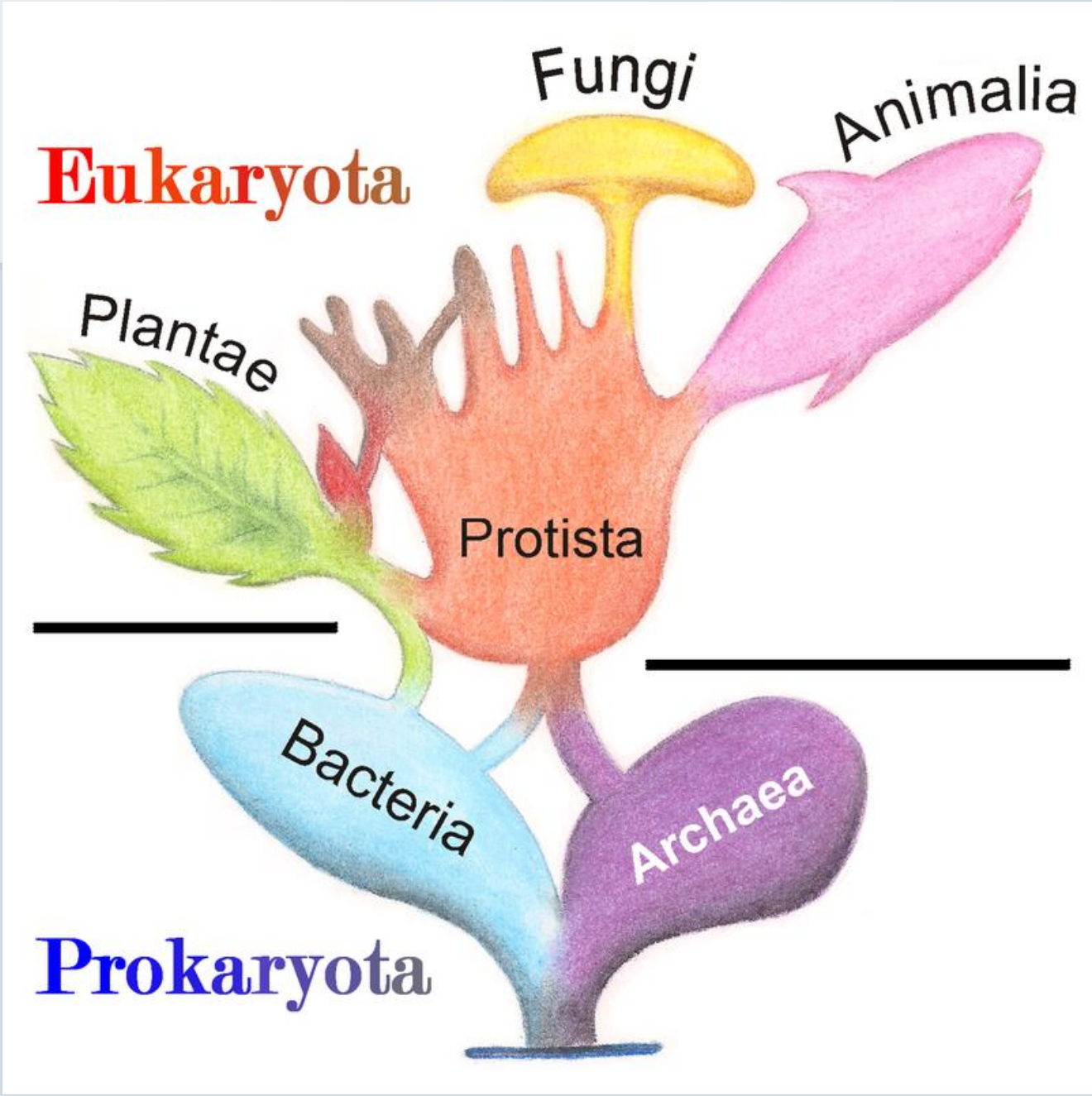
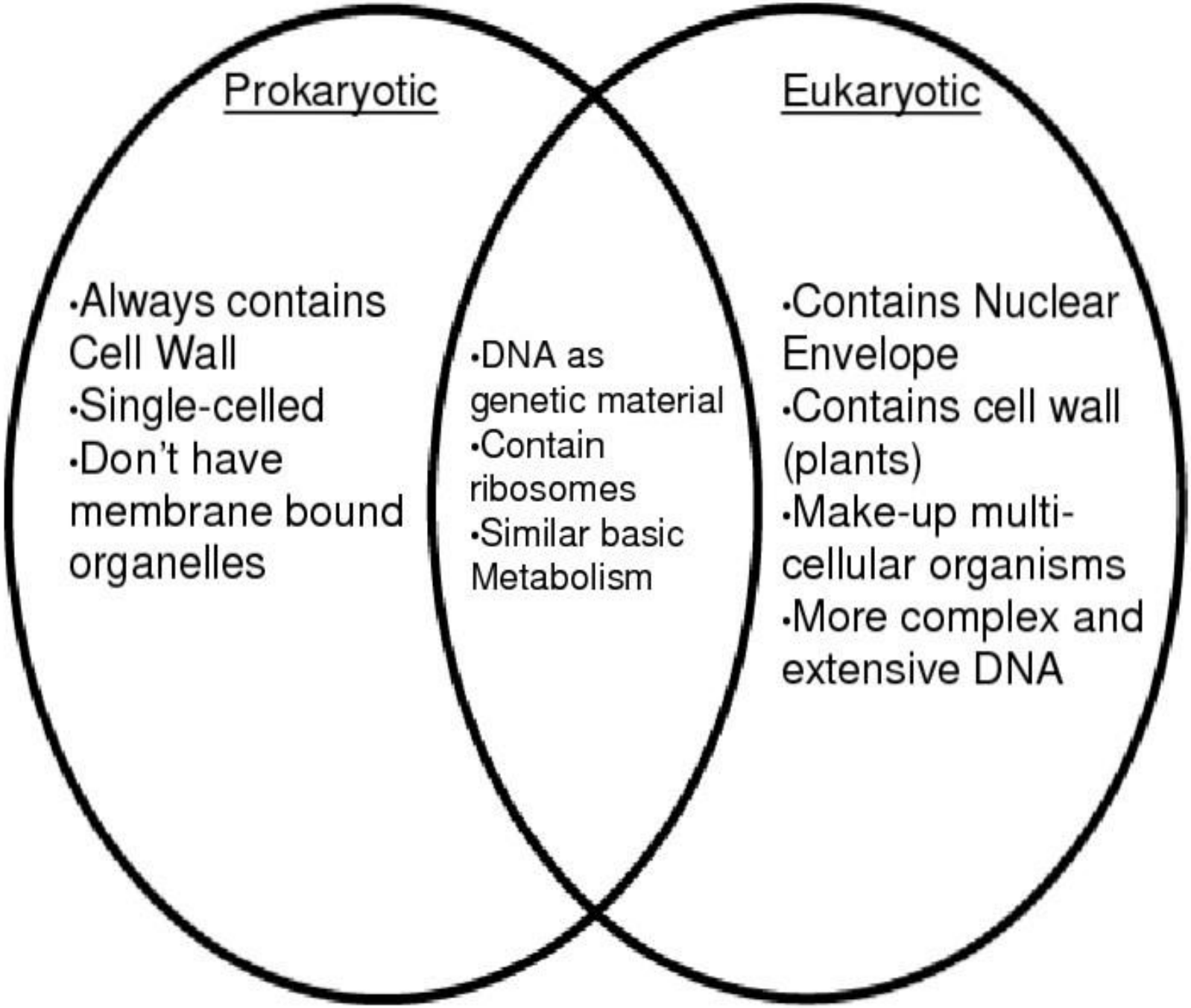
Chapter Two

General properties of bacteria

Introduction

- Prokaryotes (pro = primitive; karyote = nucleus) include bacteria
- Eukaryotes (eu = true; karyote = nucleus) include: Algae, Fungi, Protozoa, Plants and Animals.


