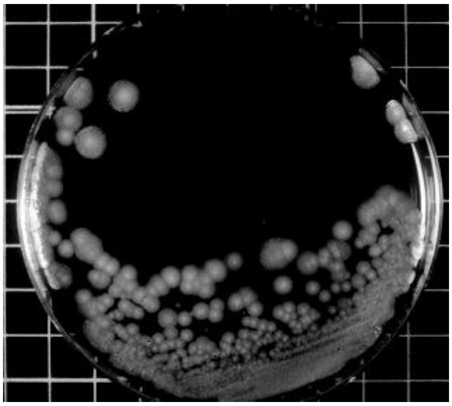

Figure (99): *Bacillus cereus* colonies on blood agar

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(10) Diseases caused by some actinomycetes (Corynebacteria)

Corynebacteria are Gram +ve, aerobic, nonmotile, rod- shaped bacteria related to the Actinomycetes.

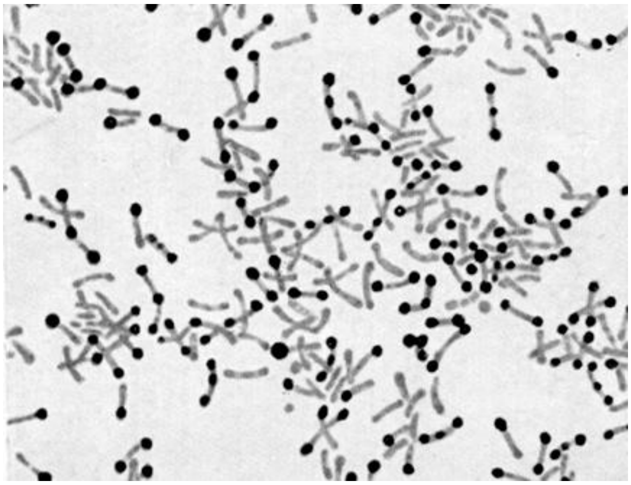

Figure (100): Stained *Corynebacterium* cells: note the presence of terminal polyphosphate inclusions called metachromatic granules. Note also the characteristic "Chinese- letter" arrangement of cells.

They do not form spores or branch but they have the characteristic irregular club- shaped or V- shaped arrangements in normal growth. They undergo snapping movements just after cell division which brings them into characteristic arrangements resembling Chinese letters.

The genus *Corynebacterium* consists of a diverse group of bacteria including animal and plant pathogens, as well as saprophytes. Some corynebacteria are part of the normal flora of humans, finding a suitable niche in virtually every anatomic site. The best known and most widely studied species is *Corynebacterium diphtheriae*, the causal agent of the disease diphtheria.

No bacterial disease of humans has been as successfully studied as diphtheria. The modes of transmission, pathogenic mechanism and molecular basis of exotoxin structure, function, and action, have been clearly established. Consequently, highly effective methods of treatment and prevention of diphtheria have been developed.

The disease

Diphtheria is "an upper respiratory tract illness characterized by sore throat, low- grade fever, and an adherent membrane of the tonsil (s), pharynx, and/ or nose". Diphtheria is a rapidly developing, infection involving both local and systemic pathology. A local lesion develops in the upper respiratory tract and involves necrotic injury to epithelial cells. As a result of this injury, blood plasma leaks into the area and a fibrin network forms, which is interweaved with rapidly- growing *C. diphtheriae* cells. This membranous network covers over the site of the local lesion and is referred to as the **pseudomembrane**.At this site they produce the toxin that is absorbed and disseminated through lymph channels and blood to the susceptible tissues of the body. Degenerative changes in these tissues, which include heart, muscle, peripheral nerves, adrenals, kidneys, liver and spleen, result in the systemic pathology of the disease.

In parts of the world where diphtheria still occurs, it is primarily a disease of children, and most individuals who survive infancy and childhood have acquired immunity to diphtheria. In earlier times, when nonimmune populations were exposed to the disease, people of all ages were infected with mortality rate of about 10%. Diphtheria is more severe for those under 5 and over 40 years of age.

Immunity to Diphtheria

Acquired immunity to diphtheria is due primarily to toxin-neutralizing antibody (antitoxin). Passive immunity is acquired transplacentally and can last at most 1 or 2 years after birth for babies. In areas where diphtheria is endemic and mass immunization is not practiced, most young children are highly susceptible to infection. Probably active immunity can be produced by a mild or inapparent infection in infants who retain some maternal immunity, and in adults infected with strains of low virulence.

Because of the high degree of susceptibility of children, artificial immunization at an early age is universally advocated. Toxoid is given in 2 or 3 doses (1 month apart) for primary immunization at an age of 3- 4 months. A booster injection should be given about a year later, and it is advisable to administer several booster injections during childhood.

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(11) Diseases caused by *Mycobacterium tuberculosis*

Tuberculosis (TB) is the leading cause of death in the world from a bacterial infectious disease. The disease affects 1.7 billion people/ year which is equal to one- third of the entire world population.

Mycobacterium bovis is the etiologic (disease- causing) agent of TB in cows and rarely in humans. Both cows and humans can serve as reservoirs. Humans can also be infected by the consumption of unpasteurized milk. This route of transmission can lead to the development of **extrapulmonary TB,** exemplified in history by bone infections that led to curved backs. Other human pathogens belonging to the *Mycobacterium* genus include ***Mycobacterium avium*** which causes a TB- like disease especially prevalent in AIDS patients, and ***Mycobacterium leprae,*** the causative agent of **leprosy**.

History and Present Day Importance

Mycobacterium tuberculosis (M. TB.) was the cause of the "White Plague" of the 17th and 18th centuries in Europe. During this period nearly 100 percent of the European population was infected with M. TB., and 25 percent of all adult deaths were caused by M. TB. (Note: The White Plague is not to be confused with the "Black

Plague", which was caused by *Yersinia pestis* and occurred about 3 centuries earlier).

