Bacterial taxonomy Lab:5

Flagella Stain

Flagella (singular flagellum) are threadlike, long, thin helical filaments measuring 0.01-0.02nm in diameter. These appendages extend outward from the plasma membrane and cell wall. Flagella are so thin that they cannot be observed directly with a bright field microscope, but must be stained with special techniques (example: Fontana's silver staining technique) that increase their

thickness. The detailed structure of a flagellum can only be seen in the electron microscope

The bacterial flagellum is composed of three parts: a basal body (associated with the cytoplasmic membrane and cell wall), a short hook and a helical filament (which is usually several times as long as the cell). Filament is external to cell wall and is connected to the hook at cell surface; the hook and basal body are embedded in the cell envelope (Figure 7.4). Hook and filament are composed of protein subunits called as flagellin.

One can generalize that all spirilla, about half of the bacilli and a small number of cocci are flagellated. Some bacteria do not have flagella. Flagella vary both in number and arrangement on the cell surface. Flagella are arranged generally in two patterns.

1. In polar arrangement, the flagella are attached at one or both ends of the cell. Bacteria with polar flagellar arrangement are

further classified into monotrichous, lophotrichous, and amphitrichous.

2. In lateral arrangement, flagella are arranged randomly all over the surface of the cell. Bacteria with lateral flagellar arrangement are called peritrichous.

Various types of mobility are observed based on the arrangement of the flagella. Serpentine motility is seen with *Salmonella*, darting motility with *Vibrio* and tumbling motility with *Listeria monocytogenes*. Some bacteria like *Cytophaga* exhibit a gliding motility, which is slow sinuous flexing motion. This occurs when the cells come in contact with solid surface.

Some bacteria have the ability to move toward or away from chemical substance. This movement is called chemotaxis. Positive chemotaxis is the movement of a cell in the direction of a favorable chemical stimulus (usually a nutrient). Negative chemotaxis is the

movement away from a chemical substance (usually harmful compound). Some photosynthetic bacteria exhibit phototaxis, movement in response to light rather than chemicals.

The presence of motility is one piece of information used to identify a pathogen in the laboratory. One way to detect motility is to stab a tiny mass of cells into soft (semi solid) medium in a test tube. Growth spreading rapidly through the entire medium is indicative of motility. Alternatively, cells can be observed microscopically by a hanging drop method. As shown in the table (7.1).