Fungi
Animalia
Eukaryota
Plantae
Protista
Bacteria
Archaea
Prokaryota


Prokaryotic
•Always contains Cell Wall
•Single-celled
•Don't have membrane bound organelles
•DNA as genetic material
•Contain ribosomes
•Similar basic Metabolism
Eukaryotic
•Contains Nuclear Envelope
•Contains cell wall (plants)
•Make-up multi-cellular organisms
•More complex and extensive DNA

Bacteria differ from viruses by:

Property	Bacteria	Viruses
Approximate size	1 μm diameter	< 150 nm
Both DNA and RNA present	Yes	either
Growth on ordinary media	Yes	No
Intracellular replication	some can	Yes
Binary fission	Yes	No
Muramic acid	Yes	No
Sensitivity to antibiotics	Yes	No

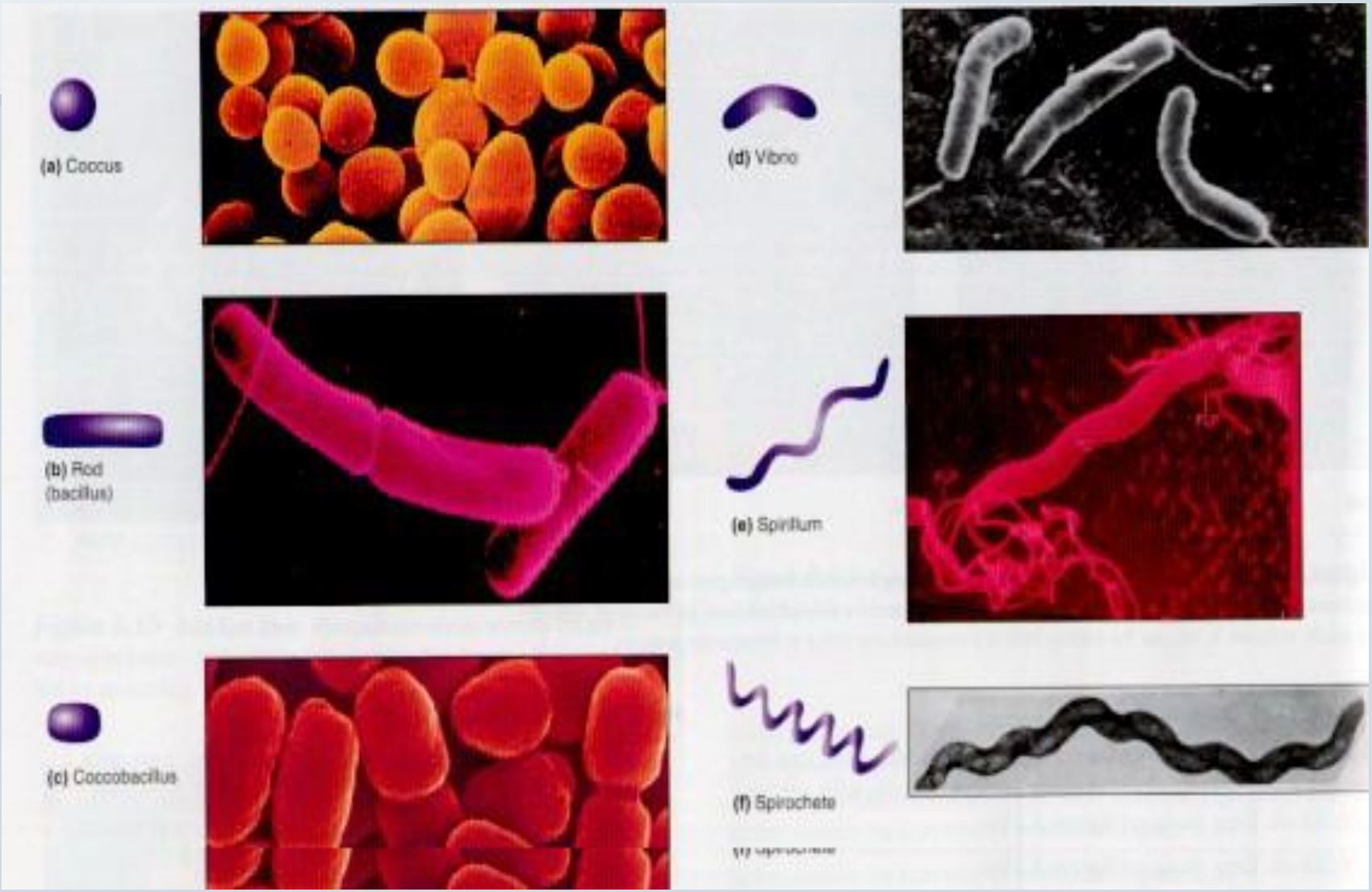
Structure of bacterial cell

- **Nuclear apparatus** (Loose arrangement of DNA), surrounded by amorphous cytoplasm containing ribosomes, mesosomes, and inclusion granules
- **Cytoplasm** enclosed within **cytoplasmic membrane**
- The structure and integrity of bacteria is maintained by a rigid **cell wall**, which have certain appendages:
  - **Fimbriae** (pili) and **Flagella** are surface adherents
- Some have **capsule** or **loose slime** around the cell wall.

Shape and size of bacteria

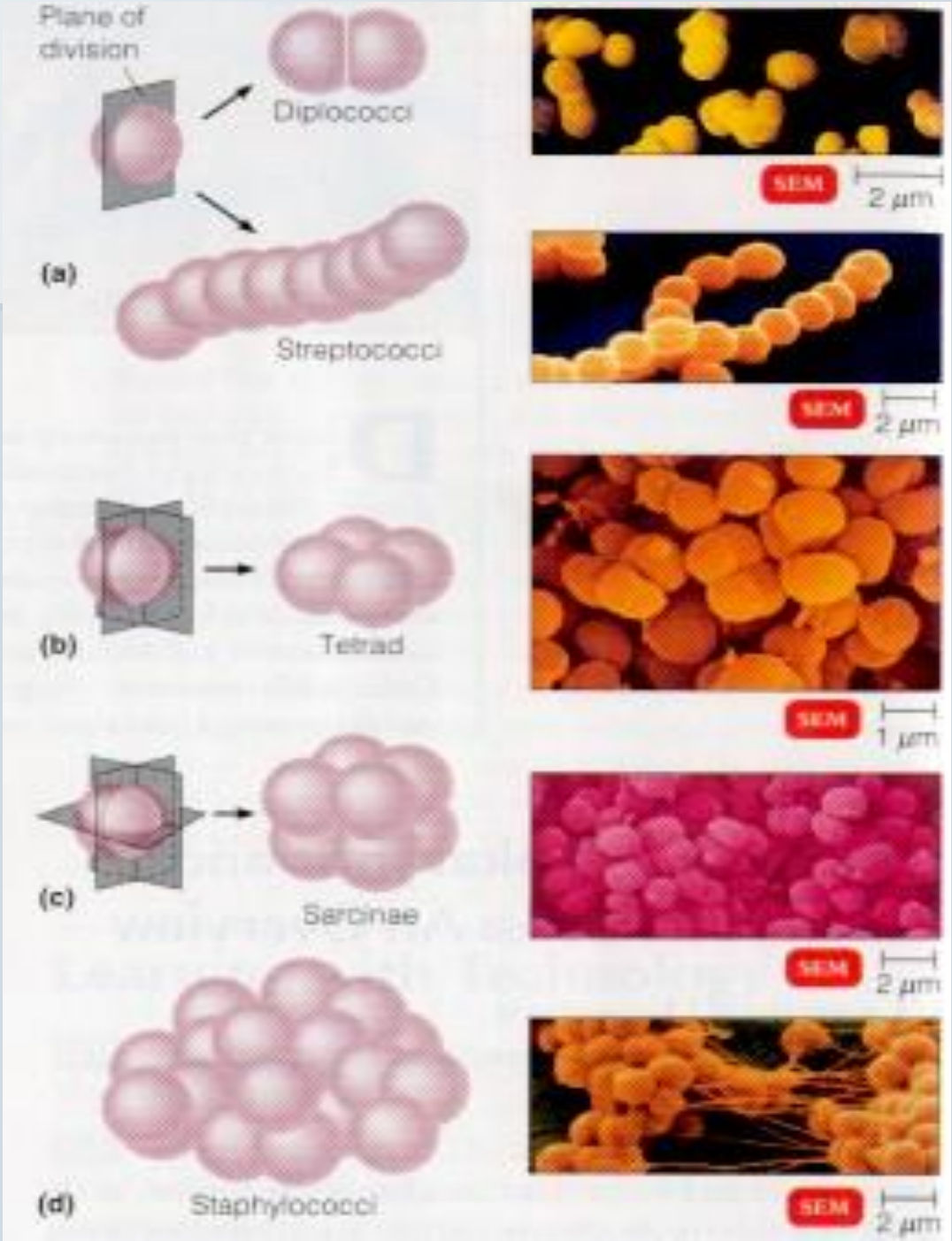
- Bacteria can be:-
  - Spherical (cocci)
  - Rod or cylindrical (bacillus)
  - Coccobacilli (short rod or bacilli)
  - Curved bacilli (vibrio)
  - Long threads of bacilli which have not separated (filamentous)
  - Spirillum
  - Spirochaetes:- long and sinuously curved

Shape and size of bacteria



Arrangement of bacterial cells

- **Cocci** are:-
  1. In pairs → (diplococci) eg. Pneumococci, gonococci or meningococci
  2. In chains → streptococci
  3. In clusters (grape like) → staphylococci
  4. Tetrads
  5. Sarcina


Plane of division
Diplococci
(a)
Streptococci
(b)
Tetrad
(c)
Sarcinae
(d)
Staphylococci
SEM
2 μm
SEM
2 μm
SEM
1 μm
SEM
2 μm
SEM
2 μm

Arrangement of bacterial cells

Bacilli are:-

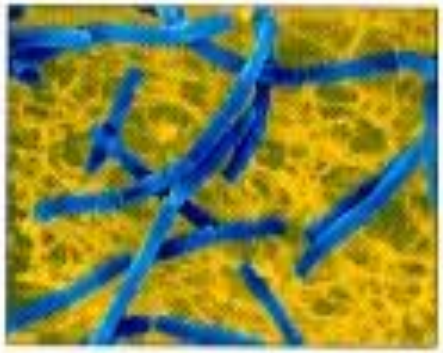
1. Single
2. Diplobacilli
3. Streptobacilli
4. Coccobacilli

