PW2: Other Gram-negative bacilli bacteria

Pseudomonas aeruginosa

1- Introduction

The genus *Pseudomonas* comprises a diverse group of Gram-negative bacilli that are widely distributed in nature, especially in soil, fresh water, and marine environments. These bacteria are strictly aerobic, motile due to the presence of polar flagella, and oxidase positive. They are characterized as non-fermenters**,** meaning that instead of fermenting carbohydrates, they rely primarily on oxidative metabolism for energy production. Their remarkable metabolic versatility enables them to survive in nutrient-poor and even hostile environments, which explains their success as both environmental organisms and opportunistic pathogens (Wu et al., 2015).

One of the most studied species, *Pseudomonas aeruginosa*, has important clinical significance. It is considered an opportunistic pathogen, frequently associated with hospital-acquired infections such as pneumonia, urinary tract infections, wound and burn infections, and septicemia. It is also capable of producing distinctive pigments, including pyocyanin (blue-green) and pyoverdine (yellow-green, fluorescent), which are useful laboratory markers. In addition, *P. aeruginosa* shows a high level of intrinsic resistance to antibiotics and disinfectants, largely due to its impermeable outer membrane and active efflux pumps, making infections difficult to treat and a serious concern in immunocompromised patients (Wu et al., 2015).

Beyond human disease, *Pseudomonas* species also play beneficial roles. Some are important in agriculture, acting as plant growth-promoting rhizobacteria or as biocontrol agents due to their ability to produce antimicrobial substances. Others are used in biotechnology and bioremediation, where their ability to degrade hydrocarbons and other pollutants makes them valuable in cleaning contaminated environments (Krell and Matilla, 2024).

Pseudomonas spp. represents a group of Gram-negative bacilli that are ecologically versatile, medically significant, and industrially useful. Their dual importance, both as opportunistic pathogens and as beneficial organisms, illustrates their central role in microbiology and applied sciences (Krell and Matilla, 2024).

2- Objectives

The objective of this workshop is to enable students to:

- Recognize the main morphological and biochemical characteristics of *Pseudomonas aeruginosa* as a model of non-fermentative Gram-negative bacilli.
- Apply key laboratory tests (oxidase, catalase, motility, O/F glucose utilization, pigment production, etc.) for its identification.
- Develop skills to differentiate *P. aeruginosa* from Enterobacteriaceae and other Gram-negative bacilli using standard microbiological techniques.

3- Techniques

3-1- Gram staining

The Gram staining technique, already explained in detail during PW1, is a fundamental method used to classify bacteria based on their cell wall structure. It differentiates organisms into Gram-positive (thick peptidoglycan layer, retaining the crystal violet stain) and Gram-negative (thin peptidoglycan, decolorized by alcohol and counterstained with safranin).

Expected result for *Pseudomonas* spp.: Gram-negative rods, appearing as pink to red bacilli, typically isolated and slender.

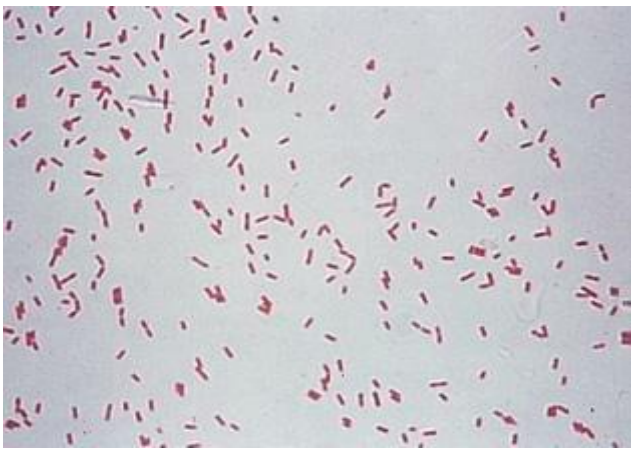


Figure 11. Gram staining of *Pseudomonas aeruginosa* (Showkat et al., 2016)

3-2- Catalase and oxidase tests

The oxidase test (positive in *Pseudomonas* spp.) and the catalase test (also positive) are important biochemical reactions used to differentiate Gram-negative bacilli. These techniques were already explained in detail during PW1, and in this session they serve mainly as

confirmatory tools. A positive oxidase reaction is a key criterion that distinguishes *Pseudomonas* spp. from Enterobacteriaceae, which are oxidase negative, while the catalase reaction confirms the presence of the enzyme that breaks down hydrogen peroxide into water and oxygen (Stewart et al., 2000).

