Bacterial Structure (Lab 4)

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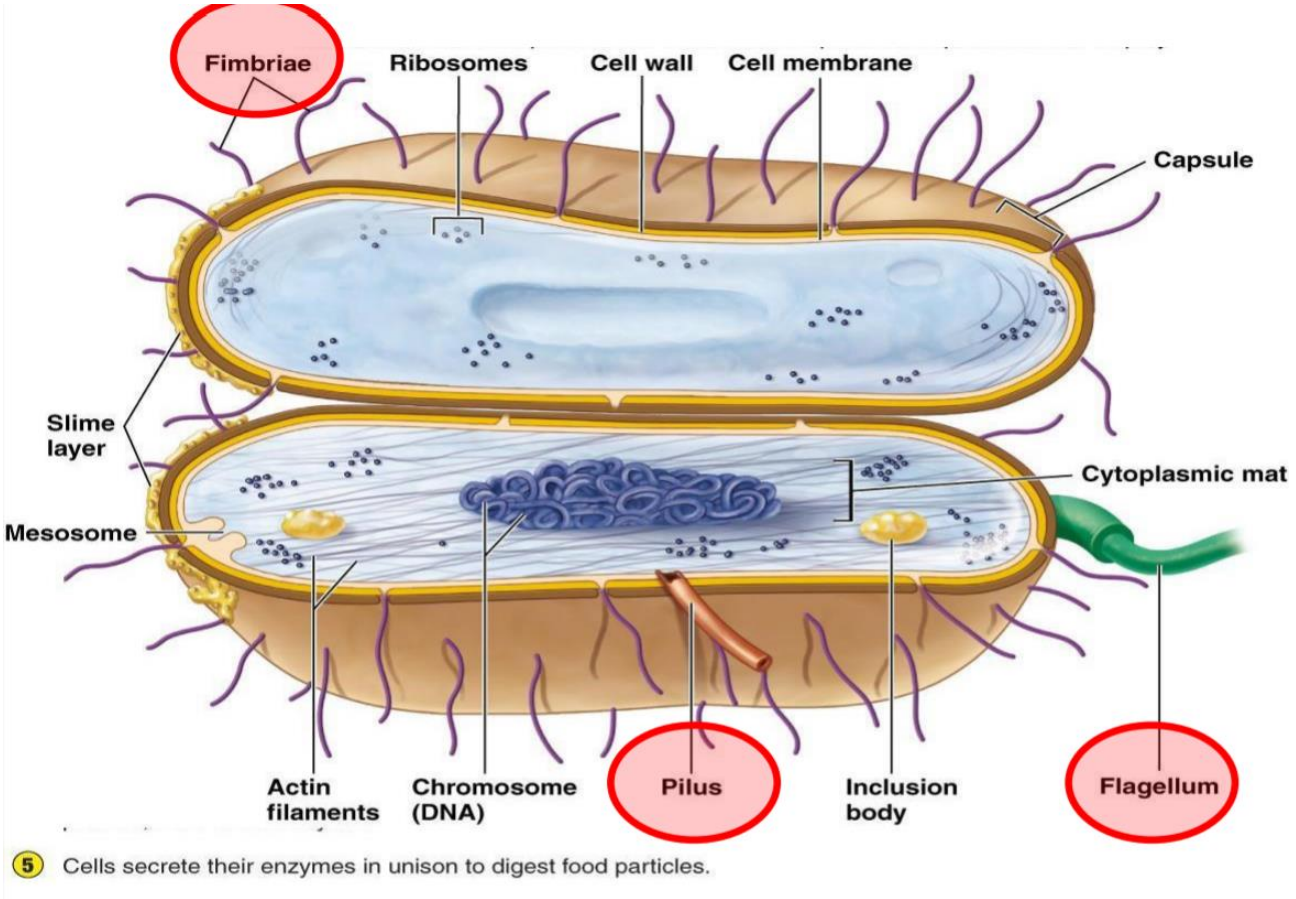
Essential Bacterial Structures

- Cell wall.
- Cell membrane.
- Cytoplasm.
- Nuclear material.

Particular Bacterial Structures

- Capsule.
- Flagella.
- Pili.
- Fimbriae.
- Spore.

