

# Flagella staining

Microbiology 1



4.2.21

# Introduction

- Many bacteria are **motile** due to the presence of **flagella**
- it originate in the cytoplasm and project out from the cell wall
- They are fine, threadlike structures made up of protein called flagellin
- They cannot be easily observed under microscope.
- So a special technique is designed to increase **thickness** of flagella and to **stain** it.

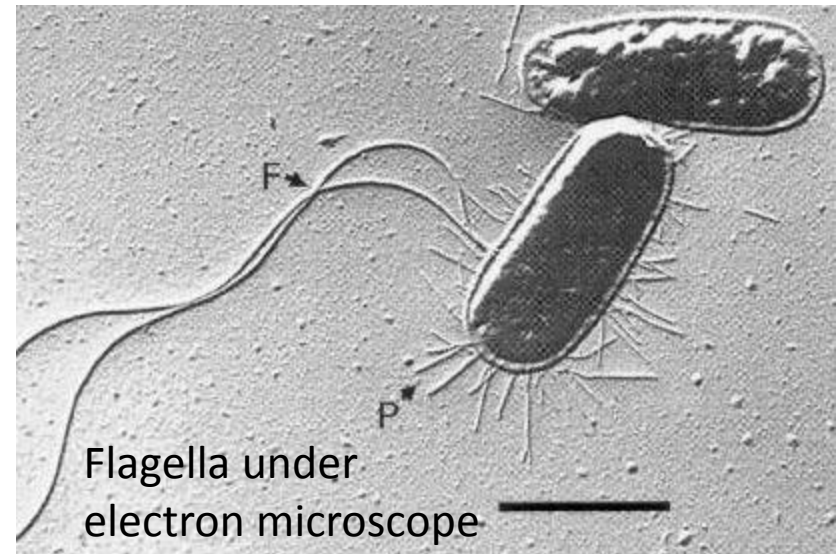




Figure: Monotrichous flagella



Figure: Lophotrichous flagella



Figure: Amphitrichous flagella

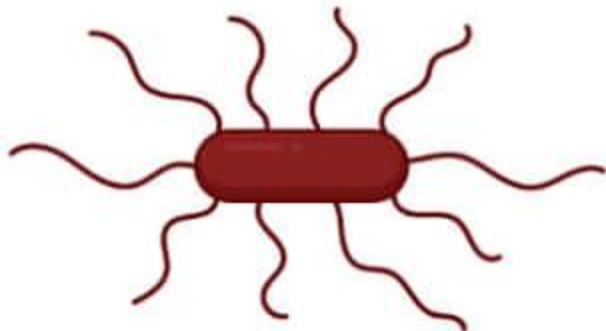


Figure: Peritrichous flagella

# Flagella

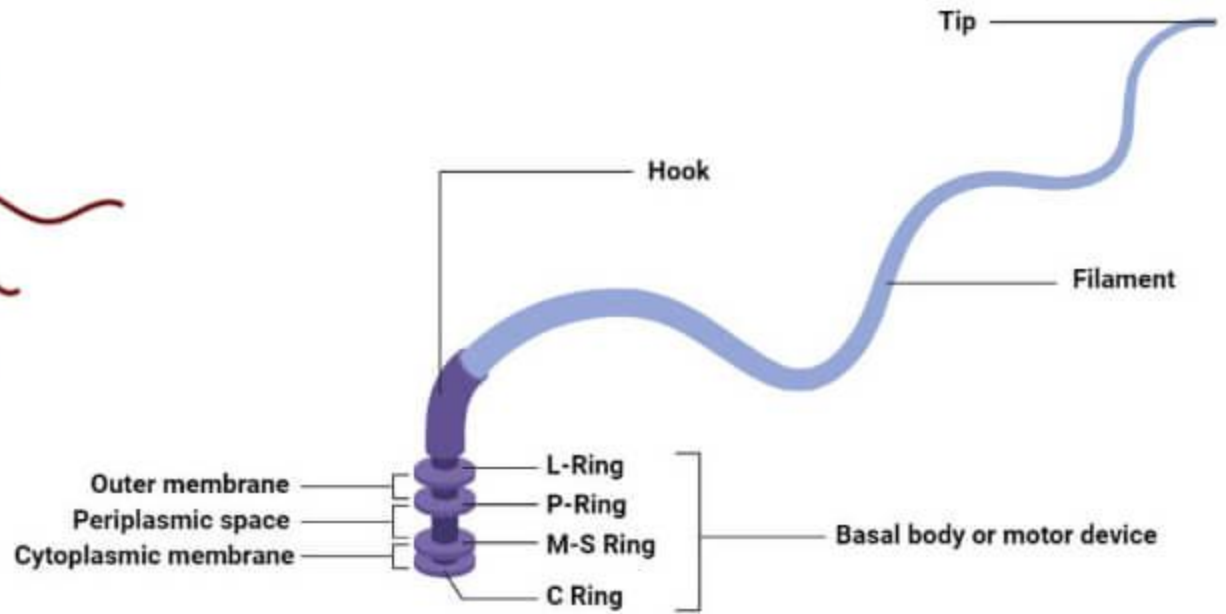


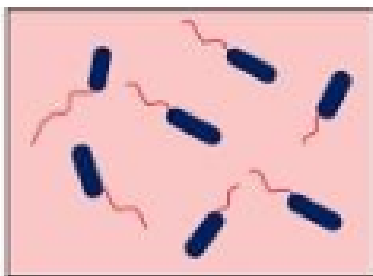
Figure: Structure of Flagellum of Gram Negative Bacterium