(a) Single bacillus

SEM 2 μm

(b) Diplobacilli

(c) Streptobacilli

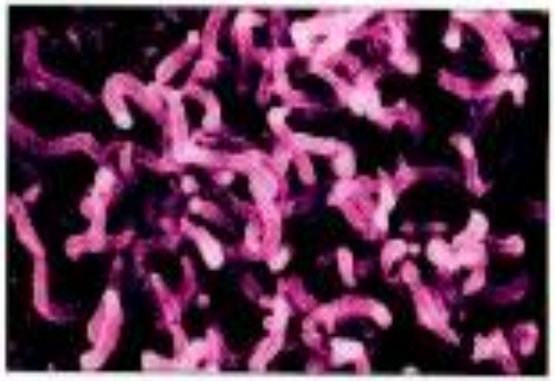


SEM 5 μm

(d) Coccobacillus

SEM 1 μm

(a) Vibrio

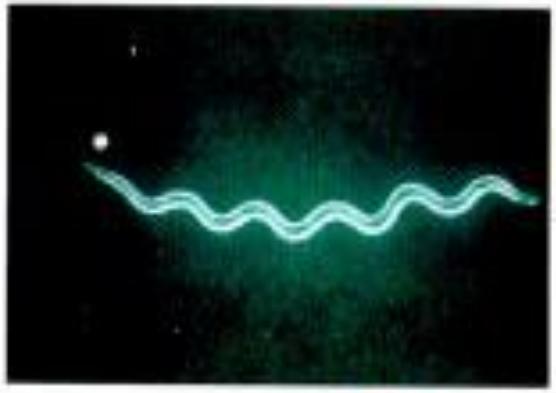


SEM 4 μm

(b) Spirillum

SEM 2 μm

(c) Sprochete



SEM 1.5 μm

Techniques to study morphology of bacteria

- **Microscopy** and **staining** techniques are basic tools for visualizing and studying bacteria
- Ordinary light microscope gives a resolving power of about 200 nm under optimal conditions
- Objects smaller than this cannot be delineated to reveal their true size or structure
- Resolving power of optical system can be greatly increased by using short wave-length (UV)

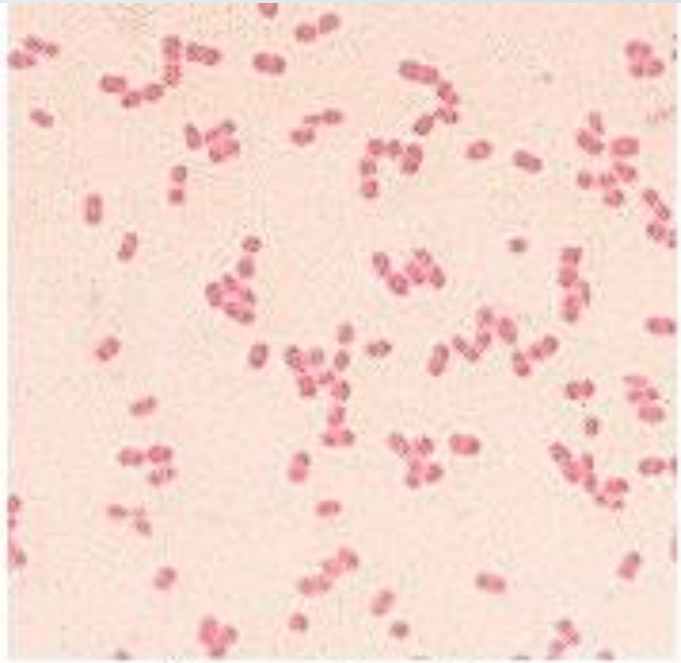
- This principle is used in fluorescent microscopy (ultraviolet rays are used to examine cells after being treated with fluorescent dyes)
- Phase contrast microscope enhances the refraction index differences of cell components
- This microscopy can be used to reveal details of internal structures, capsules, endospores and motility

Types of microscopy in bacteriology

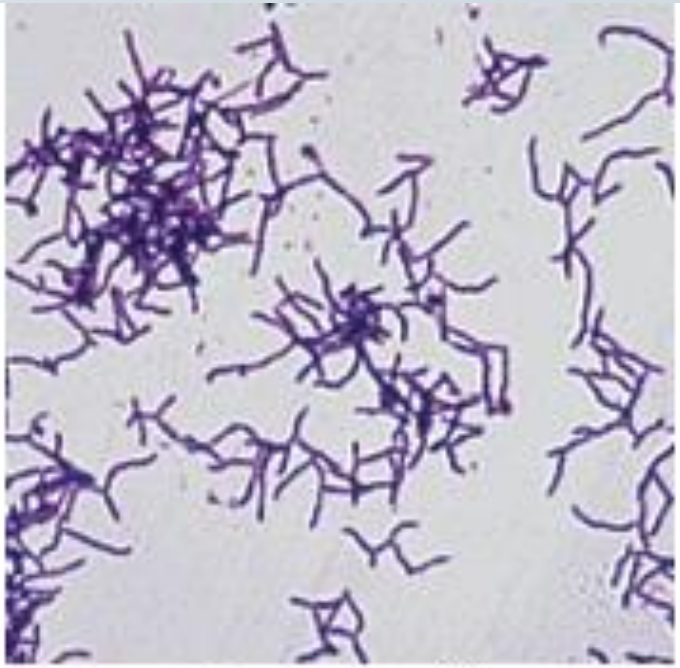
1. Light microscopy
2. Fluorescent microscopy
3. Phase contrast microscopy
4. Darkfield microscopy
5. Scanning electron microscopy
6. Transmission electron microscopy

Staining of bacteria

- Many combinations of dyes and mordants have been used to study the structure and arrangement of bacteria as well as identify them.
- Some of the commonly used techniques are:-
- Gram's staining
- Ziehl-Neelsen (acid-fast) staining
- Albert's staining



Gram Negative

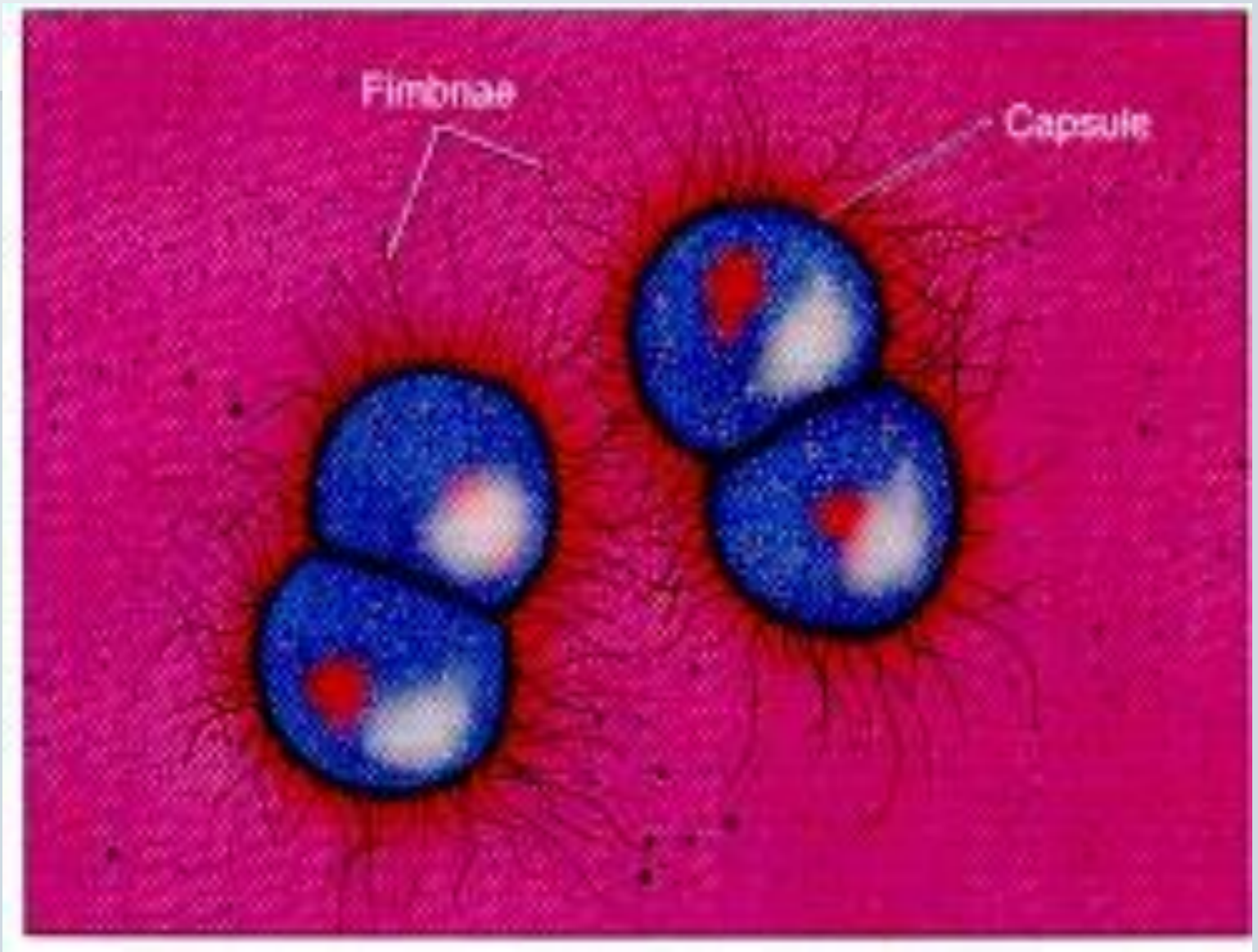


Gram Positive

Surface adherents and appendages

- **Capsule and slime:-**
  - Capsule and slime surround the bacteria
  - The basic differences between capsule and slime is their property of firm attachment to the cell
  - Capsule form gel by which it adhere to the cell, slime can be easily washed off
  - Capsule detected by India ink (negative staining) and appear as halo, or by specific antiserum and observing the capsular swelling phenomenon called ***Quellung reaction***