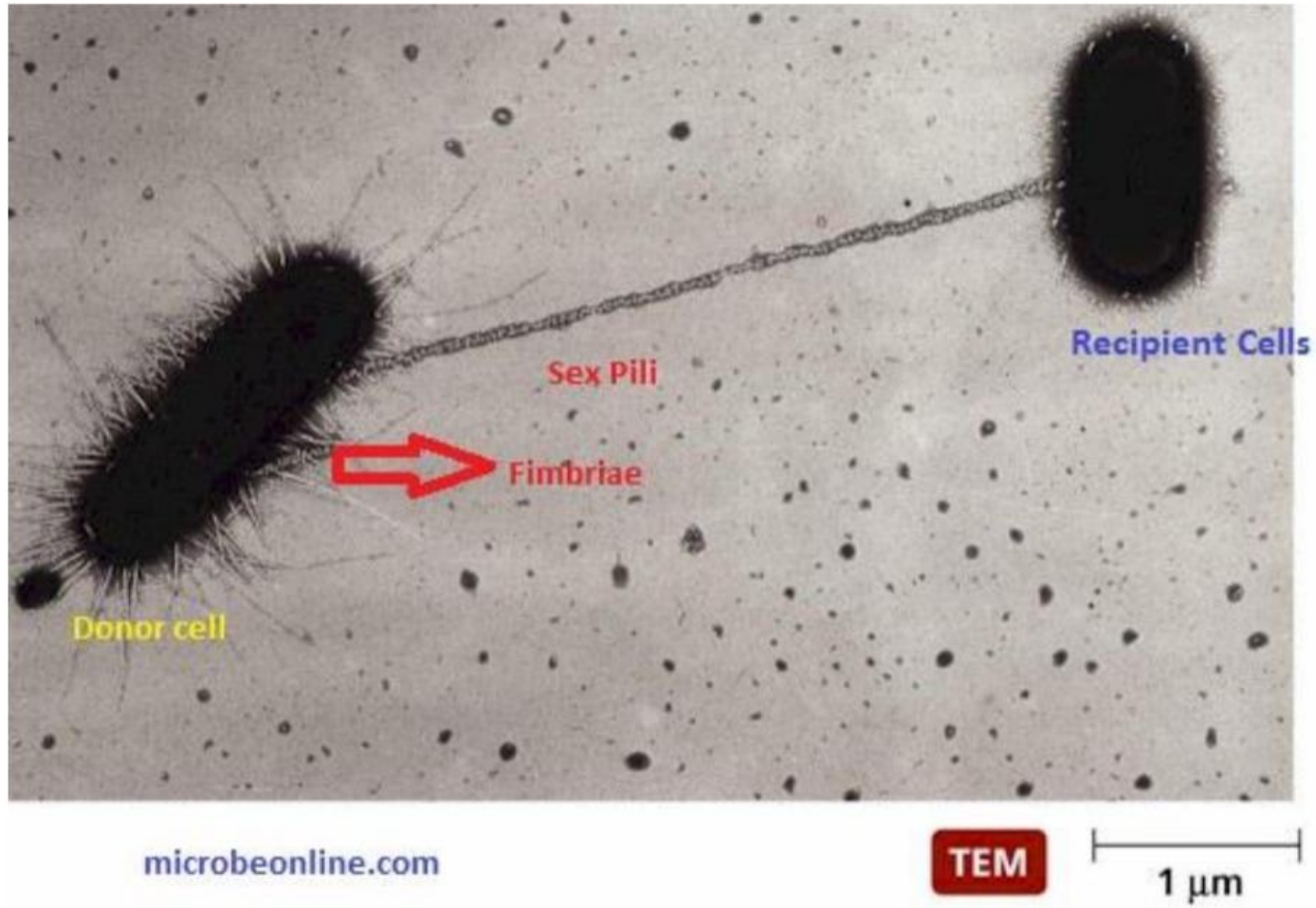
Bacterial Structure



Bacterial Appendages (Pili)

- Only found in gram negative bacteria
- Tubular, hair like structures of protein larger and more rare than fimbriae.
- 2 types of pili :

1. Attachment pilus - allow bacteria to attach to other cells
2. Sex pilus, - transfer from one bacterial cell to another.