General Characteristics

Mycobacterium tuberculosis is a large nonmotile rod- shaped bacterium related to the Actinomycetes. Many non pathogenic mycobacteria are components of the normal flora of humans, found most often in dry and oily locales. The rods are 2- 4 µm in length and 0.2- 0.5 µm in width.

Mycobacterium tuberculosis is an **obligate aerobe**. For this reason, in the classic case of tuberculosis, the M. TB. complexes are always found in the well- aerated upper lobes of the lungs. The bacterium is a **facultative intracellular parasite**, usually of macrophages, and has a **slow generation time**, 15- 20 hours. Two media are used to grow M. TB. **Middlebrook's medium** which is an agar based medium and **Lowenstein- Jensen medium** which is an egg based medium. M. TB. colonies are small and beige coloured when grown on either medium. It takes 4- 6 weeks to get visual colonies on either type of media.

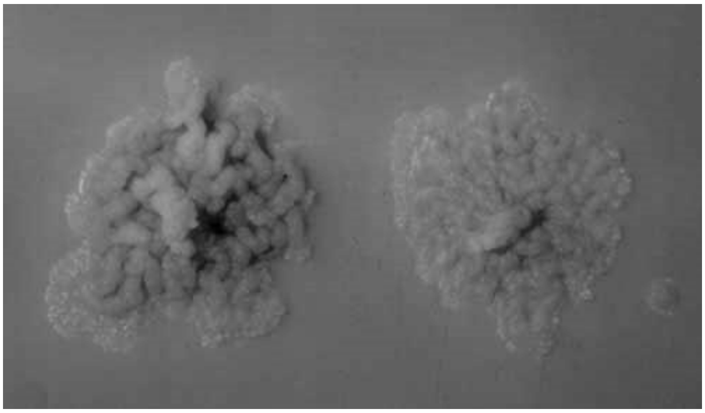


Figure (101): Colonies of *M. tuberculosis* on Lowenstein- Jensen medium

Chains of cells in smears made from *in vitro*- grown colonies often form distinctive **serpentine cords**. This observation was first made by Robert Koch who associated **cord factor** with virulent strains of the bacterium. M. TB. is not classified as either Gram +ve or Gram -ve because it does not have the chemical characteristics of either, although the bacteria do contain peptidoglycan (murein) in their cell wall. If a Gram stain is performed on M. TB., it stains very weakly Gram +ve or not at all (ghosts). *Mycobacterium* species, along with members of a related genus *Nocardia*, are classified as **acid- fast bacteria** due to their impermeability by certain dyes and stains. Despite this, once stained, acid- fast bacteria will retain dyes when heated and treated with acidified organic compounds. One acid- fast staining method for *Mycobacterium tuberculosis* is the **Ziehl-Neelsen stain**. When this method is used, the M.TB. smear is fixed, stained with carbol-fuchsin (a pink dye), and decolorized with acid-alcohol. The smear is counterstained with methylene- blue or other dyes. Acid- fast bacilli appear pink in a contrasting background.

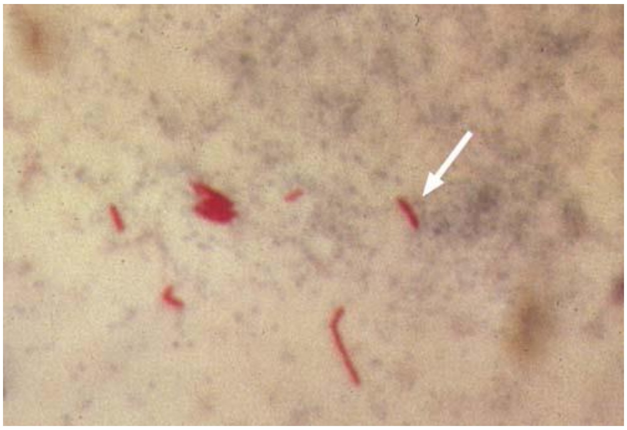


Figure (102): Acid- fast staining of *M. tuberculosis*

Cell Wall Structure

The cell wall structure of *M. tuberculosis* deserves special attention because it is unique among procaryotes and it is a major determinant of virulence for the bacterium. The cell wall complex contains **peptidoglycan**, but otherwise it is composed of complex lipids. Over 60% of the mycobacterial cell wall is lipid. The lipid fraction of the cell wall consists of three major components: 1) **Mycolic acids** that are strong hydrophobic molecules forming a lipid shell around the organism and affect permeability properties at the cell surface, 2) **Cord Factor** which is responsible for the serpentine cording mentioned above, and 3) **Wax- D** in the cell envelope. In summary, the high concentration of lipids in the cell wall of *M. tuberculosis* has been associated with these properties of the bacterium:

- Impermeability to stains and dyes
- Resistance to many antibiotics
- Resistance to killing by acidic and alkaline compounds
- Resistance to osmotic lysis
- Resistance to lethal oxidations and survival inside of macrophages

The Disease Tuberculosis

TB infection means that M. TB. is in the body but the immune system is keeping the bacteria under control. The immune system does this by producing macrophages that surround the tubercle bacilli. The cells form a hard shell that keeps the bacilli contained and under control. Most people with TB infection have a positive reaction to the **tuberculin skin test**. People who have TB infection but not TB disease are not infectious, i.e., they cannot spread the infection to other people. These people usually have a normal chest X- ray.

Treatment

Because administration of a single drug often leads to the development of a bacterial population resistant to that drug, effective treatment of TB must contain multiple drugs to which the organisms are susceptible. When two or more drugs are used simultaneously, each helps prevent the emergence of tubercle bacilli resistant to the others. Hence, tuberculosis is usually treated with four different antimicrobial agents. The course of drug therapy usually lasts from 6- 9 months. The most commonly used drugs are rifampin isoniazid, pyrazinamide and ethambutol or streptomycin.

Prevention

A vaccine against M. TB. is available called **BCG** (Bacillus of Calmette and Guerin, after the two Frenchmen that developed it). BCG consists of a live attenuated strain derived from *M. bovis*. This strain of *Mycobacterium* has remained avirulent for over 60 years. The vaccine is not 100% effective. Studies suggest a 60-80% effective rate in children. Some disadvatages of the vaccine are:

• The vaccine cannot avoid disease reactivation in previously exposed individuals.