Capsule and slime



Some of the important characters of capsule

- Weakly antigenic
- Not necessary for viability
- Endows (give) virulence
- Protects from phagocytosis
- Capsulated strains are invariably nonmotile
- Visualized by
  - Negative staining (India ink)
  - Special capsule staining

- Detected by Quellung phenomenon
- Have been demonstrated in pneumococci, *Klebsiella, Escherichia coli, Haemophilus inluenzae etc.*
  - Capsule is carbohydrate (polysaccharide), it is antigenic too
  - Many organisms can be grouped into types on the basis of capsular antigens, eg. Streptococci, *H. influenzae*
  - Capsule is produced better *in vivo* than *in vitro*

Flagella

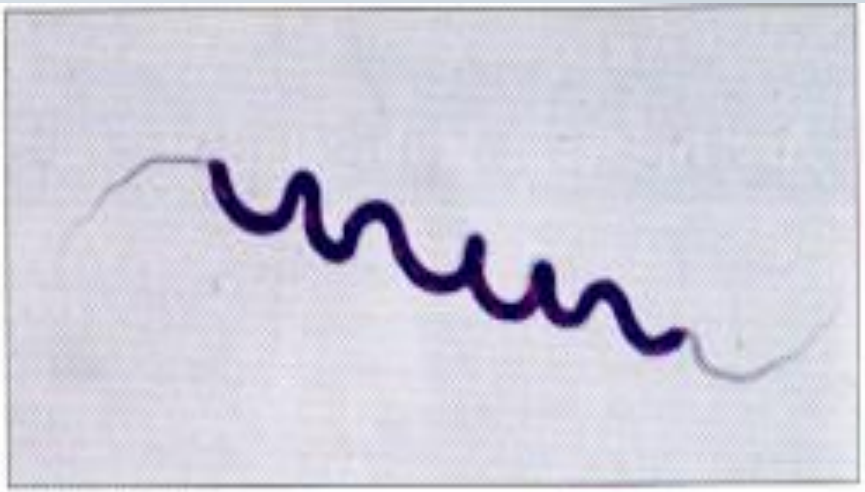
- These are the organs of locomotion and provide motility to the bacteria
- Motility can be ascertained by a simple method under the microscope or by passing the organism through a semisolid agar medium
- Examples of motile bacteria are:- *Pseudomonas*, *Escherichia coil*, *Salmonella*, *Proteus*, spirochaetes, and spirilla
- Nonmotile bacteria include:- pathogenic cocci, *Klebsiella*, and *Shigella*

Arrangement of flagella

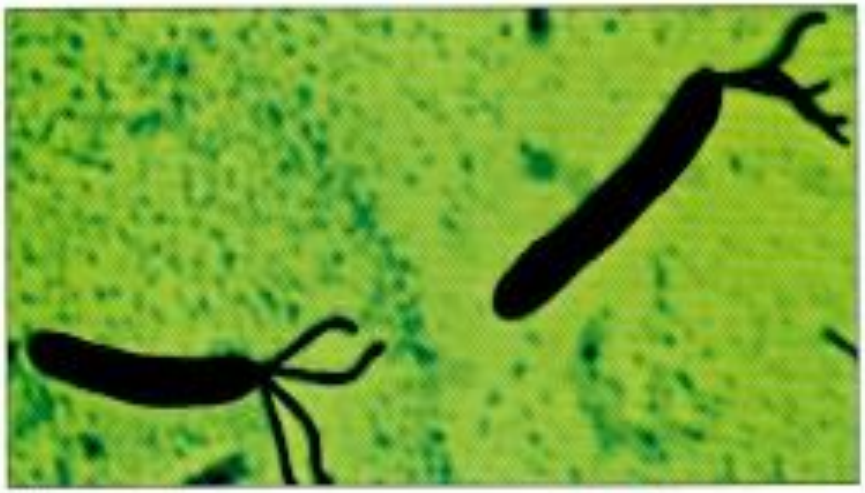
- **Monotrichous** → one polar flagellum
- **Lophotrichous** → tuft of several polar flagella
- **Amphitrichous** → flagella at both ends
- **Peritrichous** → flagella distributed all over the surface of the bacterium
- **Atrichous** → flagella absent (Figure 2.3)