Recipient Cells
Sex Pili
Fimbriae
Donor cell
microbeonline.com
TEM
1 µm

Bacterial Appendages (Fimbriae)

- Fimbriae are very fine fibrillar structures.
- Fimbriae help the bacteria to stick to surfaces.
- e.g. E.coli

Bacterial Appendages (Flagella)

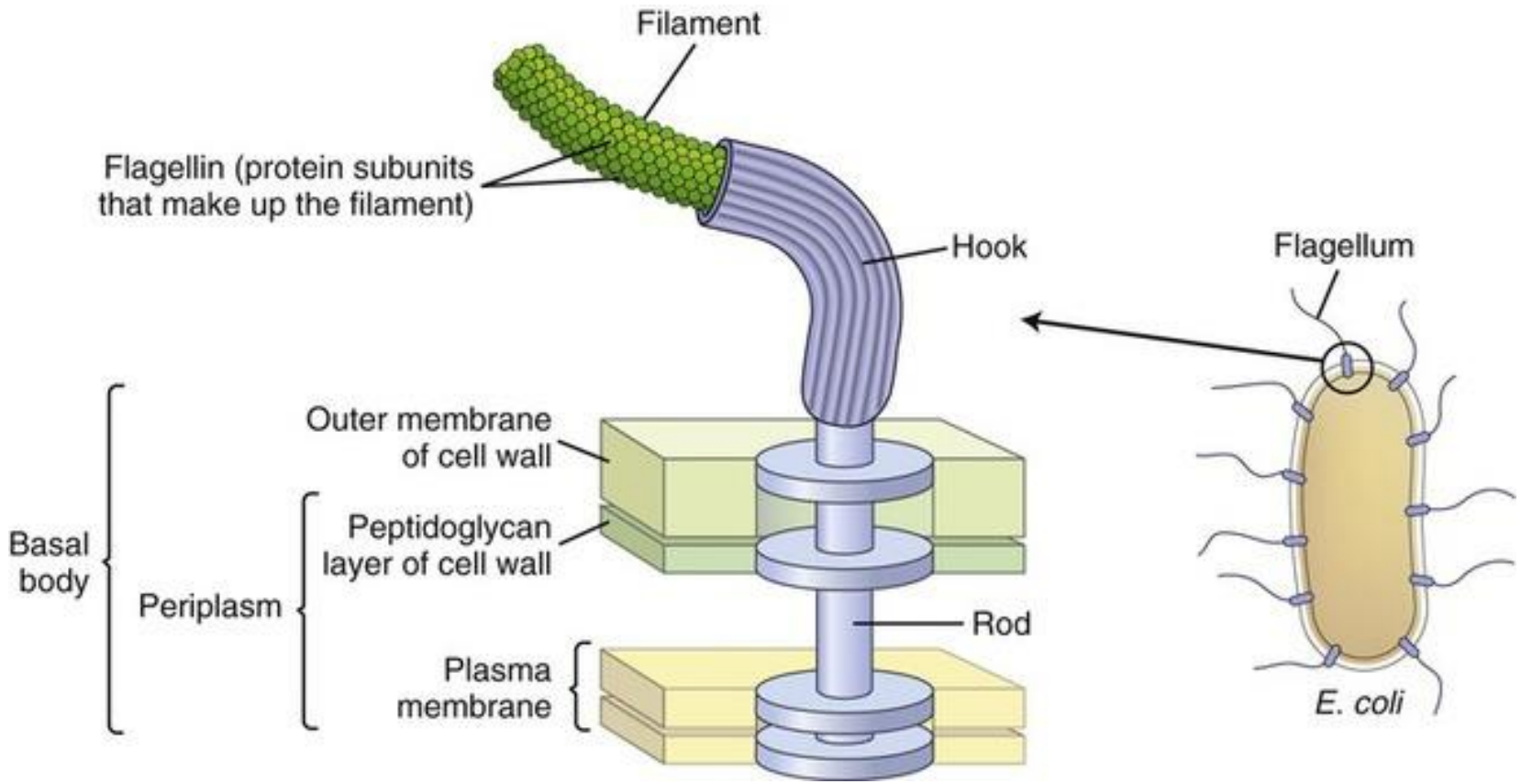
- long appendages which rotate by means of a "motor".
- Bacteria may have one, a few, or many flagella in different positions on the cell.
- Advantages:

1. Identification of Bacteria
2. Pathogenesis
3. Motility of bacteria

- All spirilla, half of bacilli, rare cocci.