• The vaccine does not prevent infection, only disease.

End of Lecture (11)

SOIL MICROBIOLOGY

STUDYING SOIL MICROBIOLOGY

Studying soil microbiology is very important because without soil microorganisms, soil would be a sterile, largely inert rooting medium. They are responsible for most of the activity in soil.

A simple definition of soil microbiology is "the study of organisms that live in the soil; their metabolic activity; their roles in energy flow; their roles in nutrient cycling". The soil Science Society of America (SSSA) defines soil microbiology as "the branch of soil science concerned with soil- inhabiting microorganisms, their functions and activities".

Studying soil microbiology can be carried out by two basic approaches:

1- Studying the organisms in soil by examining their:

- Physiology: how they grow and metabolize.
- Taxonomy: their morphology and relations to each other.
- Pathology: how they cause diseases to plants, animals and humans.
- Symbioses: how they interact with more complex organisms.

2- Microbial activities (processes) in soil:

- Biogeochemistry: how they affect our environment chemically.
- Nutrient cycling: how they recycle compounds in soil.
- Global change: how they affect global properties such as temperature and atmospheric chemistry.

- Ecology: how they interact with their environment and with other microorganisms.

Considering that these categories may overlap, we can not study soil microbiology without taking both approaches.

Current topics in soil microbiology

The following are examples of some research fields that many microbiologists adopt and pursue in many famous universities and institutes:

- Symbiotic nitrogen fixation.
- Organic matter decomposition (waste removal and composting).
- Mineral nitrogen transformations such as nitrification, denitrification and ammonification.
- Rhizosphere studies (root/ soil/ microorganism interactions).
- Soil enzymes (ureases, cellulases, ligninases, phosphatases).
- Biodegradation and bioremediation.
- Metal transformation.
- Carbon cycling.
- Greenhouse gases and atmospheric pollution (production of methane, carbon dioxide, nitric oxide, nitrous oxide).
- Release and monitoring of GEM's (genetically engineered microorganisms).
- Microbial ecology.
- Subsurface microbial activity.

BACTERIA IN SOIL

Filamentous actinomycetes

While actinomycetes are like fungi in their morphology, there are two important characteristics that distinguish them from fungi:

1. Actinomycetes have no cell nucleus (prokaryotic).
2. Actinomycetes form hyphae that are from 0.5 to 1 µm in diameter (much smaller than fungal hyphae which are 3 to 8 µm in diameter).

Actinomycetes are not photosynthetic. Most of them are saprophytes, grow by decomposing organic matter. Some actinomycetes are pathogenic to humans such as *Mycobacterium tuberculosis* that causes tuberculosis. But, on the other hand, most of the actinomycetes are harmless soil microorganisms. Some of them are beneficial such as the members of the genus *Frankia* that form associations with woody, non- leguminous (actinorhizal), woody shrubs and trees and fix atmospheric nitrogen.

Actinomycetes compose 10% to 50% of the total microbial population in soil. They are commonly found in soil, compost and sediment. They are second in abundance to bacteria in soil ranging from about 10^5 to 10^8 propagules/ gm of soil. A propagule is any part of any microorganism that can grow and reproduce.

II. Other bacteria

Bacteria are more numerous in soil than all other organisms combined, with the exception of viruses. There are over 200 identified bacterial genera and a single soil sample may have over 4000 genetically distinct bacteria. But it is estimated that less than

1% of bacterial species are culturable, so the actual diversity of soil bacteria is much greater.

- Indigenous bacteria (autochthonous bacteria) are the true permanent residents of a given soil.
- Foreign bacteria (allochthonous bacteria) are invaders or transients entering soil by precipitation, diseased tissue, manures or other human activities. They may persist and grow but rarely contribute to significant biological activities.
- Counting bacteria in soil is difficult because no single culture medium is adequate for all groups. Part of this difficulty occurs because minor deviations in the environment may have great influences on bacterial populations when grown on solid media (microenvironment). Bacterial presence in soil is less than 10% of the biomass in soil that include other microorganisms, animals and others.
- The most common bacteria isolated from soil are *Arthrobacter* (an actinomycete), *Bacillus* and *Pseudomonas*. *Arthrobacter* is common because it can grow on diverse substrates. *Bacillus* species represent 7 to 67% of the soil isolates. Species of both genera are spore- formers which explain their persistence in diverse habitats. *Bacillus* survive a pH range from 2 to 8 and temperature from –5 to 75˚C. *Pseudomonas* species represent 3 to 15% of soil isolates. They are noted for their diverse metabolism, particularly their ability to degrade organic chemicals like pesticides. Some *Pseudomonas* species are pathogenic.

- Other soil microorganisms include viruses, mycoplasmas, viroids and prions.

SOIL MICROBIAL INTERACTIONS (DEFINITIONS)

1- Neutralism

Two microorganisms behave entirely independent of one another. There is no effect of one organism on the other- no direct interaction.

2- Commensalism

Only one microorganism actually benefits from the interaction. Commensal relationships are common but not obligatory. An example is yeast that can reduce the osmotic potential for more sensitive organisms or microorganisms that dissolve minerals and release nutrients for other organisms.

3- Amensalism

In which one species is suppressed while the other is not affected. Amensalism is the opposite of commensalism. Modifying an environment to inhibit another organism's growth, for example, is amensal.

4-Mutualism (including: symbiosis, synergism, and protocooperation).

- Mutualism implies a relationship between microorganisms that is mutually beneficial.
- Symbiosis implies only a stable relationship between two organisms that is not necessarily beneficial. However, symbioses are almost always beneficial relationships. It may or may not be

obligatory. The most common examples are *Rhizobium*- legume and *Frankia*- actinorhizal symbioses.