# Flagella staining

- To observe flagella with the light microscope the **thickness** of the flagella is increased.
- **Leifson's method (Dried-smear method):**
- This technique makes the use of Leifson dye by mixing a mordant (**tannic acid**) and (stain) **basic fuschin** formed in an alcohol base.

# Principle

- Leifson's stain is treated with cell
- tannic acid gets attached to flagella and alcohol gets evaporated
- flagella thickness is increased because of deposition of tannic acid.
- After the above treatment, cells are treated with Methylene blue stain.
- This will stain the cell blue and flagella appear pink.



LEIFSON'S  
STAINING

- **Requirement**


- Flagellated cell culture slant.
- Leifson's stain.
- 1 % Methylene blue.
- Distilled water.

# Preparation of Leifson's Stain


Reagent	Vol.
Ammonium or potassium alum saturated water solution:	20 ml
Tannic acid in 20% water solution:	10 ml
Distilled water:	10 ml
95% of ethyl alcohol:	15 ml
Saturated ethyl alcohol solution of basic fuschin:	3 ml

# Procedure


Take slant of flagellated bacterial culture + Add 2-3 drops of sterile distilled water




Incubate for 20 mins  
Air dry the smear



Slide is flooded with Leifon's stain till a thin film of shiny surface appear  
wash the slide with water



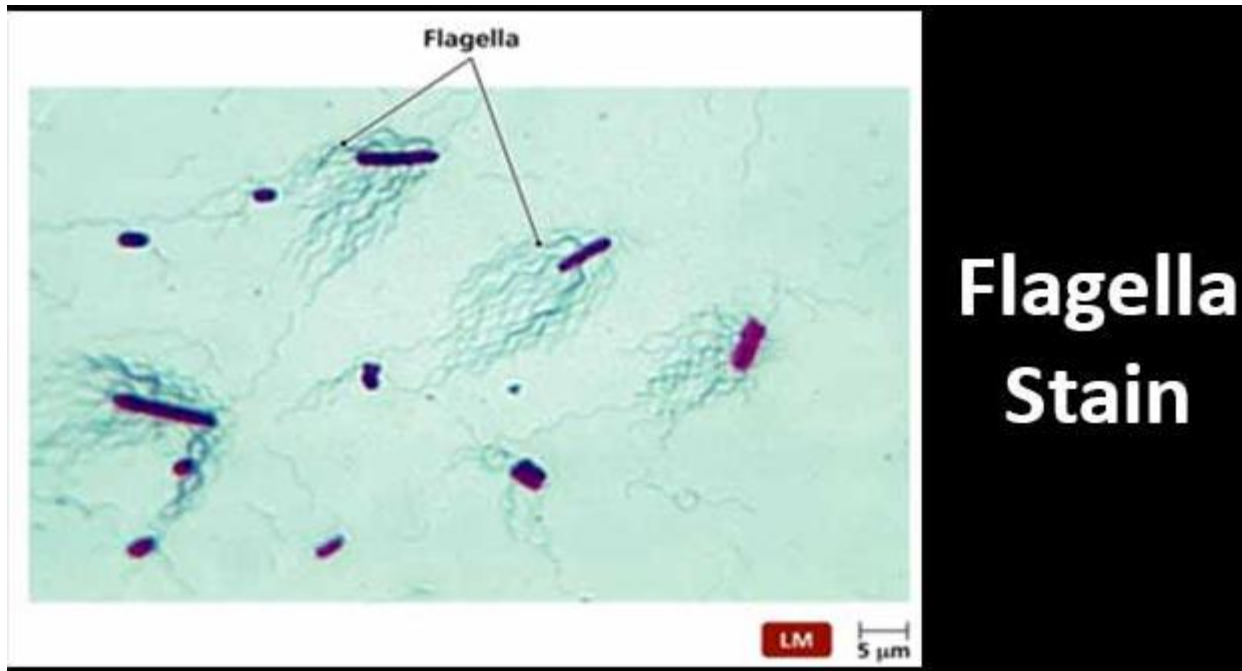
treat the slide with 1 % methylene blue treatment for 1 minute



slide water wash treatment ,air dry  
and observe under oil immersion lens



# Observation



Motile bacteria ex:

- *Escherichia coli*,
- *Salmonella species*,
- *Pseudomonas aeruginosa*,
- *Vibrio cholerae*

## Uses:

- Flagella staining is used to check the motility in the bacteria
- to classify them depending upon its:
  - Presence or absence
  - number and
  - arrangement
- on the bacterial cell, under the light microscope.