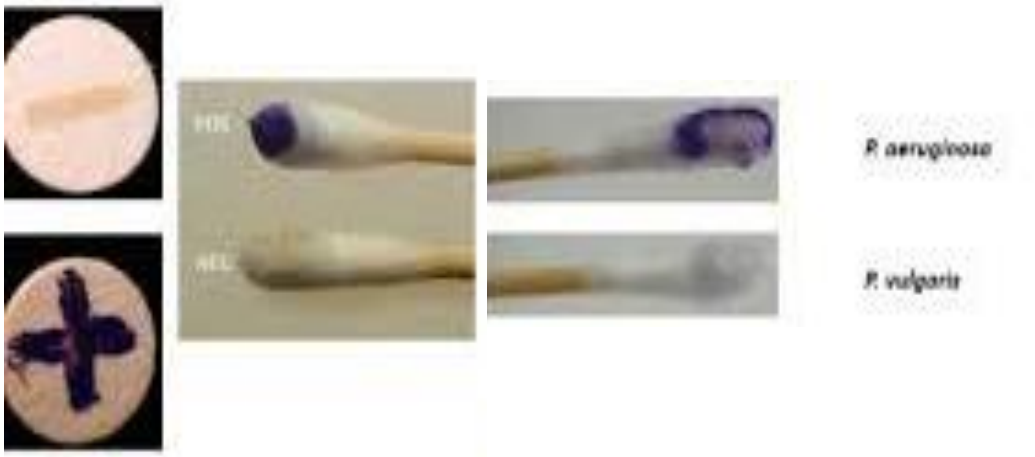


Figure 12. Oxidase results in case of *Pseudomonas* species

3-3- Mobility test

The motility test is used to determine whether bacteria are capable of active movement. For *Pseudomonas* spp., this test is particularly important because they are usually motile thanks to a polar flagellum, which helps differentiate them from non-motile Gram-negative bacilli such as *Klebsiella* or *Shigella*.

The test is performed in a semisolid agar medium (motility agar), which contains nutrients to support growth and a low concentration of agar (0.3–0.4%) to allow the free movement of motile bacteria. Some formulations include TTC (triphenyltetrazolium chloride), a colorless indicator that turns red when reduced, making bacterial growth more visible (Yoon and Jeong, 2021).

- ✓ **Principe**

When a motile bacterium is inoculated into the semisolid medium, it moves away from the inoculation line by means of its flagella, producing diffuse, hazy growth in the agar. In contrast, non-motile bacteria remain confined to the stab line where they were inoculated (Turnbull and Whitchurch, 2014).