- Synergism Synergistic relationships are not obligatory. These are associations of mutual benefit to both species, but their cooperation is not obligatory for their existence or their performance. Herbicides are often degraded synergistically; one microorganism may degrade one part, while another microorganism may degrade another part.
- Protocooperation Microorganisms can cooperate with one another (protocooperation). The fact that microbial populations form colonies is probably evidence that they have made adaptations based on cooperative interactions. Two *Escherichia coli* colonies merging together eventually begin enzyme induction at the same time. Extracellular enzyme production also results in better substrate use in colonial population compared to individual cells.

5-Competition

It is a condition in which two organisms suppress each other's growth as they compete for limited nutrients.

6-Predation and parasitism

Each major group in the soil community has parasites living on or in its cells. Bacteria have bacteriophages, for example, while fungi and bacteria both parasitize themselves. Predation is the attack of one organism on another. Bacteria are preyed upon by protozoa, viruses, slime molds and other bacteria such as *Myxobacteria*. Nematodes, also, are preyed upon by nematode- trapping fungi and other soil animals.

7- Succession

It is very common in microbial communities. It occurs in the degradation of complex polymers. It occurs in compost piles and in the fermentation of silage. It can be confused with synergistic relationships.

NITROGEN FIXATION

In nitrogen fixation, atmospheric N (N_2) is converted into organic N by the enzyme complex nitrogenase. Nitrogen fixation is carried out by microorganisms both free- living and in association with higher plants

Symbiotic nitrogen fixation

Nitrogen fixation occurs in legume and non- legume nodules. The most important nodule- forming associations are the actinorhizal nodules that play an important role in forest fertility and the rhizobial nodules that form on legumes in cropland.

3- *Frankia*- actinorhizal symbiosis

Actinomycetes of the genus *Frankia* form N_2- fixing nodules on about 80 species of trees and shrubs. They are very important for rehabilitation and cultivation of harsh environments such as mine spoils and desertified areas. Based on plant- trapping studies, *Frankia* populations can range from 0 to 4600 infectious units (IU) per gram of soil. Their populations tend to be higher in slightly alkaline soils and in the rhizosphere of some plants that may never form nodules. Typical plants that *Frankia* nodulate are pioneering species such as *Alnus* that grow in moist environments. Other plant species include *Ceanothus* (California), Myrica that colonize eroded slopes and mined areas and *Casuarina* that dominate in the southern hemisphere.

- Nitrogen- fixing actinorhizal nodules are not studied as much as other N_2- fixing associations because, until recently, it was impossible to grow the infective *Frankia* apart from their host. The first isolate of *Frankia* was obtained in 1978. It is also difficult to get *Frankia* isolate to re- infect their hosts. Growing host plants is also time- consuming and it is difficult to work with perennial shrubs and trees than with legumes.
- Three distinct host groups (host infectivity groups) can be identified based on the infection pattern: strains that form nodules on *Alnus*, strains that form nodules on *Elaeagnus*, and strains that form nodules on *Casuarina*. There are some interactions between these groups as indicated by a thorough study in 1987.
- *Frankia* cells has three characteristic shapes namely: hyphae, spore- bearing sporangia, and vesicles. Vesicles are the main sites for nitrogenase and nitrogen fixation. Some *Frankia* strains are infective (form nodules) but ineffective (can not fix nitrogen). The capacity for nitrogen fixation varies widely among *Frankia*- host combinations.

4- *Rhizobium*- legume symbiosis

- The best- studied symbiosis in agricultural ecosystems comes form the association of rhizobia and higher plants such clover, soybeans and broad beans. It is worth mentioning that there are 7000 genera and 14000 species of legumes, and perhaps 100 agricultural important legumes are used, but not all legumes were studied for their ability to fix nitrogen.

SOIL MICROORGANISMS AND THE ENVIRONMENT

1- COMPOSTING

- Composting is a microbial process in which organic waste is converted into stable humus- like substances suitable for land applications.

Figure (103): A finished (ripened) compost

- Initial sorting of waste separates organic and inorganic fractions (compostable and non-compostable waste). Magnets can remove ferrous materials; mechanical separators can separate glass, aluminum, and plastic into recyclable and non- recyclable fractions. Recycling these fractions helps improving the composting process (a minor advantage) and reduces the amount of solid waste disposal in landfills (a major advantage). One of the principal advantages that cities have for composting is to extend the working lives of their landfills.
- The remaining organic waste is ground up to increase the surface area, amended with sludge, soil or old compost (as an inoculum),

mixed bulking agents such as shredded newspapers, wood chips, and anything that decomposes slowly, to provide porosity.

- Composting is accomplished in windrows, aerated piles and continuous feed reactors. Aerated piles method is the faster and less- costing method. It is appropriate for countries where sophisticated technologies are not available. In this method, perforated pipes are buried in a pile and air is either pumped inside or drawn through the piles by vacuum. The air stream oxygenates and cools the compost. This is important, since composting is an aerobic rather than an anaerobic process. The heat generated by a compost pile can be used to dry the final product.