Arrangement of flagella

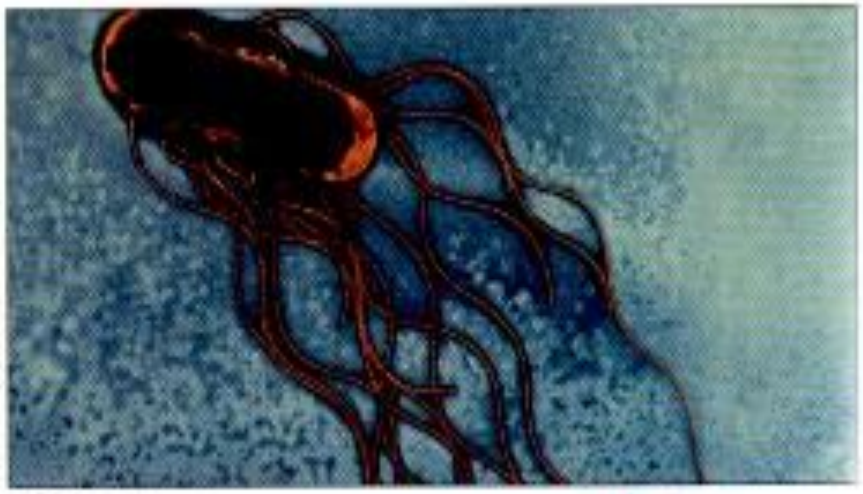
(a) Monotrichous SEM 1 μm



(b) Amphitrichous SEM 10 μm



(c) Lophotrichous SEM 1 μm

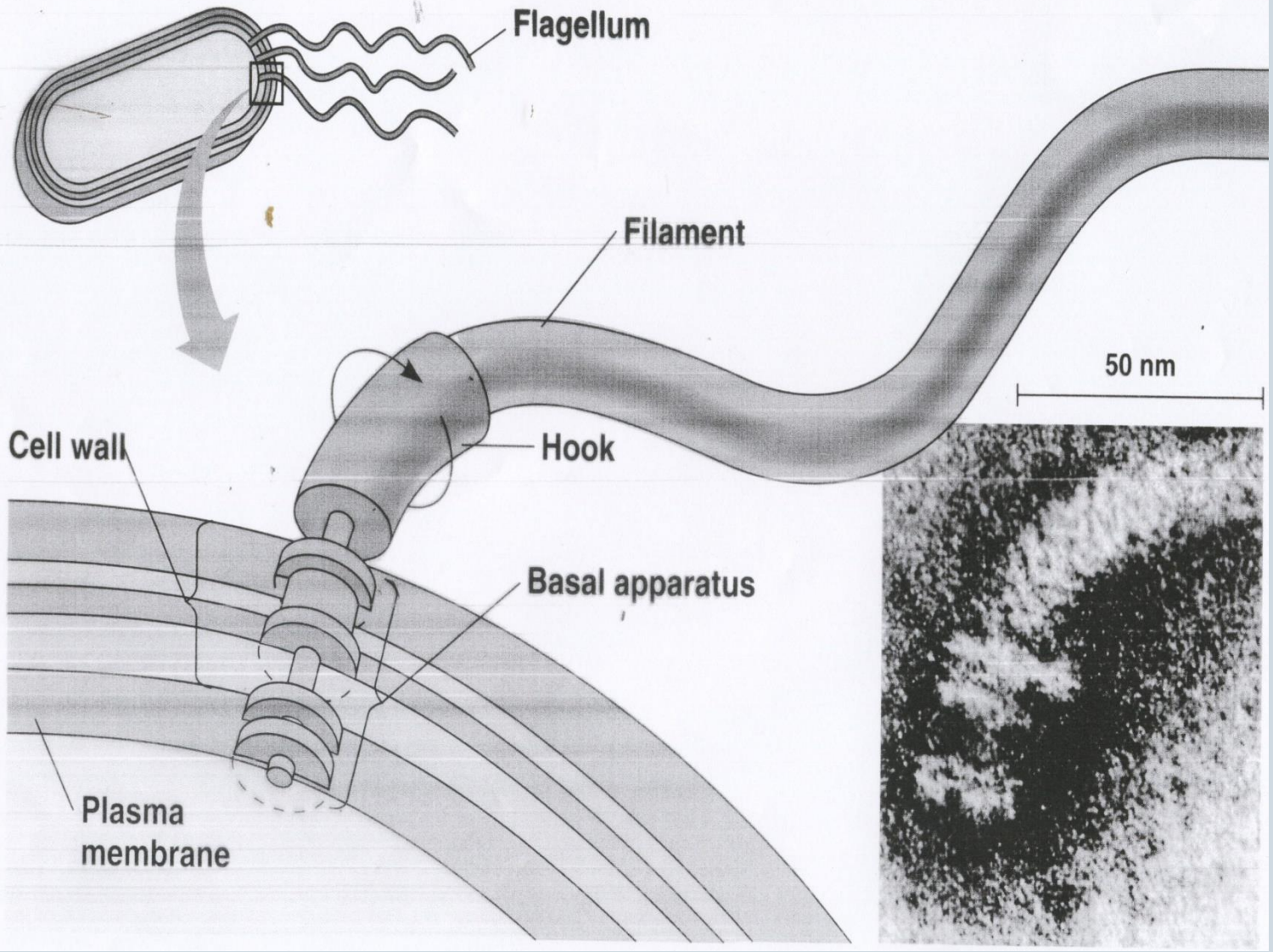


(d) Peritrichous TEM 1 μm

Anatomy of flagella

- consist of 3 parts:- the filament, the hook and the basal body (figure 27.7)
  - The **filament** → composed of protein called ***flagellin***
  - The flagellar antigen is called **H** (***Hauch***) antigen in contrast to somatic antigen which is called as **O** (***Ohne hauch***)

Fig. 27.7 Form and function of prokaryotic flagella



Pili (Fimbriae)

- Hair-like structures located on the surface of certain Gram-negative bacteria
- The only Gram-positive bacteria having pili is *Corynebacterium renale*
- Pili composed of protein called pilin arranged in helical strands
- Are shorter and straighter than flagella

- They have two important roles:-
  - Help for attachment of bacterium to the cell surface of human eg. RBC, and epithelial cells
  - Sex-pili → forms the attachment for the transfer of genetic material from one bacterium to another during conjugation

The cell wall

- Is a multilayered structure
- Constitutes 20% of the dry weight of the bacterium
- Average thickness is 0.15 to 0.50 µm
- Chemically composed of mucopeptide scaffolding formed by **N-acetyl glucosamine** and **N-acetyl muramic acid** cross linked by **peptide chains**

- In gram-positive bacteria various proteins and polysaccharides are attached to peptidoglycan present in cell wall
- The envelope of **gram-negative** bacteria composed of 3 layers:-
- **Outer membrane** → lipopolysaccharide + lipoprotein + phospholipids
- **Middle layer** → periplasmic gel or area between outer membrane and cytoplasmic membrane
- **Plasma membrane** → in some species is site of enzymes called β-lactamases, that degrade penicillin and other β-lactam antibiotics

Gram-positive cell wall

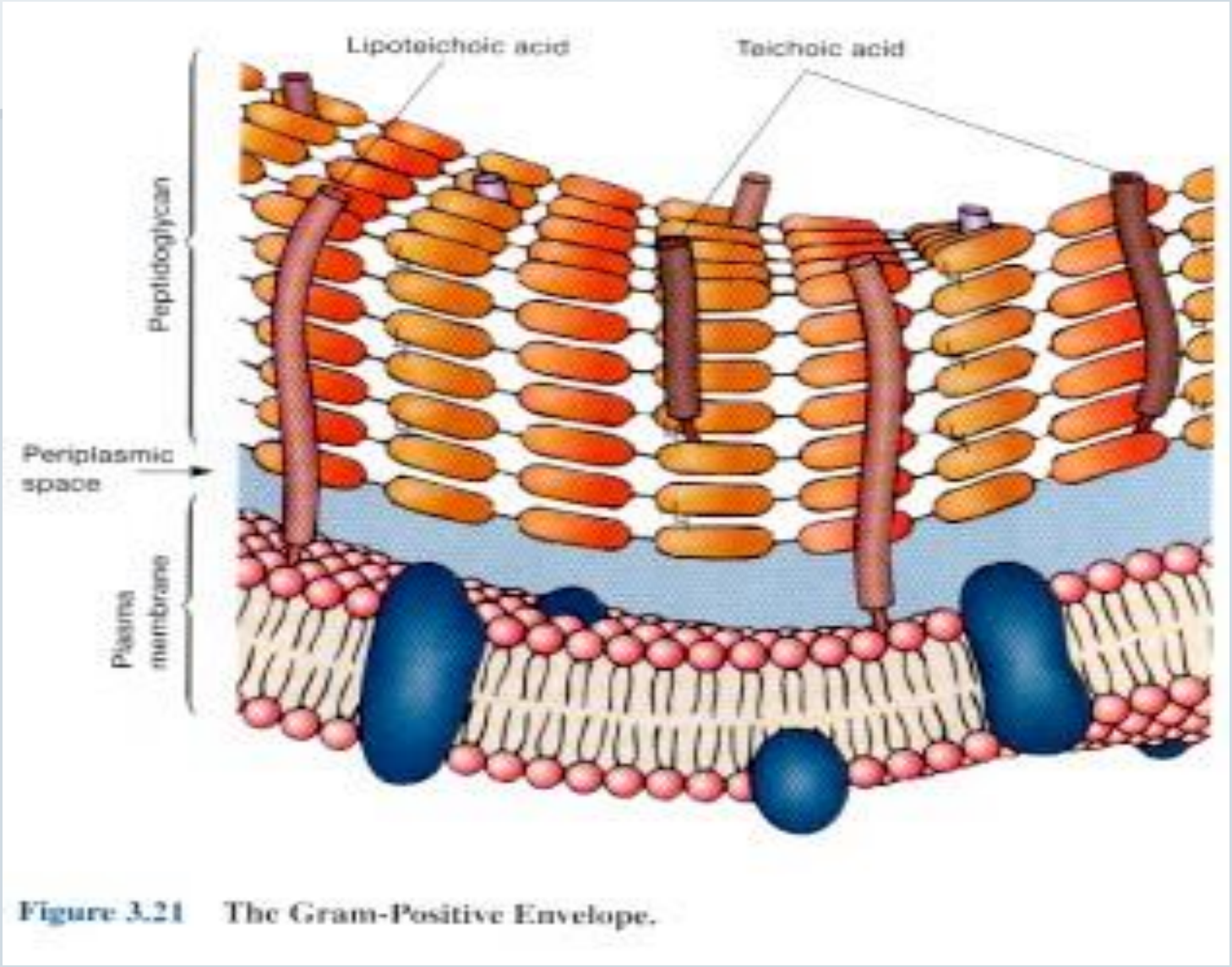


Figure 3.21 The Gram-Positive Envelope.

Gram-negative cell wall

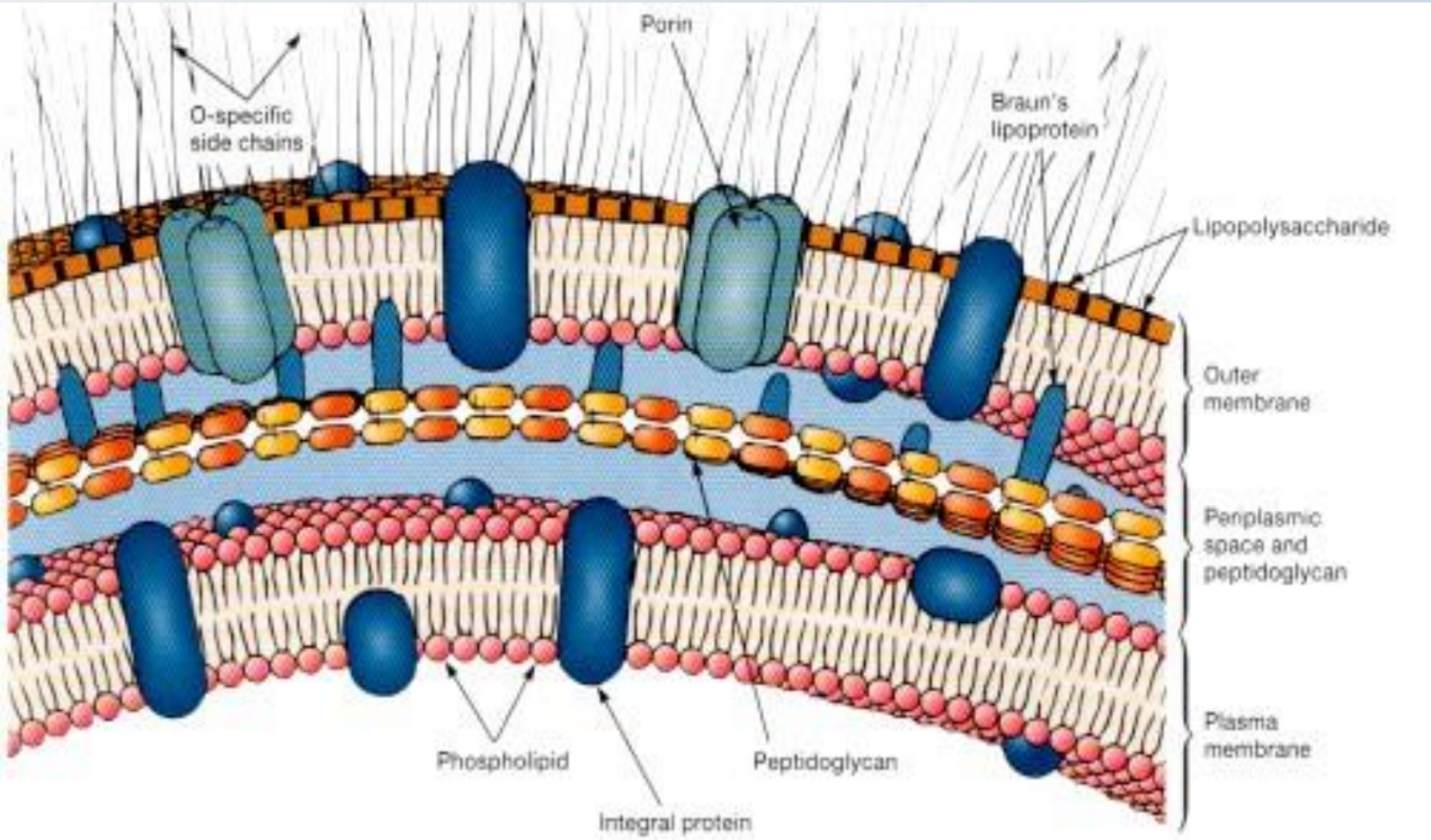


Figure 3.23 The Gram-Negative Envelope.