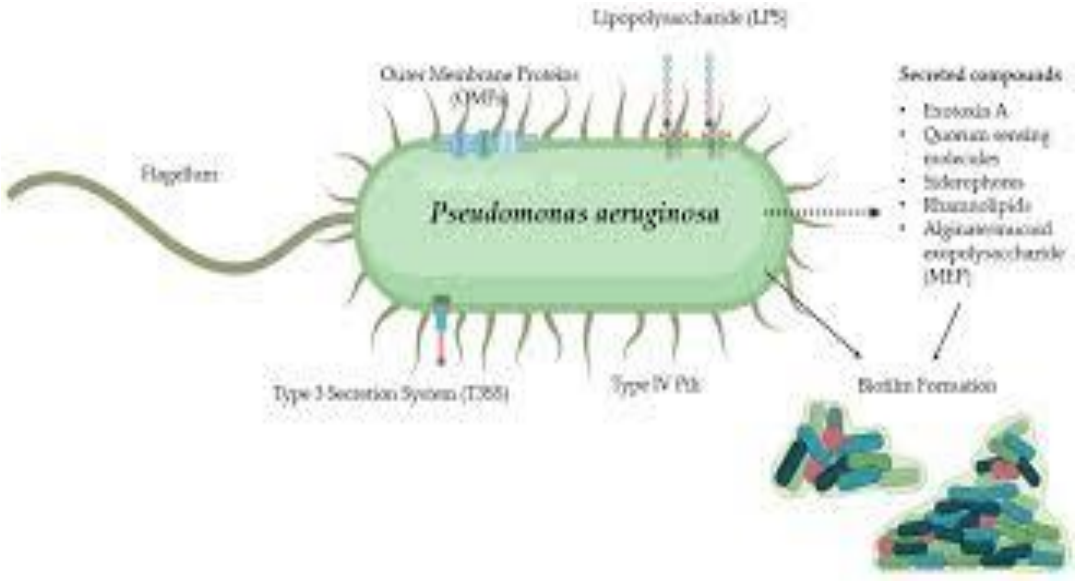
- ✓ **Inoculation**

Using a sterile straight needle, a single stab is made in the center of the medium, from the surface to about two-thirds of the depth of the tube. The tube is then incubated at 37 °C for 24 h under aerobic conditions.

- ✓ **Results interpretation**

Positive (motile)**:** Diffuse or cloudy growth spreading outward from the stab line; the medium often appears turbid (typical of *Pseudomonas* spp.).

Negative (non-motile): Growth is restricted to the stab line, with the surrounding medium remaining clear (Turnbull and Whitchurch, 2014).



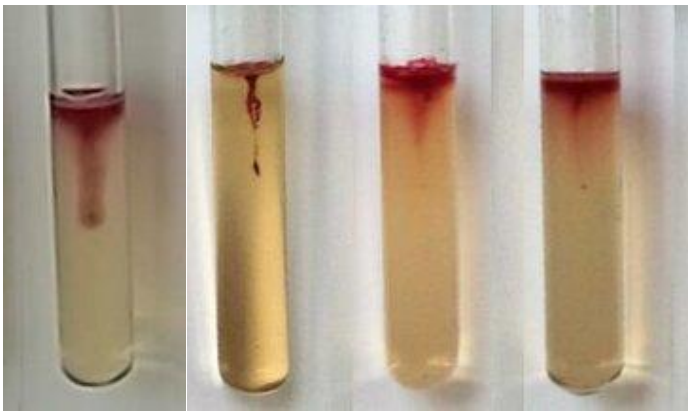


Figure 13. Motility test. *Pseudomonas aeruginosa* -motile strain; *Pseudomonas veronii*; *Pseudomonas gessardii* and *Pseudomonas* sp (from left to right) (Baoune et al., 2017)

3-4- Pigment production test

Pigment production is a useful test for the identification of *Pseudomonas aeruginosa*, since this bacterium produces characteristic diffusible pigments that are not commonly found in other Gram-negative bacilli. These pigments, particularly pyocyanin (blue-green) and pyoverdine (fluorescent yellow-green), are distinctive features that help differentiate P. aeruginosa from other Pseudomonas species (Antonic et al., 2013).

- ✓ **Principe**

The test is based on the ability of *P. aeruginosa* to synthesize pigments under specific nutritional conditions.

- Pyocyanin is a blue-green, water-soluble pigment produced in King's A medium.
- Pyoverdine (fluorescein) is a yellow-green fluorescent pigment produced in King's B medium. These pigments diffuse into the medium and can be seen visually, with pyoverdine also observable under UV light due to its fluorescence (Antonic et al., 2013).

- ✓ **Composition**

King's A medium: contains peptone, magnesium chloride, potassium sulfate, enhances production of pyocyanin.

King's B medium: contains glycerol, peptone, potassium sulfate, magnesium chloride, favors the production of pyoverdine (fluorescein) (Rice et al., 2012).

- ✓ **Inoculation**

The medium is inoculated with a pure culture of *Pseudomonas* using a sterile loop to streak the surface of the agar. Plates are incubated at 37 °C for 24-48 hours under aerobic conditions, since pigment production is oxygen-dependent (Rice et al., 2012).

- ✓ **Results interpretation**

Positive for *P. aeruginosa*:

- On King's A: Blue-green pigment (pyocyanin) diffuses into the medium.
- On King's B: Yellow-green fluorescent pigment (pyoverdine) visible under normal light and showing bright fluorescence under UV light.

Negative / Other *Pseudomonas*: No pigment or only pyoverdine production without pyocyanin (Rice et al., 2012).