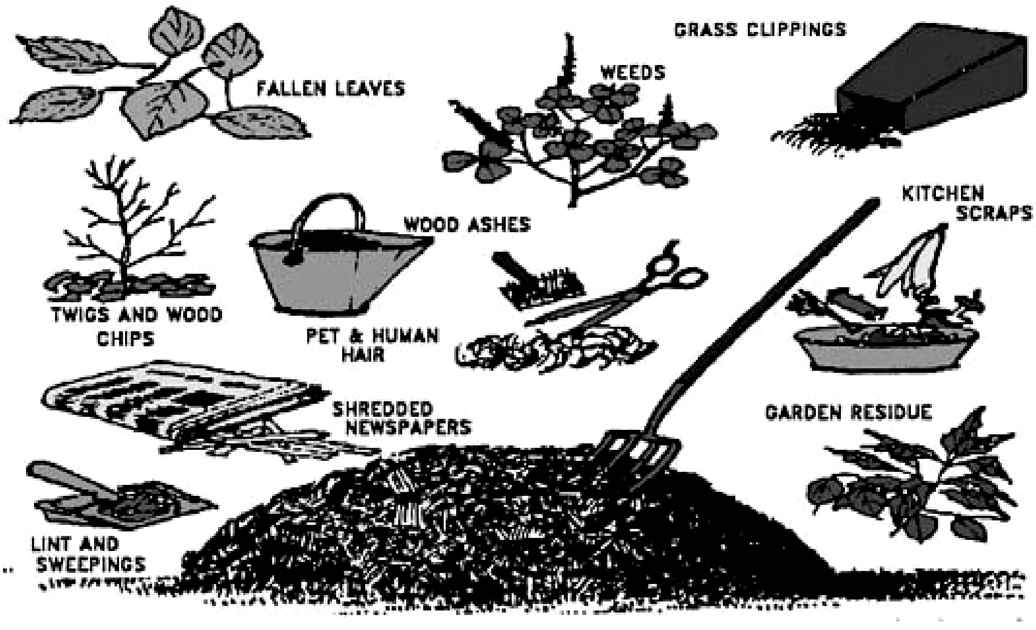


Figure (104): Different components for composting

The microbiology of composting

Composting is initiated by mesophilic chemoheterotrophs (mainly bacteria and fungi). As they respire, the temperature in the pile increases and they are replaced by thermophilic organisms. The heat is produced by aerobic oxidation of the waste. Eventually, the temperature of the compost declined as the available substrates are consumed and mesophiles re- establish themselves. The temperature may rise to 76 to 80ºC. This temperature is too high and kills the organisms that are responsible for the composting process. On the other hand it is advantageous in killing the pathogenic organisms. Thus, aerating, watering, or turning a compost pile prevents excessive self- heating and speed up composting (for every 10ºC rise, microbial activity increases two to three- fold). It also kills the pathogenic organisms in compost which is particularly important for composted sewage sludge and manure. Turning compost piles is also important for making the final product more uniform, or otherwise, the thermophilic decomposition would be restricted to the pile core.

- Examples of the important bacteria in compost are species like ***Bacillus stearothermophilus, Clostridium thermocellum***, the genera *Thermomonospora*, and *Thermoactinomyces*.
- **Optimal composting** The optimal composting requires considering the following factors:
- The type and composition of the organic waste.
- The availability of microorganisms.
- Aeration.
- The C, N and P ratios.
- Moisture content.
- Temperature.

- The pH and time.

Table (43): Carbon/ nitrogen ratios for composting organics

Sandy loam (fine)	7:1
Humus	10:1
Food scraps	18:1
Alfalfa hay	10:1
Grass clippings	12-25:1
Coffee grounds	20:1
Vegetable trimming	12-20:1
Cow manure	20:1
Horse manure	25:1
Horse manure with litter	60:1
Rotted manure	20:1
Poultry manure (fresh)	10:1
Poultry manure with litter	18:1
Sandy loam (coarse)	25:1
Oak leaves (green)	26:1
Leaves, varies	35-85:1
Peat moss	58:1
Corn stalks	60:1
Straw	80:1
Pine needles	60-110:1
Farm manure	90:1
Newspaper	50-200:1
Douglas fir bark	491:1
Sawdust, weathered 2 months	625:1

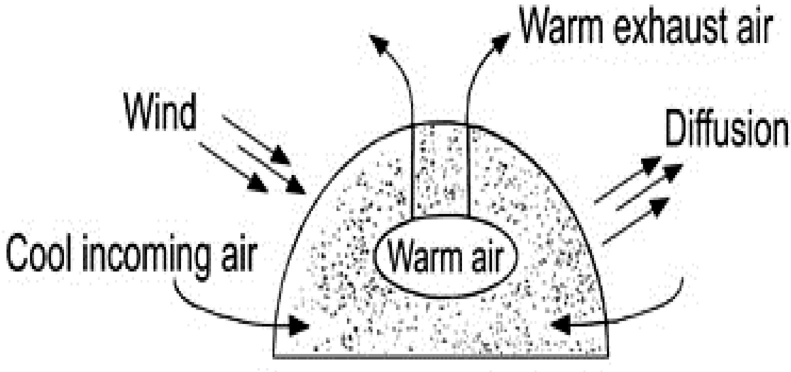
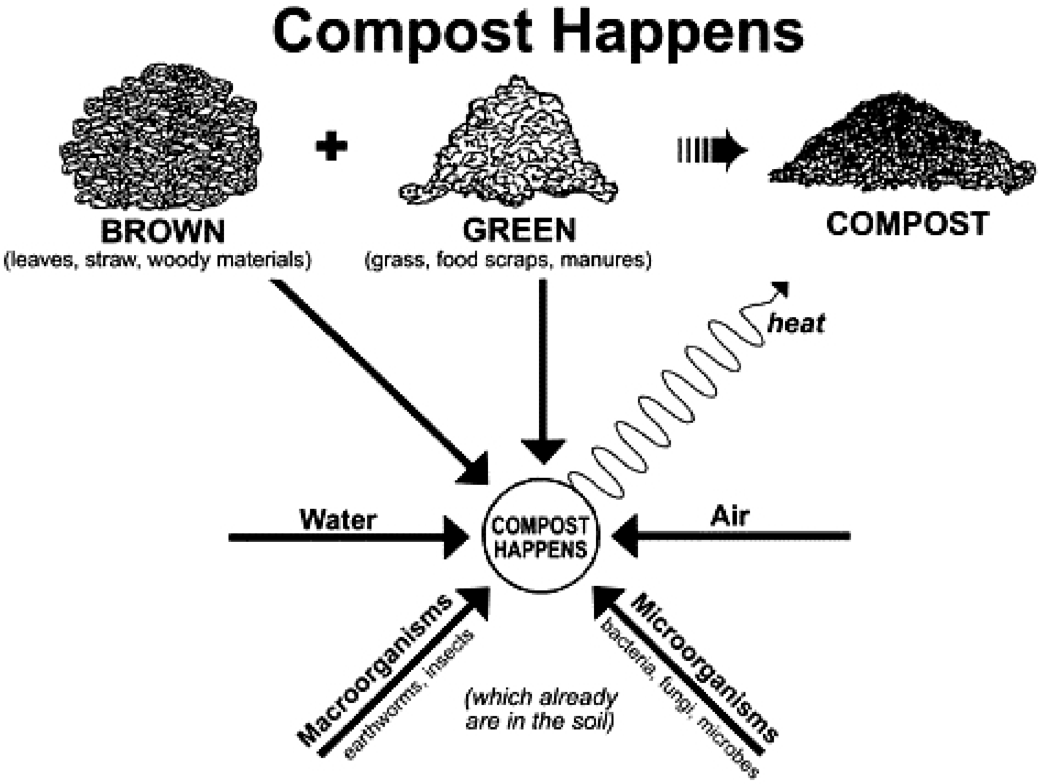