Function of bacterial cell wall

- Provides shape of the bacterium
- Gives rigidity to the organism
- Protects from environmental factors
- Contains receptor sites for phages
- Site of action of antibody
- Provides attachment to complement
- Contains components toxic to host
- Site of action of colicine (toxins)

Cytoplasmic structures

- **The plasma membrane** is a delicate membrane separates rigid cell wall from the cytoplasm
- Various structures are attached to it, hence it constitute 30% of total cell weight
- Chemical composition is 60% proteins, 29-30% lipids, and the remaining is carbohydrates

- Acts as effective osmotic barrier under normal conditions
- Helps in establishing the movement of certain essential nutrients eg. amino acids regardless to concentration gradient
- Secretion of enzymes and toxins
- Energy generation

Mesosomes

- Principle site of respiratory enzymes
- Is analogous to mitochondria in higher forms of life
- Are attached to DNA chromatin and cell membrane
- Play important role in cell division

Ribosomes

- Are small cytoplasmic particles
- Are site of protein synthesis
- Composed of RNA and protein (40% of total cell protein and 90% of total cellular RNA)
- Are 70S unit, which can be split into 2 subunits 50S and 30S

Cytoplasmic granules

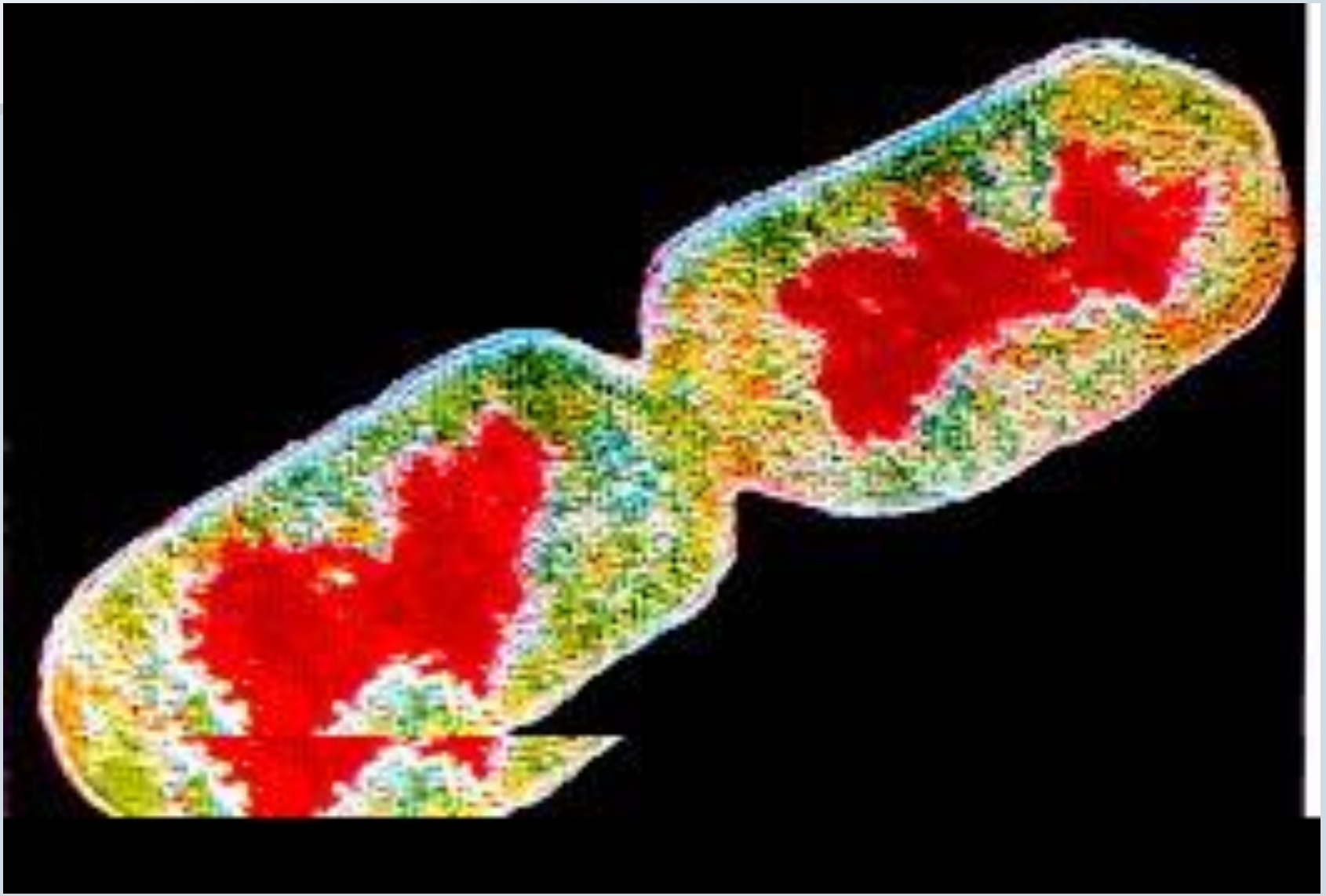
- Granules are accumulated food reserves
- Their amount in bacterial cell vary depending on the nature of medium and functional state of cell

nuclear apparatus

- Bacteria do not posses a well-defined nucleus, so nuclear region is referred to as **nuclear body** or **nucleoid**
- Bacterial DNA represents 2-3% of cell weight and 10% of bacterial volume
- DNA is a single circular molecule, which may open under certain conditions to form a long chain of about 1000 μm

- Bacterial chromosome is haploid and replicate by binary fission. It contains about 2000 genes
- Do not have introns (noncoding, intervening sequence within eukaryotic gene)
- In addition to nucleoid, DNA, bacteria may have some extra-chromosomal genetic material in the form of DNA known as **plasmid**

nuclear apparatus



Bacterial spores

- Gram positive bacilli and actinomycetes form highly resistant and dehydrated forms called **endospores**
- These are formed under conditions of limited supply of nutrients in vegetative forms of bacteria
- The surrounding mother cell which give rise to them is known as **sporangium**