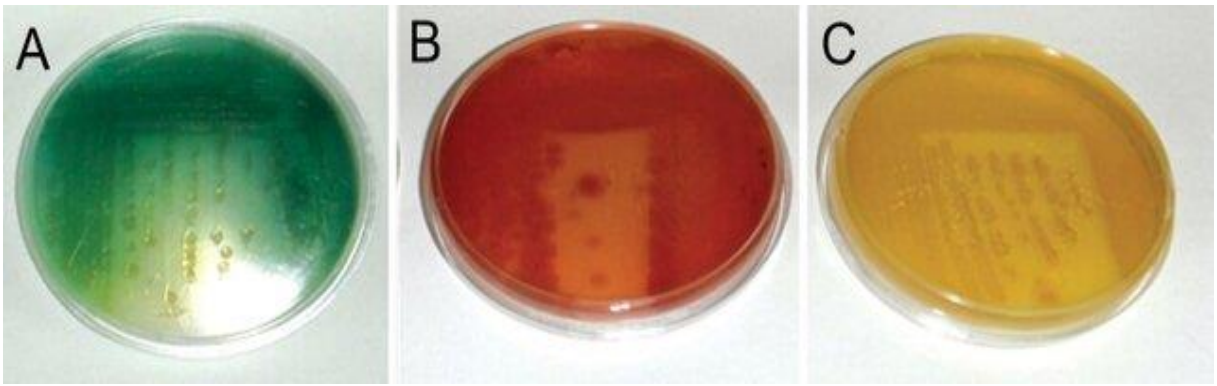


Figure 14. Pigments produced by three different strains of *P. aeruginosa* (Nowroozi et al., 2012).

3-5- Glucose utilization test (O/F test of Hugh & Leifson)

Many Gram-negative bacilli can be classified according to their ability to use glucose either by fermentation (anaerobic breakdown) or by oxidation (aerobic metabolism). This is important because *Pseudomonas* spp. are non-fermentative bacteria, and the O/F (oxidation–fermentation) test allows clear differentiation from Enterobacteriaceae, which are glucose fermenters (Hamill et al., 2008).

- ✓ **Principle**

The test uses two tubes containing Hugh & Leifson's O/F medium, which includes glucose and the pH indicator bromothymol blue.

One tube is left open (aerobic conditions), while the other is covered with a layer of mineral oil (anaerobic conditions).

- ✓ **Composition**

The medium contains glucose as the fermentable carbohydrate, which serves as the substrate to test whether the bacterium can metabolize sugars oxidatively or fermentatively. Peptone provides nitrogen and essential nutrients for bacterial growth. A very low concentration of agar (about 0.2–0.3%) gives the medium a semisolid consistency, allowing both aerobic and anaerobic conditions to be assessed. The pH indicator, bromothymol blue, changes color depending on acid production: it is green at neutral pH, turns yellow under acidic conditions (due to glucose metabolism), and shifts to blue under alkaline conditions (from peptone utilization) (Hamill et al., 2008).

✓ **Inoculation**

Two tubes of the medium are required for each bacterial strain. Using a sterile inoculating needle or loop, the tubes are inoculated by stabbing or gently streaking through the center of the medium to introduce the organism. One tube is left open to provide **aerobic conditions**, while the second tube is overlaid with a thin layer of sterile mineral oil to create an anaerobic environment. Both tubes are then incubated at 37 °C for 24-48 h. This dual setup allows the differentiation between oxidative and fermentative metabolism of glucose.

✓ **Results interpretation**

- Oxidative metabolism (O): Only the open tube turns yellow, the oil-covered tube stays green (typical of *Pseudomonas* spp.).
- Fermentative metabolism (F): Both tubes turn yellow (typical of *E. coli* and Enterobacteriaceae).
- Non-utilizer: Both tubes remain green (e.g., *Alcaligenes*) (Hamill et al., 2008).

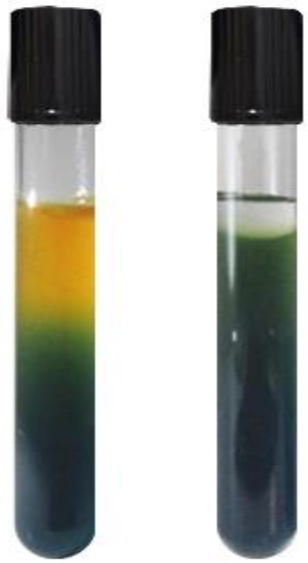


Figure 15. Appearance of *P. aeruginosa* on Hugh and Leifson medium, which exhibits negative fermentation. In the unsealed tube (left), it appears yellow at the top, while in the sealed tube (right), there is no observable change (Hamill et al., 2008)