Figure (105): Aerated pile composting



An overall illustration of the composting process

2- <u>BIOREMEDIATION</u>

Bioremediation is also called biodegradation enhancement and includes any purposful use of microbes to degrade unwanted substances in the environment.

- All natural compounds are biodegraded in favourable environmental conditions. The term “xenobiotic” refers to artificial compounds that are foreign to biological systems (containing structures and bonds that do not occur in biological systems). Xenobiotics may be polymers, gases, polychlorinated or polybrominated compounds or pesticides.
- Biodegradation refers to the process in bioremediation by which xenobiotics are transformed into less toxic states. Just because a compound is biodegraded does not mean that the breakdown products are less toxic. Mineralization usually means decomposition of a xenobiotic to inorganic ions and CO_2. This is the most desirable situation because the end products are usually non- toxic.

<u>Natural products</u>

- **Petroleum**: certain bacteria (some cyanobacteria, pseudomonads, corynebacteria, mycobacteria), green algae and fungi (several molds and yeasts) oxidize hydrocarbons at aerobic water/ oil interfaces (with optimal conditions, up to 80% removal within 1 year after a spill).
- **Biodegradable plastics including**:

1. **photobiodegradable-** structure of polymer altered by UV light in sunlight so that it is now amenable to biodegradation.
2. **biochemically biodegradable starch- linked polymers-** starch- digesting bacteria in soil attack the starch, releasing polymer fragments which are degraded by other microbes.

- **Xenobiotics**
- Chemically synthesized compounds not found in nature (pesticides, synthetic polymers, etc.) and thus are unlikely degradable by naturally existing microorganisms. These products tend to persist in nature and many nations are working to ban the use of many of them. **Microbes that can degrade xenobiotics are rather diverse and typically include both bacteria and fungi.** Recalcitarnt molecules are fossil organic matter (humus), polyaromatic compounds (tannins and lignins), persistent microorganisms (endospores and melanin- rich fungi), synthetic molecules (fungicides, nematicides, herbicides, insecticides), polhalogenated biphenyls (flame retardants and solvents), plastics, and detergents. The persistence of xenobiotics ranges from days to years and minor alterations in biodegradable compounds can render them recalcitrant.
- **PCBs-** certain *Pseudomonas* species have been engineered to accelerate breakdown of polychlorinated biphenyls (formerly used by electric industry as transformer insulation).
- **PAHs** (poly aromatic hydrocarbons) can be difficult to degrade, but there are microbes in the environment that can accomplish this task, especially when working together (synergistically).
- **Pesticides** – **herbicides, insecticides and fungicides**: these are typically rather complex molecules.
- Some xenobiotics are good carbon sources and electron donors for soil microbes, so they are more readily degraded than others.

- Other xenobiotics, such as chlorinated insecticides, are recalcitrant to degradation; thus they have rather long persistence times in the environment.
- **Lindane** – 3 years for 75-100% disappearance
- **DDT** – 4 years for 75-100% disappearance
- **Chlordane** – 5 years for 75-100% disappearance
- To degrade these xenobiotics, microbes may employ co-metabolism, in which an organic material other than the xenobiotic is used as the primary energy source and the xenobiotic is degraded as a secondary process.

Genetically engineered microbes in bioremediation

- Microbes can be "engineered" to carry out the biochemical processes needed for bioremediation. There are concerns about long- term of the effects of genetically engineered microbes on the environment.
- Naturally- occurring microbes bioremediate just as well as engineered microbes in many cases and it is important to adjust environmental conditions to favor their growth.
- **Approaches to bioremediation**: There are two general approaches to bioremediation:

1. Environmental modification- for example, improving the potential activity of existing microorganisms through fertilization and aeration.
2. Addition of appropriate and sometimes selective microorganisms.

- There are several advantages to bioremediation compared to waste disposal. The end products are generally non- toxic if complete mineralization occurs. Other biological activity in the contaminated site is left relatively undisturbed. Bioremediation is inexpensive compared to physical methods for cleanup, and bioremediation requires simple equipment.
- There are many disadvantages of bioremediation. A high waste concentration is sometimes needed to stimulate growth of the bioremediating microorganisms, and therefore, getting rid of more quantities of the waste. There are limits to the materials that can be treated because they may be inherently toxic or recalcitrant. Bioremediation is limited by the environmental conditions existing at the contaminated site. The process is also limited by many factors such as temperature, humidity, acidity and the availability of specific organisms. Bioremediation is limited by the time available for treatment; it takes longer to bioremediate a contaminated site than to treat it by chemical or physical means.

Examples of bioremediation

5- Bioremediation of petroleum.

Bioremediation has its greatest application in treating petroleum spells in water or soil. Fertilization approaches appeared to be much successful at removing oil form beaches while some companies now specialize in developing specific microbial strains for oil degradation in soil and water. These companies supply mixtures of hydrocarbon- degrading *Pseudomonas* to bioremediate contaminated sites. In winter, they provide a mixture of psychrophilic hydrocarbon degraders.