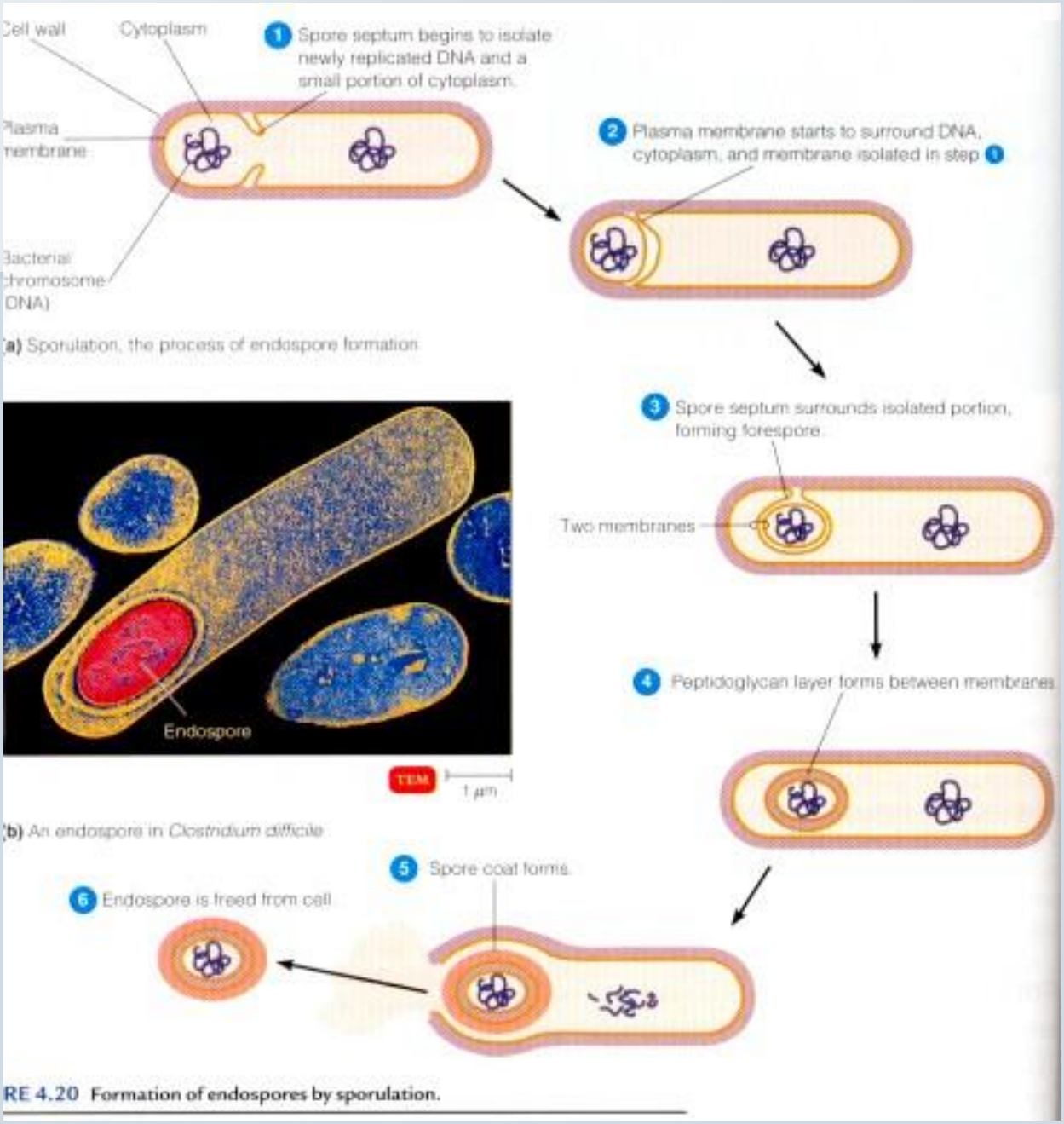
Structure

- Spores are smooth walled and ovoid, in some bacteria are spherical
- In bacillus, spores usually fit into the normal cell diameter except in *Clostridium* where these may cause a bulge
- The bulge can be terminal (drum stick) or central
- Spores do not take up ordinary stains, they look like areas of high refractivity under light microscope

Germination

- Under favorable environment spores get converted to normal vegetative forms through the process of germination
- It occurs in less than 2 hours
- It has 3 stages:

1. **Activation:** - either spontaneous or by activators, such as heat, low pH etc.
2. **Germination proper**:- require water and triggering germination agent such as alanine, and manganese
3. **Outgrowth:-** occurs following germination in a nutrient medium after which gradual resumption of vegetative life commence



(a) Sporulation, the process of endospore formation.

(b) An endospore in *Clostridium difficile*.

RE 4.20 **Formation of endospores by sporulation.**

Nutrition of bacteria

- Bacteria have well defined requirements, such as proper nutrients, oxygen, pH and Temperature
- Most of the medically important bacteria grow only if a source of organic material as a nutrient is available, such bacteria is called ***Chemoheterotrophs***

Nutrition of bacteria

- Bacteria also require a source of nitrogen and a number of salts such as: K, Mg, Fe, P, and S.
- Growth is facilitated when trace quantities of cobalt, zinc, chlorine, copper, nickel, etc. are present in the medium

Oxygen

- Bacteria are divided into 4 groups according to their oxygen requirements:-
- **Obligate anaerobes: -** grow only in environment free of oxygen; they lack catalase, peroxidase, superoxide dismutase and cytochrome system. Examples:-*Bacteroides* and *Clostridium*
- **Facultative anaerobes: -** grow under both aerobic and anaerobic conditions. Examples:- *Enterobacteriaceae* and many other bacteria

Oxygen

- **Obligate aerobes: -** can not grow unless oxygen is present in the medium. Examples:- *Pseudomonads*
- **Microaerophilic: -** can grow under conditions with low oxygen tension. Examples:- *Clostridium tetani*

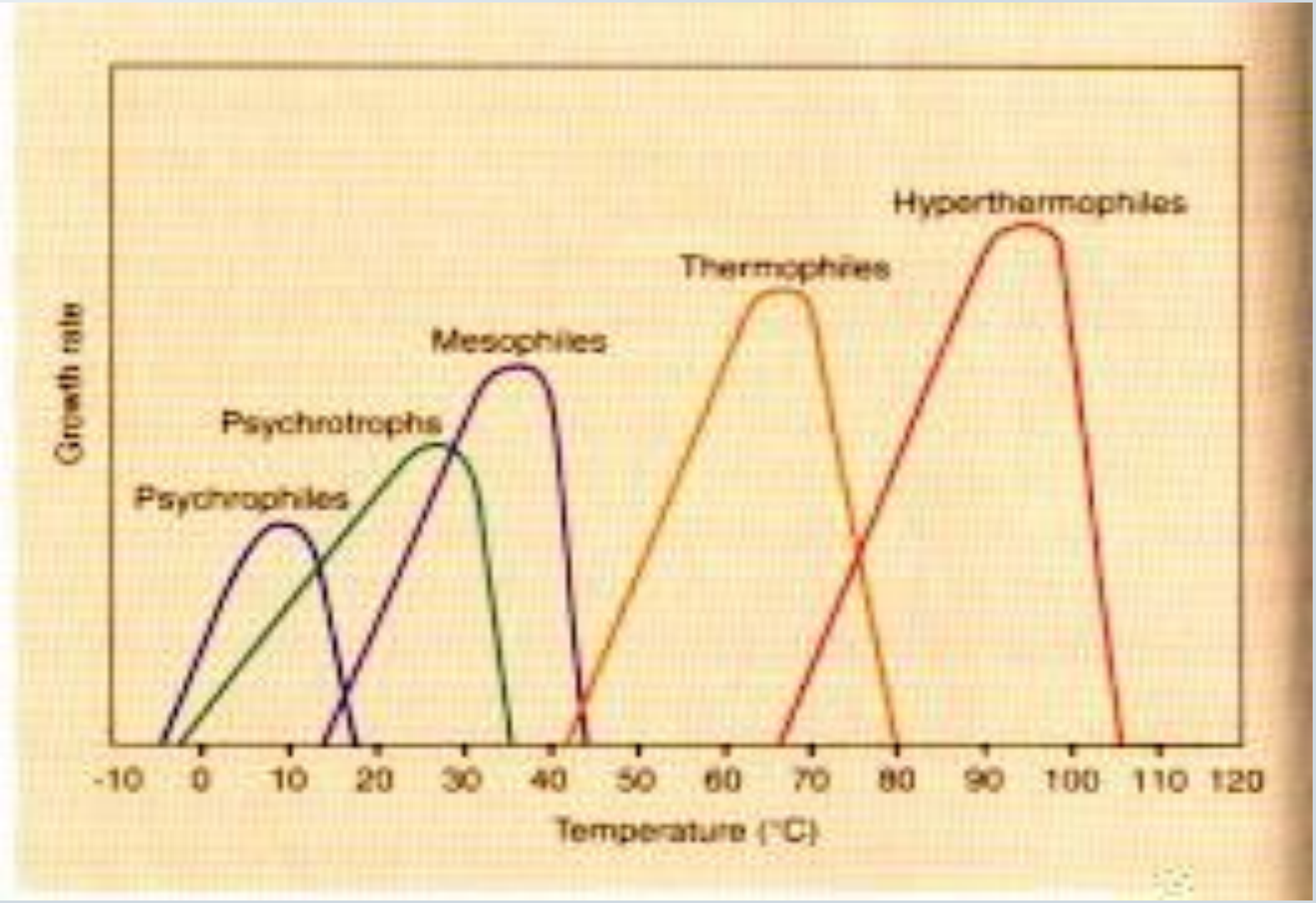
Table 6.1 *The Effect of Oxygen on the Growth of Various Types of Bacteria*

	a. Obligate Aerobes	b. Facultative Anaerobes	c. Obligate Anaerobes	d. Aerotolerant Anaerobes	e. Microaero-philes
Effect of oxygen on growth	Only aerobic growth; oxygen required.	Both aerobic and anaerobic growth; greater growth in presence of oxygen.	Only anaerobic growth; ceases in presence of oxygen.	Only anaerobic growth; but continues in presence of oxygen.	Only aerobic growth; oxygen required in low concentration.
Bacterial growth in tube of solid growth medium					

Temperature

- Most bacteria have a narrow range of temperature requirements for their optimal growth
- Bacteria are divided into **3 groups** according to temperature requirements:-
- **Psychrophilic**:- grow in the range of (5-30°C) optimum (10-20°C)
- **Mesophilic**: - grow at (10-45°C), optimum (20-40°C). All medically important bacteria belong to this group
- **Thermophilic**:- prefer high temperature (25-80°C), maximum growth occurs at (50-60°C)