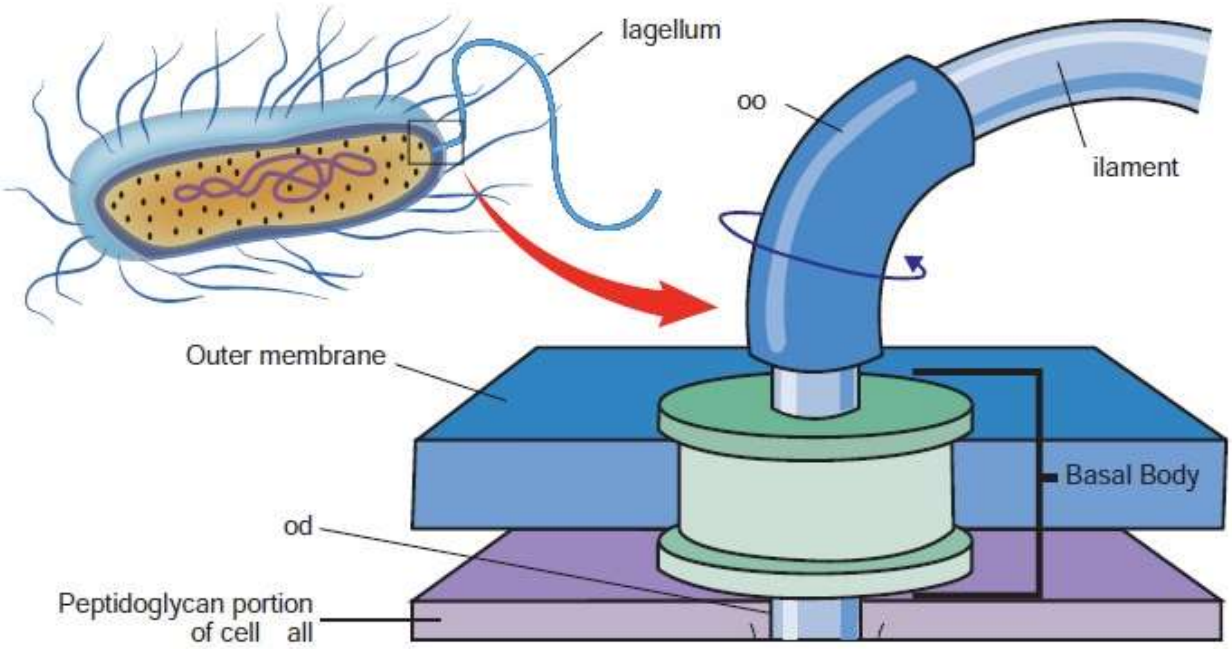


Figure 7.4: Structure of bacterial flagella

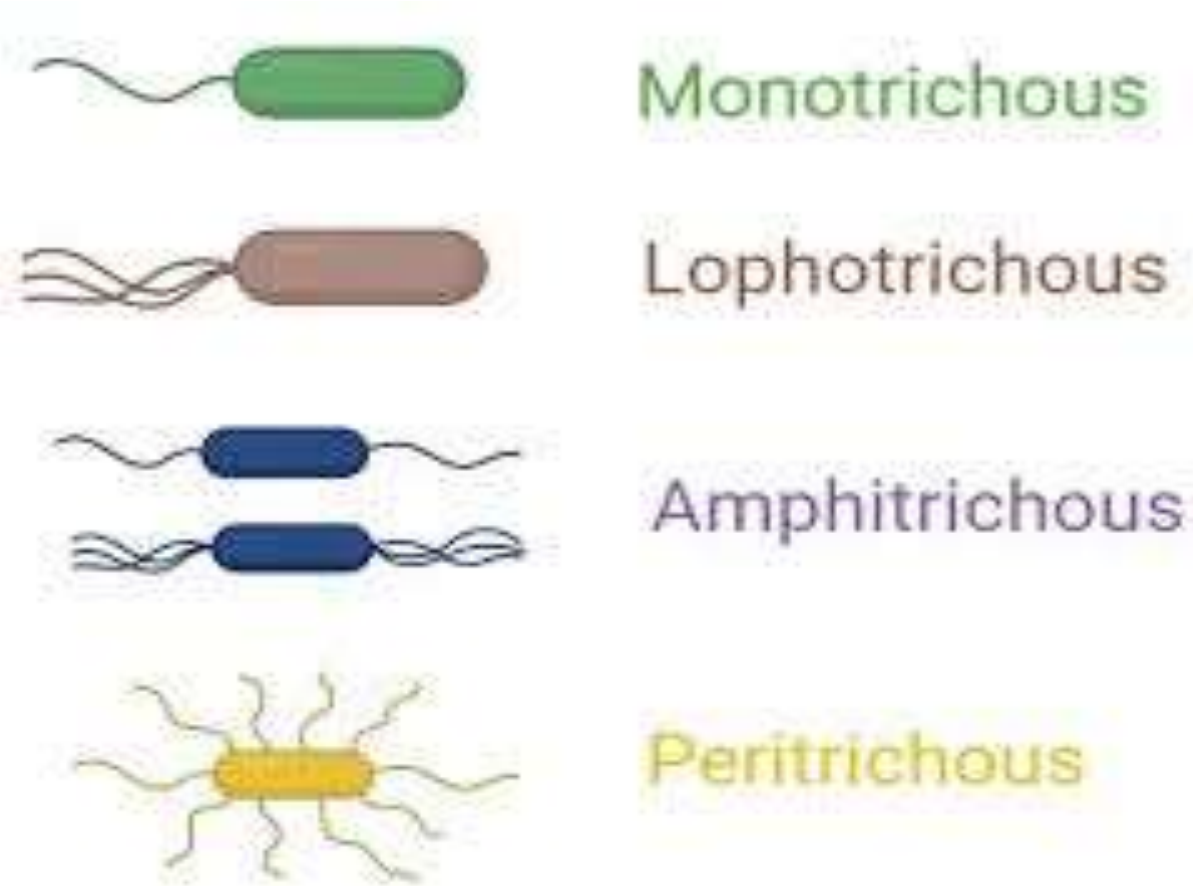
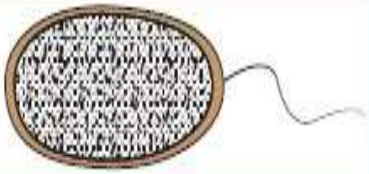
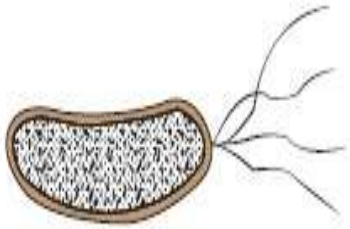
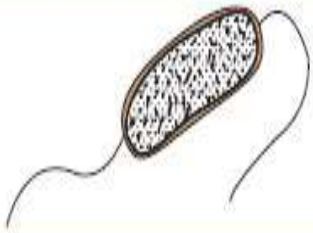
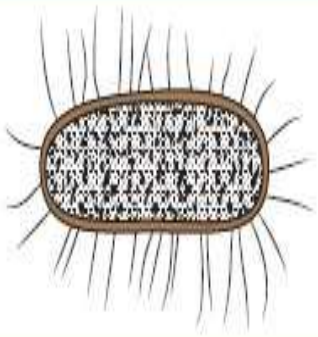