Structure of flagella

- Three morphological regions :
- **1. Helical filament :**
  - contains the protein (flagellin) arranged in several chains.
- **2. Hooked or curved area :**
  - Consists of a different protein.
- **3. Basal body:**
  - Terminal portion of the flagellum
  - Fix the flagellum to the cell wall and plasma membrane
  - Composed of a central rod inserted into a series of rings


Filament
Flagellin (protein subunits that make up the filament)
Hook
Flagellum
Outer membrane of cell wall
Peptidoglycan layer of cell wall
Basal body
Periplasm
Rod
Plasma membrane
E. coli

Flagella in G+ and G- Bacteria

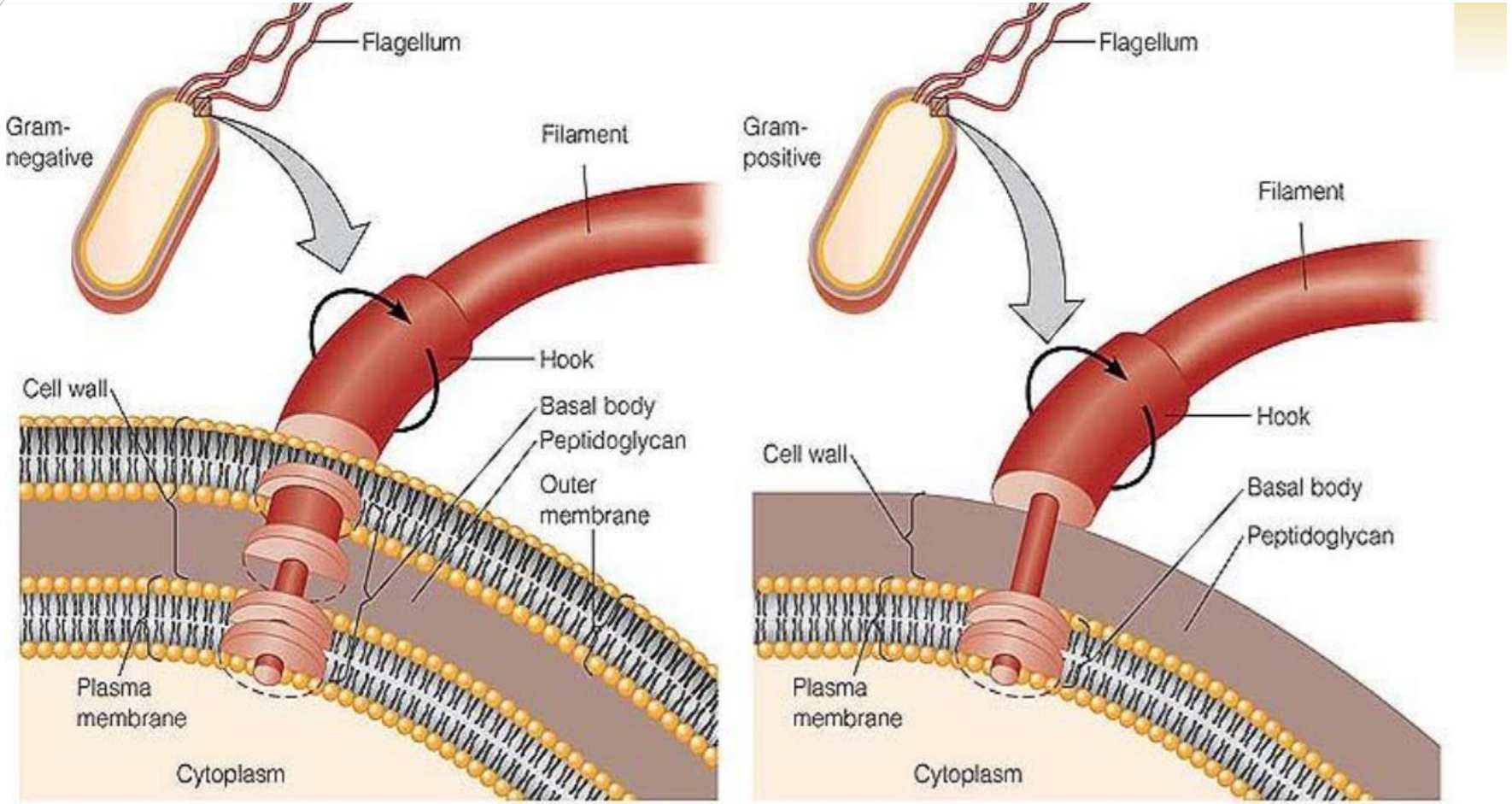
- Gram negative :
  - 2 pairs of rings :
    - Outer pair:

fixed to the outer membrane and peptidoglycan layer

- Inner pair:

fixed to the plasma membrane

- Gram positive:
  - only inner pair is present

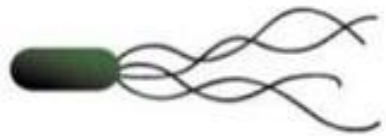


(a) Parts and attachment of a flagellum of a gram-negative bacterium

(b) Parts and attachment of a flagellum of a gram-positive bacterium

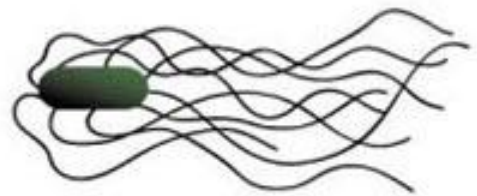
Types of Flagellar Arrangement

Polar/ Monotrichous – single flagellum at one pole

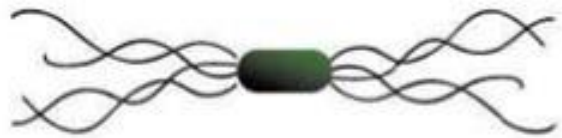


Lophotrichous – tuft of flagella at one pole

Amphitrichous – flagella at both poles



Peritrichous – flagella all over

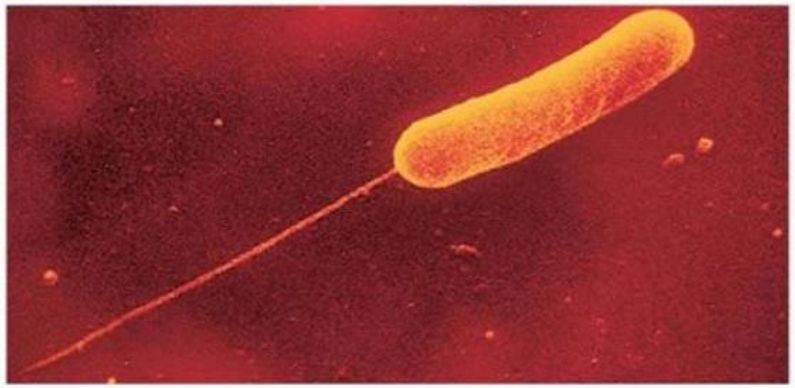