Undegraded hydrocarbons do not readily leach, so bioremediation has the potential for bioremediating fuel- contaminated soil. Although remediation os gasoline in surface soil probably occurs through volatilization as much as anything, less volatile fuel compounds can be reduced by 90% in 300 days through land farming. Three important considerations of land farming petroleum to bioremediate are: 1) The material is incorporated shallowly (such as oily sludge) because this is where the maximum microbial activity is and it makes it easier to mix in additional fertilizer N and P and aerate soil by tillage; 2) the oily sludge has an extremely high hydrocarbon/ nutrient ratio, so, unless more fertilizer N is added, the hydrocarbon degraders will immobilize all of the available N and P in soil and the biodegradation of the oily waste itself will be slowed; and 3) the application rate is kept below 10%. Application rate that start to affect the physical structure of soil, and aeration in particular, will decrease biodegradation. At higher application rates, toxic components of the waste inhibit the microbial population.

6- Bioremediation of waste gases

Bioremediation of gases in air is a common practice. To remove volatile compounds from air, biofilters, trickle filters and bioscrubbers are used. These scrubbers remove H_2S, dimethyl sulphide, terpene, orgnic sulphur gases, ethyl benzene, tetrachloroethylene and chlorobenzene from air streams. Adsorption of these gases into biofilms or beds is also used. Classic biofilter materials in use include peat, compost, bark, and soil. The soil layer placed on top of compost heaps, for example, is there to absorb foul odours that may be produced within the compost. It is nothing more than a biofilter. The waste gases are filtered by adsorption followed by biodegradation since the biofilter material becomes a rich microbial community.

____***End of Lecture (12)***

CLASSIFICATION & IDENTIFICATION OF BACTERIA

(1) CLASSIFICATION (TAXONOMY)

- Earlier methods for classifying bacteria relied on morphological characters as the main criteria (phenotypic characters). It is important to cosider the genotypic characters as well as the phenotypic ones. The morphological characteristics of colonies and cells (i. e. shape size, different staining methods, etc.), are very important as preliminary and essential identification of a bacterium.
- Classification should be phyletic or based on the natural relationships between organisms. The modern phyletic classification of prokaryotes is based on molecular criteria. Organisms should be classified according to their nucleic acids (differences and similarities). In the following paragraphs, we will discuss some of these criteria.

(1) The GC ratio of DNA (GC%).

The amount of guanine and cytosine as percentage of the total nitrogen bases is determined. This percentage is extracted from the following equation:

(guanine + cytosine) / (guanine + cytosine + adenine + thymine) × 100%.

However, similar values do not necessarily mean a close taxonomic relationship but widely differing values suggest the absence of such a relationship.

(2) <u>Sequences of nucleotides</u>

The heredity of an organism is maintained because the chromosome is copied from generation to generation successfully. Chromosomes are classified according to the unique sequences of nucleotides. If we examine a specific sequence in either DNA or RNA, we will be able to compare the differences and similarities and , may be, an evolutionary relationship.

(3) <u>DNA- DNA hybridization</u>.

Hybridization is the base- pairing between strands from different organisms. It is classified as a method of DNA sequencing as described previously. DNA hybridization compares the sequence that encode phenotypic characters and various sequences in the chromosome which are not expressed phenotypically by cell. The relationships between different organisms, therefore, are clarified by this method.

More advanced methods are used for bacterial classification now including 16s RNA, DNA fingerprinting, PCR techniques (polymerase chain reaction, or repeated replication of a given sequence), restriction fragment length polymorphism (RLFP) and PCR- RFLP, etc.

(2) <u>IDENTIFICATION OF BACTERIA</u>

The basic steps in identification are as follows:

1- Obtaining the organism in pure culture.

2- Studying morphological and metabolic characteristics.

3- Matching the results with known and named species.

The last step will be time- consuming while rapid methods are now widely used for the same purpose such as the nucleic– acid- based tests. The identification will be easier if the bacteriologist (i) has knowledge of the types of organisms usually found in a given environment and (ii) is familiar with the main distinguishing features of the common families, genera and species of bacteria.

Therefore, we can summarize the preliminary identification steps as follows:

1) Obtaining pure culture.
2) Staining with different stains, especially Gram's staining.
3) Morphology (i. e. coccus, bacillus, filamentous, etc.).
4) Motility.
5) Endospore formation.
6) Growth under aerobic or anaerobic conditions.
7) Production of the enzymes catalase.

After this preliminary identification is carried out, more tests can be done to narrow the range of organisms to which the isolated one will be compared. These methods may include both traditional and/ or advanced methods that were described previously.

BACTERIAL GROUPS

According to these above- mentioned methods, and the relation to their environment, nineteen bacterial groups were identified as follows:

(1) <u>Phototrophic bacteria</u>

Include those bacteria that perform photosynthesis. This group is mainly subdivided into oxygenic (producing oxygen as a by-product) and non- oxygenic that do not produce oxygen. Phototrophic bacteria may contain chlorophyll A as those in the higher plant and algae such as cyanobacteria while others have their own chlorophyll (bacteriochlorophyll) such as purple sulphur and non- sulphur bacteria.

(2) Gliding bacteria

Include those showing gliding movement on solid surfaces or on air- water interface, but never inside liquids. They are Gram negative such as *Myxococcus* and *Cytophaga*.

(3) The sheathed bacteria

Cells are contained in a tubular sheath such as *Sphaerotilus*.

(4) The budding and appendaged bacteria

Example on budding bacteria is *Pasteuria* and appendaged bacteria (forming appendages or prostheca) such as *Caulobacter*.

(5) The spirochetes

Include flexible bacteria that have outer envelope within which two or more fibrils are found. Examples are *Spirochaeta*.

(6) Spiral and curved bacteria

Include non- flexible bacteria but rigid spiral bacteria such as *Spirillum*.

(7) Gram negative aerobic rods and cocci

Include important genera such as *Pseudomonas*, *Azotobacter* (free nitrogen fixers), *Rhizobium* (symbiotic nitrogen fixers), *Methylomonas* (methane oxidizers) and *Halobacterium* (salt-tolerant bacteria).