Table 7.1: Arrangement of bacterial flagella

Structure	Flagella type	Example
	Monotrichous(single flagella on one side)	*Vibrio cholera*
	Lophotrichous(tuft of flagella on one end)	*Pseudomonas fluorescens*
	Amphitrichous(single or tuft on both ends)	*Aquaspirillum serpens*
	Peritrichous(flagella throughout the cells)	*Salmonella typhi*

Flagella stain

Flagella stain is a specialized microbiology technique used to visualize delicate bacterial flagella (10–30 nm) under a light microscope by coating them with mordants (like tannic acid or potassium alum) and staining with dyes (e.g., crystal violet or basic fuchsin). The stain increases the apparent diameter of the flagella, allowing for identification of their presence, number, and arrangement.

Principles and Techniques

Purpose:

To determine the motility and arrangement of flagella (e.g., peritrichous, monotrichous, lophotrichous) for bacterial identification.

Mechanism:

The method relies on a mordant to thicken the slender flagella, as they are too thin to be seen with simple staining alone.

Common Methods:

Leifson method: Uses tannic acid and a dye in an alcoholic solution to form a precipitate.

Ryu method: Uses crystal violet, tannic acid, and aluminum potassium sulfate to stain the flagella.

Note: Samples must be handled carefully (no harsh heat fixing or blotting) to avoid breaking the delicate, hair-like flagella.

The Leifson Method:

The Leifson Method is a specific staining technique used in microbiology to visualize bacterial flagella, which are generally too thin (10–30 nm) to be seen with a standard light microscope. This method involves using a mordant (tannic acid) to coat the flagella, increasing their thickness, followed by staining with basic fuchsin.

Principle

Flagella are below the visual limit of a light microscope (roughly 0.1 to 0.3μm in diameter). The Leifson stain uses an alcoholic solution containing basic fuchsin (dye), tannic acid (mordant), and NaCl.

- **Mordant (Tannic acid): Adsorbs onto the flagella, causing heavy precipitation, which increases the thickness of the flagella.**
- **Dye (Basic Fuchsin): Colors the thickened flagella.**
- **Alcohol: Facilitates the process as it evaporates, creating a deposit of stain around the flagella.**

Staining Solution

The working solution is usually prepared by mixing equal parts of three stock solutions, which should be prepared fresh or stored properly (4°C for 1 month or -20°C for 1 year):

- **Solution I: 1.2% Basic Fuchsin in 95% Ethanol.**
- **Solution II: 3% Tannic Acid in Distilled Water.**
- **Solution III: 1.5% Sodium Chloride (NaCl) in Distilled Water.**

Procedure

1. **Slide Preparation: Use a young (18–24 hour) broth culture or a light suspension of colony growth in distilled water.**
2. **Smear & Fixation: Place the suspension on a clean, preferably grease-free slide. Allow it to air-dry. Do not heat fix, as this destroys the delicate proteinaceous structure of the flagella.**

3. **Staining:** Flood the smear with the Leifson stain solution for 7–15 minutes. A shiny film indicates proper reaction.
4. **Rinsing:** Gently wash the slide with water to remove excess stain.
5. **Observation:** Air-dry and examine under oil immersion.