Amphilophotrichous – tuft of flagella at both ends

(a) Peritrichous

SEM

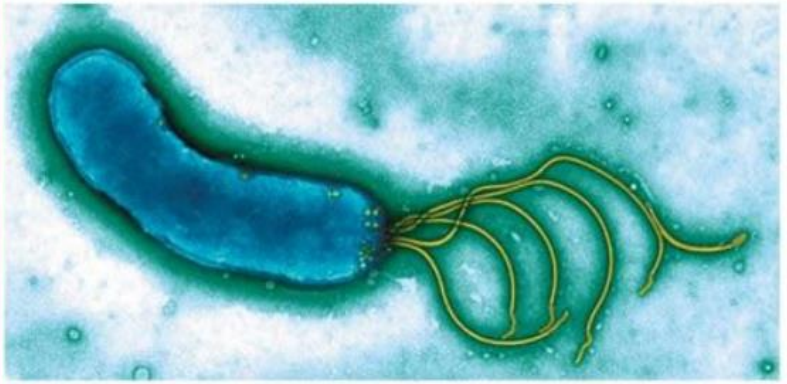
0.5 μm



(b) Monotrichous and polar

SEM

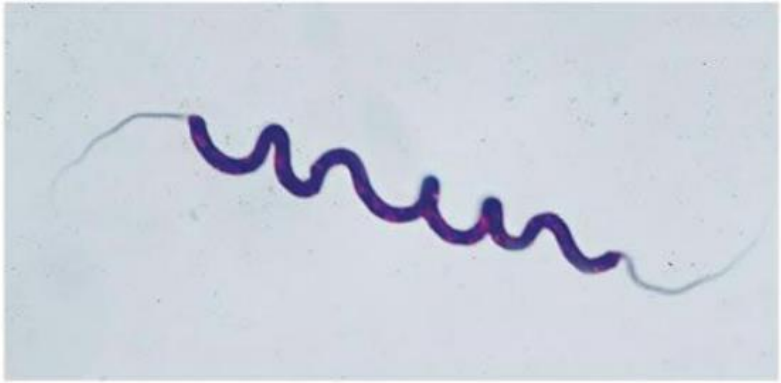
0.5 μm



(c) Lophotrichous and polar

SEM

0.5 μm



(d) Amphitrichous and polar

SEM

5 μm

Evidence of motility (Direct)

- Two ways by which motility can be demonstrated:

- **1- direct or microscopic :**

Hanging drop method.

- **Brownian movement :**

When the bacteria show molecular movement.

- **True motility**:

If a bacterium describes a rotatory, undulatory or sinuous movement.

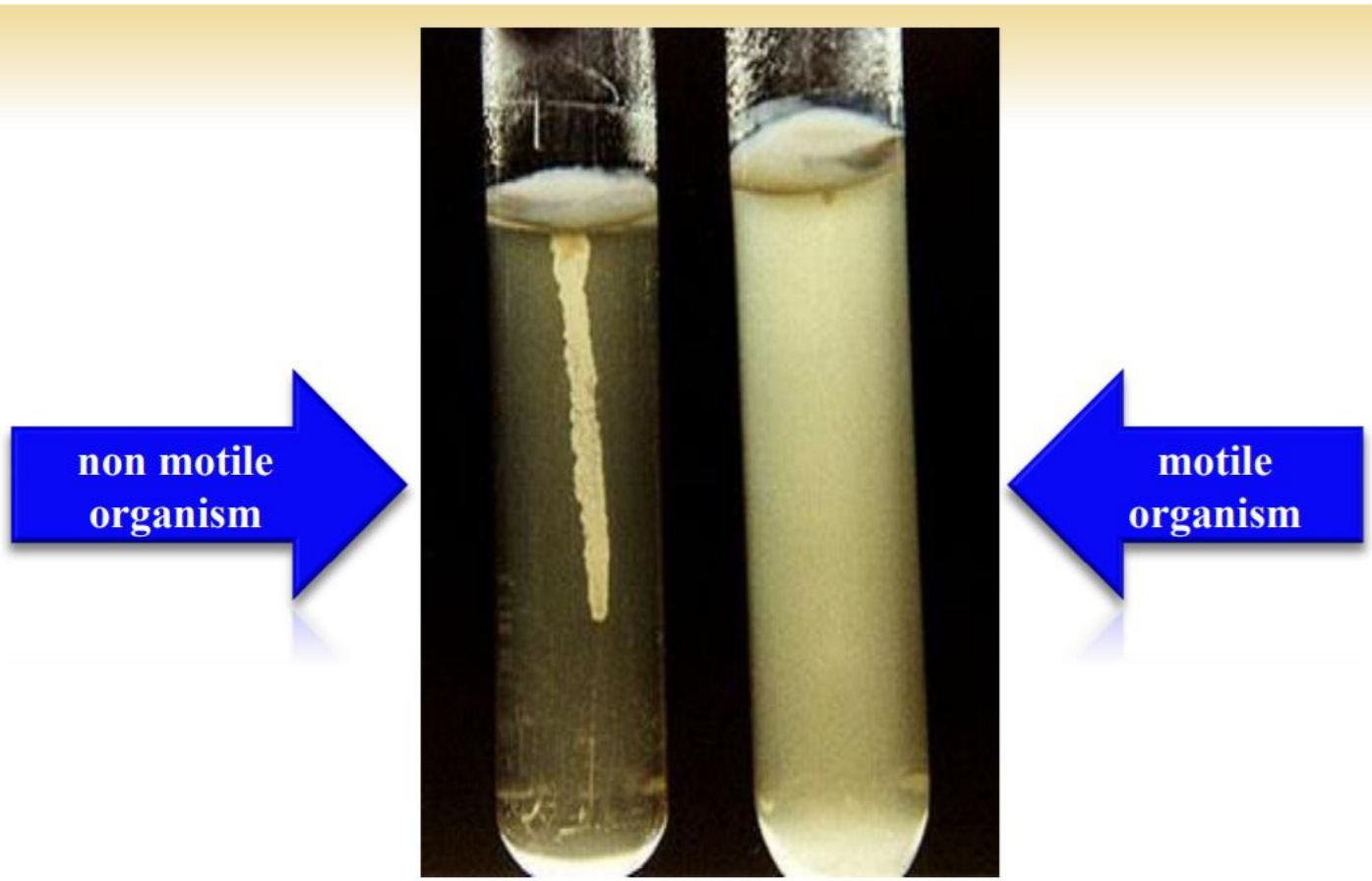
Evidence of motility (Indirect)