(8) Gram negative facultative anaerobic rods

Include for example the family Enterobacteriaceae (e.g. *Escherichia*, *Salmonella*, *Klebsiella*, *Shigella*, *Erwinia*, etc.), Vibrionaceae (e. g. *Vibrio*) and some other genera.

(9) <u>Gram negative anaerobic bacteria</u>

Some of them are found in the natural cavities of man and animals such as the genera *Bacteroides*, and *Fusobacterium*.

(10) <u>Gram negative cocci and coccobacilli</u>

e.g. *Neisseria* (aerobic or facultatively anaerobic), *Morexella* (strictly anaerobic) and *Acinetobacter*.

(11) <u>Gram negative anaerobic cocci</u>

e.g. *Veillonella* (parasitic to man and animals).

(12) <u>Gram negative chemolithotrophic bacteria</u>

Members of this group use CO_2 as the sole carbon source and, hence, they are very important for the biogeochemical cycle of carbon in nature. Examples are the nitrifying bacteria (e.g. *Nitrosomonas* and *Nitrobacter*), Bacteria that oxidize sulphur compounds (e. g. *Thiobacillus*) bacteria that oxidize iron compounds (e.g. *Siderocapsa*).

(13) <u>Methane- producing bacteria</u>:

Those bacteria are able to produce methane from CO_2 such as *Methanobacterium*.

(14) <u>Gram positive cocci</u>

Spherical bacteria that divide in more than one plane to give cluster of cells and all are chemoorganotrophic. Examples are the genera *Micrococcus*, *Staphylococcus*, *Streptococcus* and *Sarcina*.

(15) Endospore- forming rods and cocci

Include the genera of the spore- formers *Bacillus*, *Sporolactobacillus*, *Clostridium* and *Sporosarcina*.

(16) Gram positive asporogenous rod- shaped bacteria

One genus is the representative of this group, *Lactobacillus*.

(17) Actinomycetes and related organisms

Including the genera *Actinomyces* (no mycelia), *Mycobacteria* (no mycelia and cause diseases to human and animals such as tuberculosis), *Frankia* (symbiotic nitrogen fixers with non- leguminous or actinorhizal plants and spore- formers), *Dermatophilus* (transverse and longitudinal division of mycelia), *Nocardia* (cause diseases to human and animals and mycelia divide into bacillary elements), *Streptomyces* (famous of peculiar spore chains and production of antibiotics such as streptomycin and tetracycline) and *Micromonospora* (spore formed singly or in short chains).

(18) The Rickettsias

Examples for this group are *Rickettsia* (species that cause human diseases that are transmitted through vectors such as rats and flea) and *Chlamydia* (species cause diseases to animals and human such as psittacosis lymphogranuloma and trachoma).

(19) The Mycoplasmas

Include those bacterial cells without walls such as *Mycoplasma* (cause atypical pneumonia to man) and *Acholeplasma*.

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South Valley University
Faculty of Science
Department of Botany

Time: 3 hrs
3rd year/ Chem- Microbiol.
Bacteriology.

January 2011

a) Answer the following question: (75 marks)

1) A- Discuss the main differences between Gram +ve and Gm -ve cell wall.

   B) What is the role of bacterial capsule in its environment?

---

b) Answer two only of the following questions: (50 marks each)

2) Give short notes on each of the following: bacterial growth curve- synergism and antagonism between antibiotics- bacterial endospore formation- The main differences between eukaryotic and prokaryotic cells.
3) Write briefly on antiseptics, disinfectants, preservatives and the different methods of controlling bacterial growth with one example in details.
4) Discuss each of the following: antibiotic sensitivity test- greenhouse effect- different methods of bacterial taxis- methods of bacterial motility- methods of bacterial growth determination.

BEST WISHES

South Valley University
Faculty of Science
Department of Botany

Time: 3 hrs
3rd year/ Chem- Microbiol.
Bacteriology.

January 2012

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a) Answer the following question: (75 marks)

Discuss each of the following:

5) The role of Gm -ve bacterial outer membrane in pathogenicity.
6) The basis of bacterial resistance to antibiotics
7) The differences between batch and continuous cultures
8) The modes of energy- generating metabolism in prokaryotes that does not exist in eukaryotes.

---

b) Answer two only of the following questions: (50 marks each)

9) Give short notes on two different methods for bacterial staining and three methods for detecting bacterial motility.
10) A) Explain how bacteria build up peptidoglycan macromolecules?

    B) Discuss the importance of both bacterial cell wall and membrane.
11) Define each of the following: the main differences between fermentation and respiration- biomining- PCR technique- types of solute transport systems in bacteria- mutation, mutagen and mutant.

BEST WISHES

South Valley University
Faculty of Science
Department of Botany

Time: 3 hrs
3rd year/ Chem- Microbiol.
Bacteriology.

January 2014

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a) Answer the following question: (75 marks)

Discuss each of the following:

12) Explain how bacterial growth can be reactivated after being stopped by X ray or UV radiation?.
13) The role of capsular material in the environment
14) The structure of flagella, its role and types of flagellation in motile bacteria
15) The synthesis of peptidoglycan.
16) The effect of water availability on bacterial growth

---

b) Answer two only of the following questions: (50 marks each)

17) Give short notes on bacterial growth curve and different growth phases.
18) A) Explain the difference between disinfectants and antiseptics and discuss two different methods of sterilization?

    B) Discuss the bases of bacterial resistance to antibiotics.
19) Discuss in brief three different types of fermentation in bacteria.

BEST WISHES

جامعة جنوب الوادى

<u>كلية العلوم</u>

الرؤيـــة

كلية العلوم بقنا تقدم خدمات تعليمية وبحثية ومجتمعية متميزة

الرســالة

تلتزم كلية العلوم بقنا باعداد خريجين متميزين طبقا للمعايير الأكاديمية القومية وتقديم بحوث علمية متميزة وتطوير مهارات وقدرات الكوادر البشرية بها وتوفير خدمات مجتمعبة وبيئية تلبى طموحات كجتمع جنوب الوادى وذلك من خلال مشاركة مجتمعية فاعـلة