Appearance

- **Flagella:** Appear red, pink, or sometimes dark brown.
- **Bacteria:** Appear red or blue-black.

Factors Affecting Results

- **Culture Age:** Young cultures (18-24 hours) are essential for active motility and presence of flagella.
- **Slide Cleanliness:** Slides must be perfectly clean to prevent high background debris.
- **Suspension Medium:** Use distilled water; avoid media with excessive proteins.

- **Temperature & pH:** The pH should be adjusted (e.g., pH 5.0) for optimal results, as the stain is sensitive to temperature and time.

Applications

- Identifying the presence, number, and arrangement of flagella (e.g., polar, peritrichous) for bacterial classification.
- Diagnosis of *Helicobacter pylori* in gastric biopsy specimens.

The Ryu method

The Ryu method is a rapid, wet-mount flagella staining technique that uses a stable, pre-mixed solution of tannic acid (mordant) and crystal violet (stain) to coat and thicken bacterial flagella, making them visible under a light microscope. It involves

adding the stain to a wet mount of motile bacteria, allowing 5–15 minutes of incubation, and observing with oil immersion.

Procedure for Ryu Method (Wet Mount):

1. **Sample Prep:** Place a small drop of sterile water on a clean slide. Using a sterile loop, gently transfer bacteria from a 16-24 hour culture into the water.
2. **Coverslip:** Place a coverslip over the suspension; it should barely reach the edges.
3. **Attachment:** Allow the slide to sit for 5-10 minutes to allow the bacteria to attach to the glass.
4. **Stain Application:** Apply 1-2 drops of Ryu stain (1 part crystal violet, 10 parts mordant) to the edge of the coverslip, letting it flow underneath via capillary action.

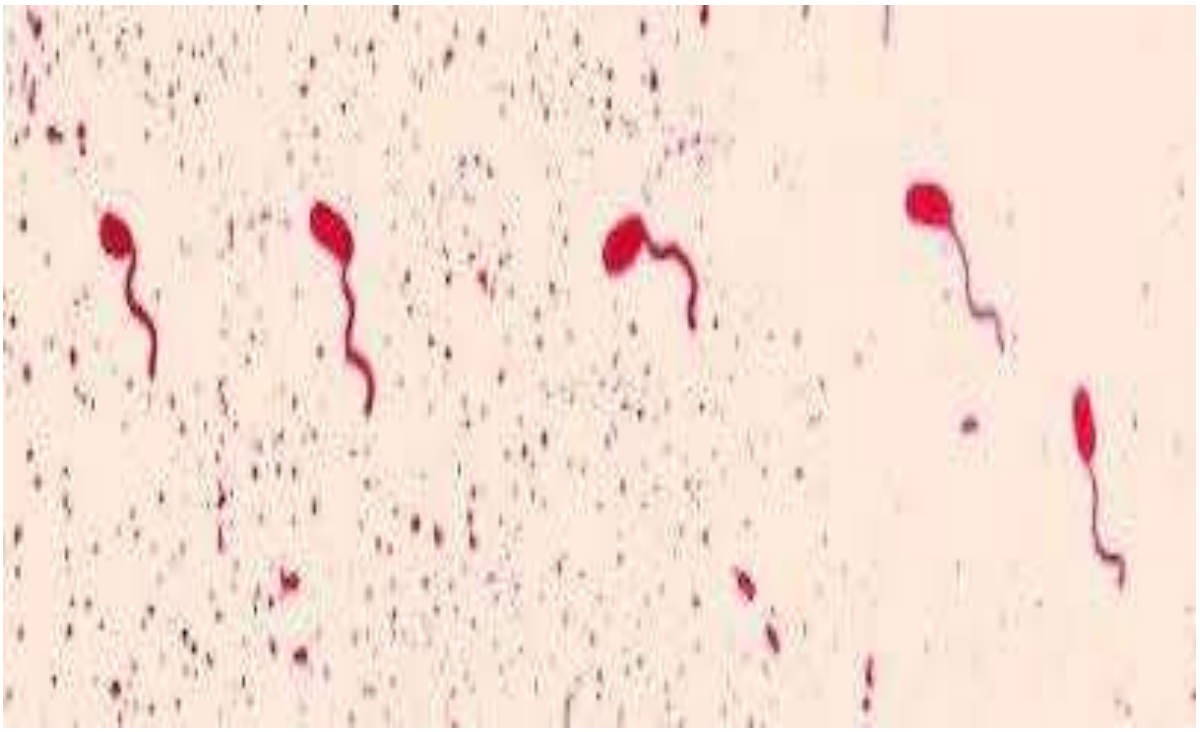
5. **Incubation:** Incubate for 5 to 15 minutes at room temperature.
6. **Observation:** Examine under 100x oil immersion; flagella will appear purple.