❖ **2- indirect or macroscopic :**

- Stab inoculation of the semisolid media
- Non motile : growth is limited at the point of inoculation
- Motile: growth is diffuse or moves away from the line of inoculation; turbidity of the medium

Evidence of motility (Indirect)

- Bacterial cells can swim in a semisolid medium.
- A semisolid medium such as O.75% agar 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, that indicates the incubated bacteria are motile bacteria.


non motile
organism
motile
organism

Experiment
(Hanging drop method)

- Materials:
  - Cultures:

24-hour broth cultures of motility bacteria.

  - Equipment:

1. Bunsen burner.
2. Inoculating loop.
3. Depression slides.
4. Coverslips.
5. Microscope.
6. Petroleum jelly.
7. Cotton swabs.

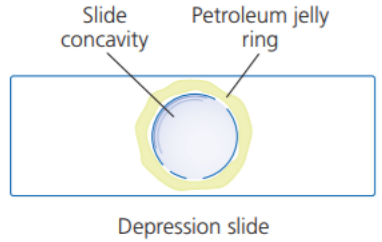
Experiment (Hanging drop method)

- Procedure:

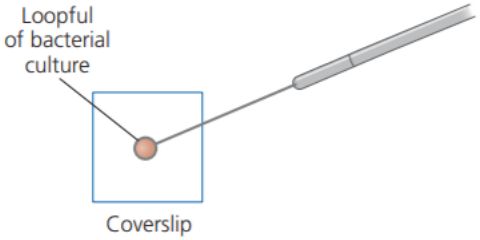
Steps 1–4 are illustrated in Figure 6.2.

1. With a cotton swab, apply a ring of petroleum jelly around the concavity of the depression slide.
2. Using aseptic technique, place a loopful of the culture in the center of a clean coverslip.
3. Place the depression slide, with the concave surface facing down, over the coverslip so that the depression covers the drop of culture. Press the slide gently to form a seal between the slide and the coverslip.
4. Quickly turn the slide right side up so that the drop continues to adhere to the inner surface of the coverslip.
5. For microscopic examination, first focus on the drop culture under the low-power objective (10x). Repeat using the high-power objective (40x).

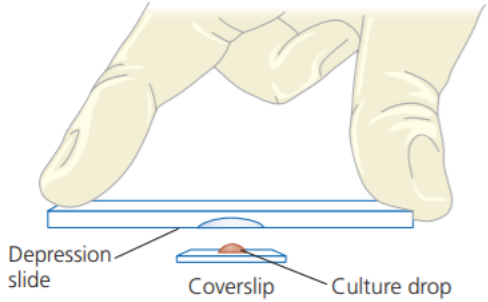
PROCEDURE



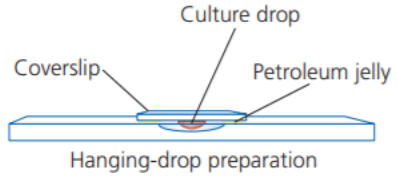
1 Spread a ring of petroleum jelly around the concavity of the depression slide.



2 Place a loopful of the bacterial culture in the center of the coverslip.



3 Lower the depression slide, with the concavity facing down, onto the coverslip. Press gently to form a seal.



4 Turn the hanging-drop preparation over so that the culture drop adheres to the coverslip.

Figure 6.2 Hanging-drop preparation

Reference

- https://fac.ksu.edu.sa/sites/default/files/362-lab4.pdf
- **James G. Cappuccino, Natalie Sherman. 2014. Microbiology a laboratory manual. 10th ed.**