Temperature

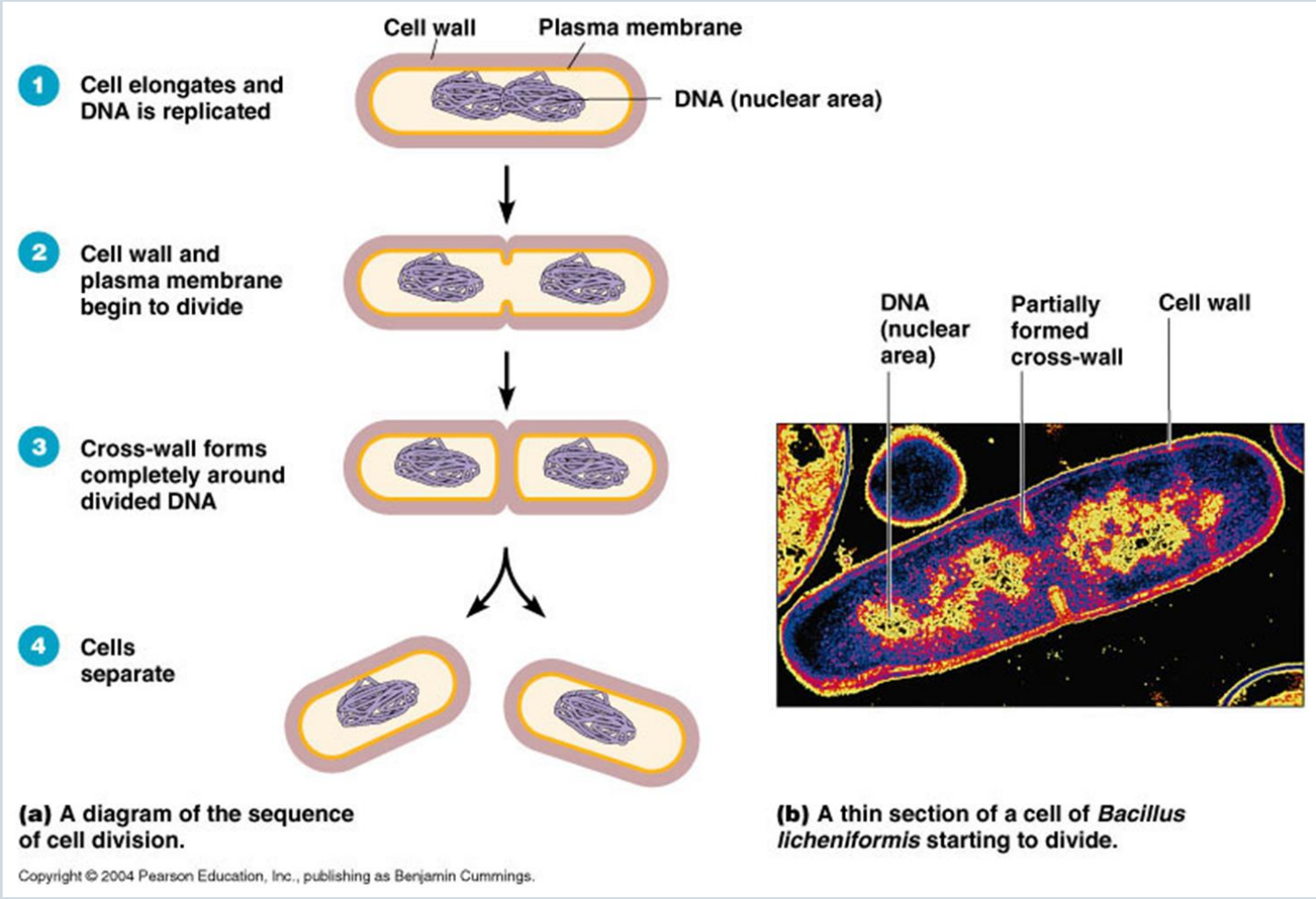


pH

- The pH of the medium has a profound effect upon multiplication of microorganisms.
- most pathogenic bacteria require a pH of 7.2-7.6 for optimum growth.
- pH of growth medium keeps on changing due to bacterial reactions with the medium.

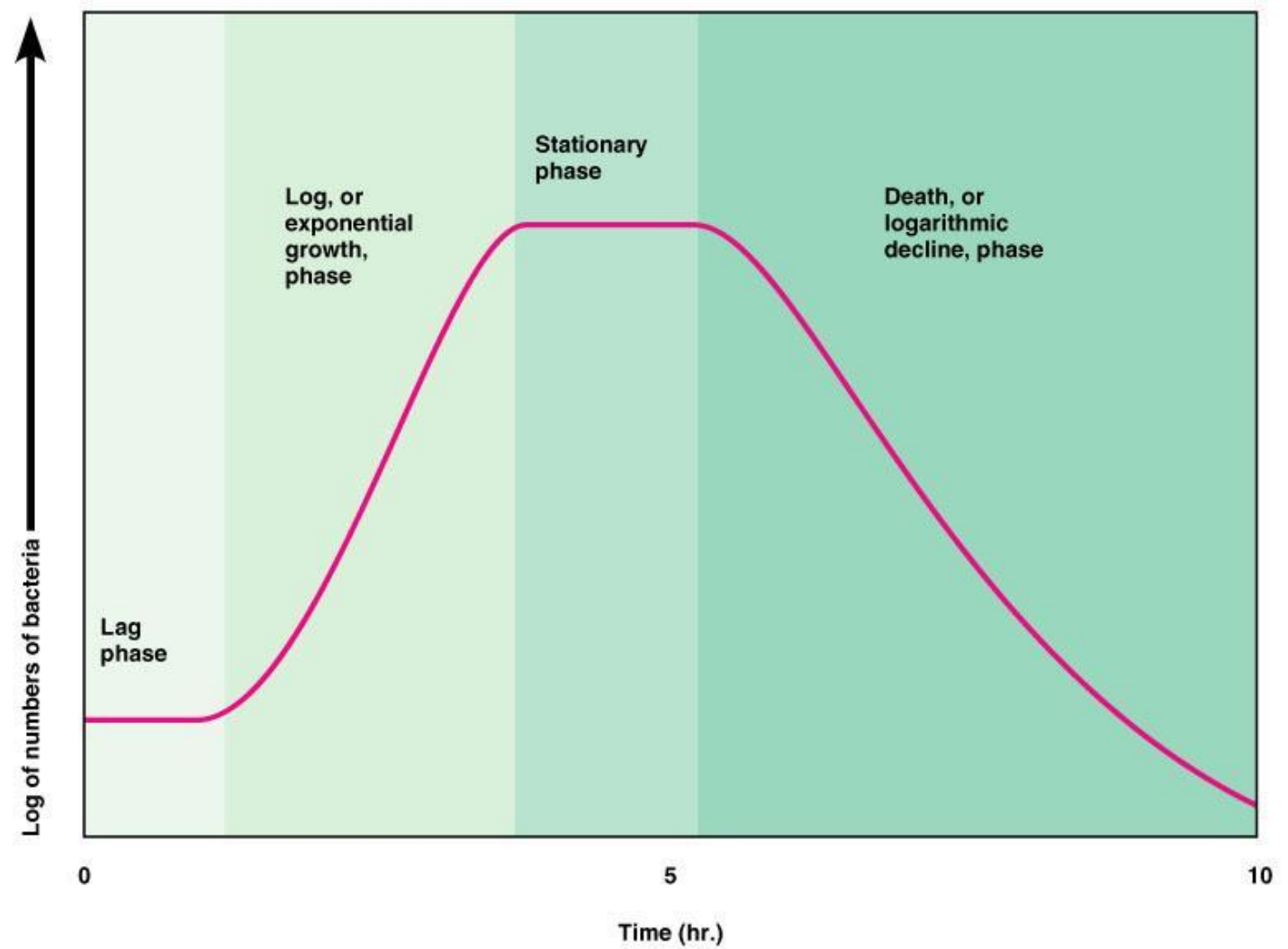
Bacterial growth

- Under favorable environmental conditions and adequate nutrition, bacterium enlarge and divides by binary fission into two daughter cells.



Bacterial growth curve

- Bacterial growth curve is in 4 phases:-



1. The lag phase:- short duration, bacteria adapted to new environment in a way that its machinery become adapted with the nutrition available

2. The log phase (exponential phase):-

- Regular growth of bacteria occurs, with short duration because of the utilization of nutrients in the medium.
- When nutrients exhausted, deceleration of growth occur and bacteria pass to stationary phase.
- The morphology of bacteria is best developed in this phase and organisms manifest typical biochemical characters.

3. The stationary phase: - starts when culture conditions are so changed, that further balanced growth and cell division can not be sustained. The viable cell count quickly declines

4. Decline phase (Death phase):-

- The number of dying bacteria is much more than the number of dividing ones.
- This is due to depletion of nutrients and accumulation of toxic end products.
- There is a general decline in the total number of organisms.

Measurement of bacterial growth

- It can be either defined in terms of mass of cellular material or cell number depending on the type of study
- **Cell mass** can be measured in terms of:
  - Dry weight
  - Packed cell volume
  - Nitrogen content
- Determining ***turbidity*** by photoelectric colorimeter or spectrophotometer

Measurement of bacterial growth

Cell number can be counted as:

- total cell number
- viable count

- Viable number of bacteria can be counted by inoculating the suspension onto solid growth medium and counting the number of colonies
- Since each colony is the end product of one viable cell (bacterium)

Measurement of bacterial growth

- Their count gives the number of viable bacteria in the suspension
- Total number of bacteria can be estimated in specially designed chamber such as **coulter counter**