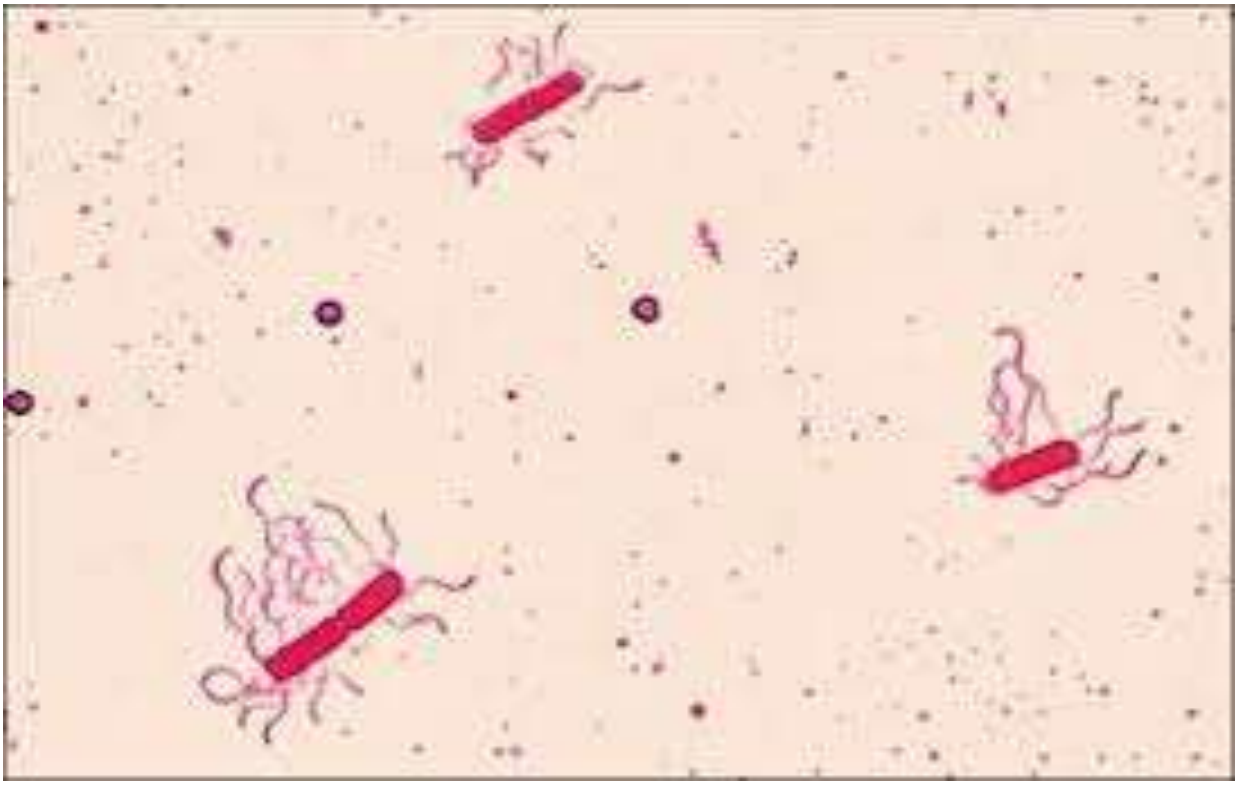
Key Aspects of the Ryu Method:

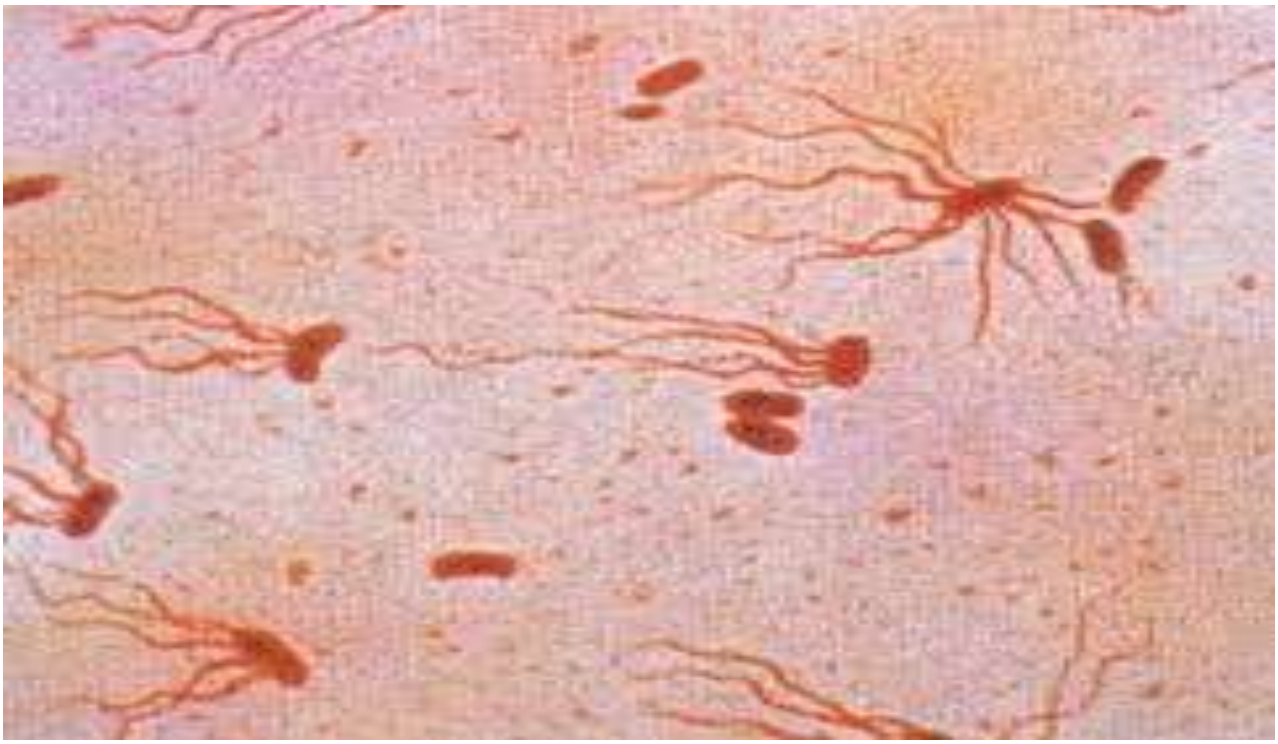
- **Ready-to-Use:** The stain is stable at room temperature indefinitely.
- **No Heat Fixing:** The wet-mount technique avoids the delicate, heat-sensitive nature of traditional, dried flagella smears.

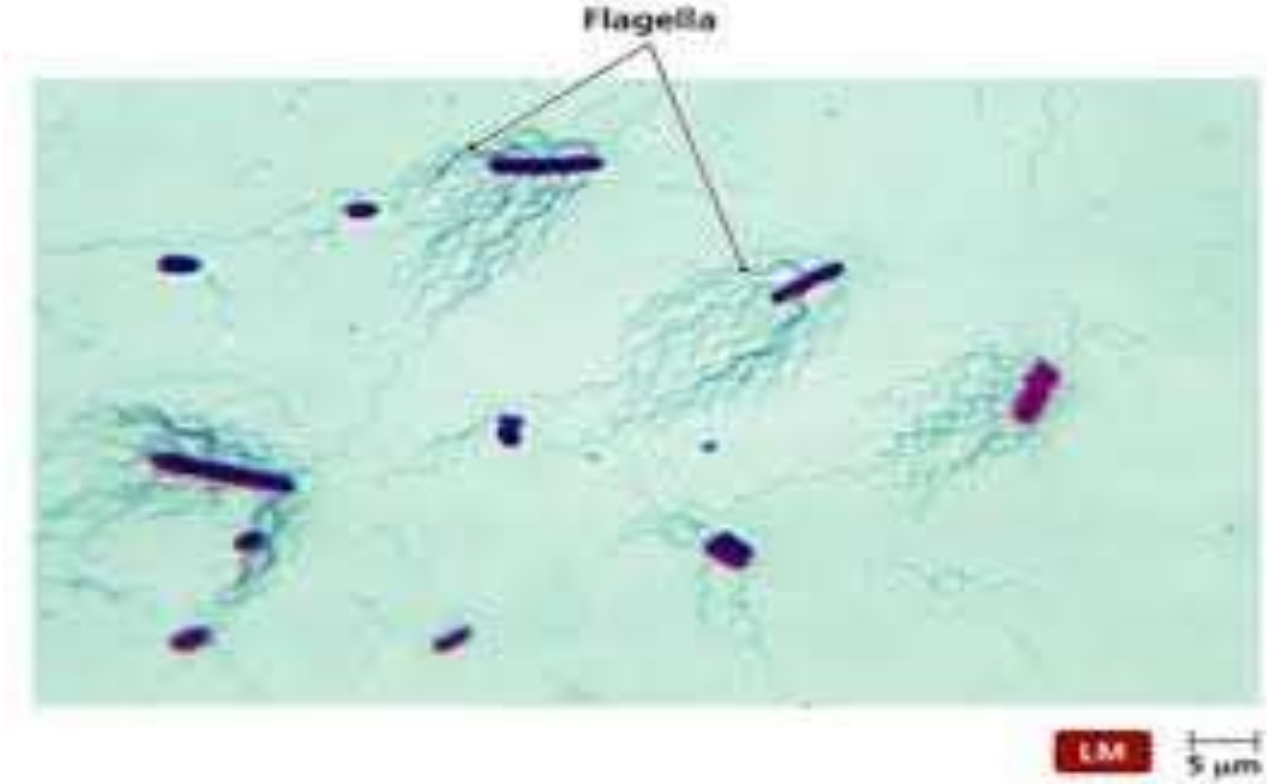
Mechanism:

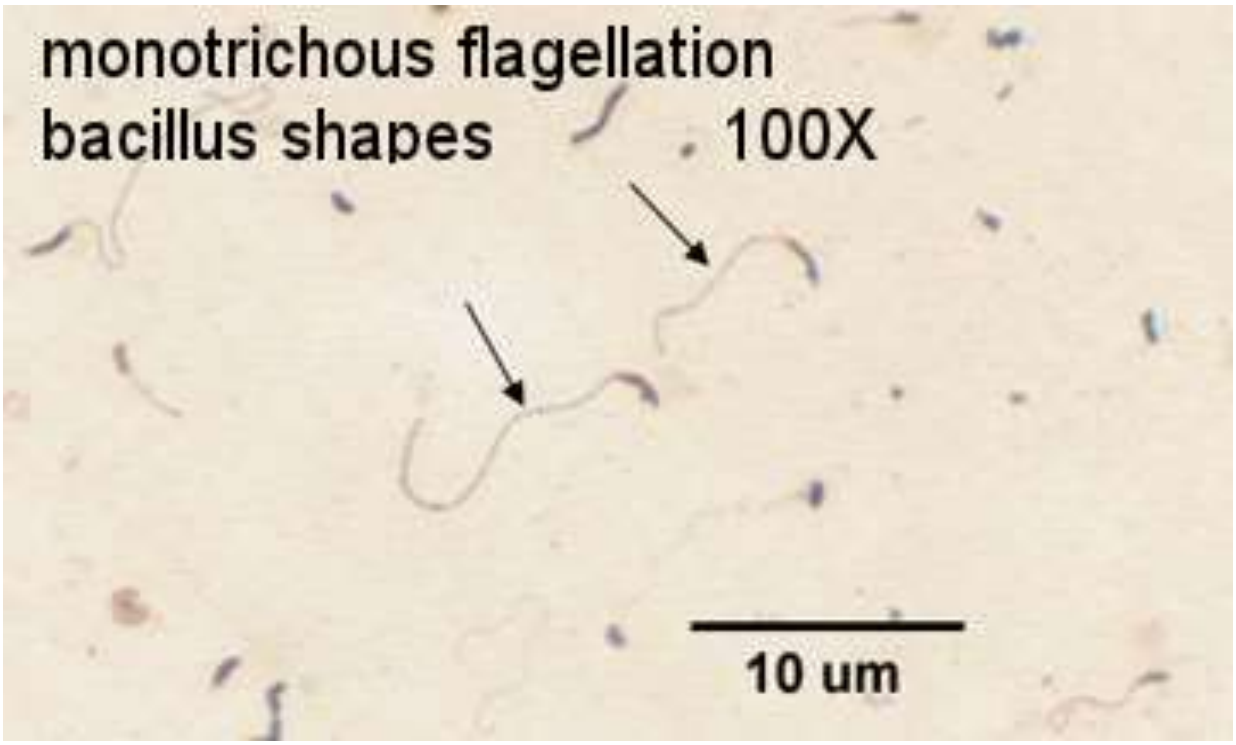
The tannic acid acts as a mordant to precipitate onto the flagella, while crystal violet stains the thickened appendage, allowing visualization of the number and arrangement of flagella.

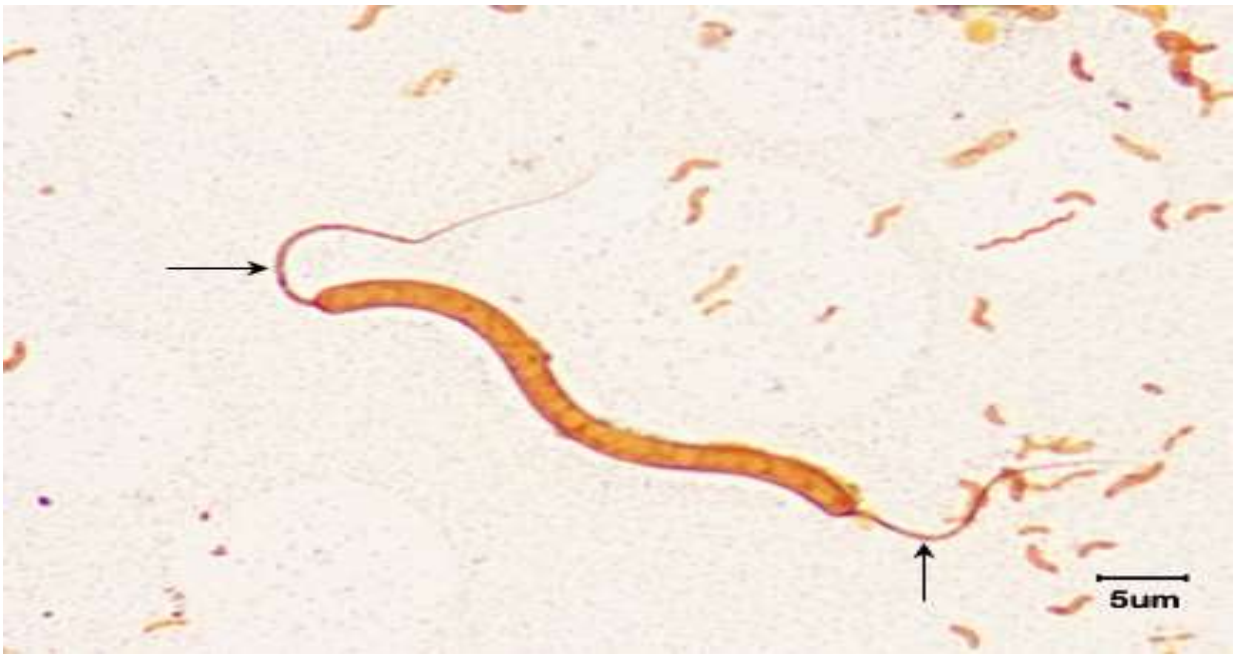







Flagella
LM
5 µm


monotrichous flagellation
bacillus shapes
100X
10 um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