Family: Pseudomonadaceae

Phylum: Proteobacteria

Class: Betaproteobacteria

Order: Pseudomonadales

Family: Pseudomonadaceae

Genus: *Pseudomonas*

Pseudomonas **Fluorescent group**	***Pseudomonas aeruginosa***
	Pseudomonas fluorescens
	Pseudomonas putida
Nonfluorescent group	***Pseudomonas stutzeri***
	Pseudomonas mendocina

Pseudomonas aeruginosa

General Characteristics :

1- gram-negative rods.

2- **strict aerobic**.

3- *P. aeruginosa* is widely distributed in nature and is commonly present in moist environments in hospitals.

4- Motile by one or several polar flagella .

5- It is occurs as single bacteria, in pairs, and occasionally in short chains.

6- It is **catalase-positive**, **oxidase-positive**..

Culture and Growth Characteristics:

P. aeruginosa is an obligate aerobe that grows readily on many types of culture media, sometimes producing a sweet or grape-like or corn taco-like odor. Some strains hemolyze blood. *P. aeruginosa* forms smooth round colonies with a fluorescent greenish color. It often produces the nonfluorescent bluish pigment **pyocyanin,** which diffuses into the agar. Other *Pseudomonas* species do not produce pyocyanin. Many

strains of *P. aeruginosa* also produce the fluorescent pigment **pyoverdin,** which gives a greenish color to the agar. Some strains produce the dark red pigment **pyorubin** or the black pigment **pyomelanin.** *P. aeruginosa* grows well at 37–42 °C; its growth at 42 °C helps differentiate it from other *Pseudomonas* species in the fluorescent group. It does not ferment carbohydrates, but many strains oxidize glucose. Specimens are plated on blood agar and the differential media commonly used to grow the enteric gram-negative rods. Pseudomonads grow readily on most of these media, but they may grow more slowly than the enterics. *P aeruginosa* does not ferment lactose and is easily differentiated from the lactose-fermenting bacteria.

Pathogenesis:

1. *P. aeruginosa* produces infection of wounds and burns, giving rise to blue-green pus.
2. Meningitis.
3. Urinary tract infection,
4. Necrotizing pneumonia.
5. It may cause invasive (malignant) otitis externa in diabetic patients.
6. Infection of the eye, which may lead to rapid destruction of the eye, occurs most commonly after injury or surgical procedures.
7. *P aeruginosa* may invade the bloodstream and result in fatal sepsis.

Enzymes and Toxins:

Most *P. aeruginosa* isolates from clinical infections produce extracellular enzymes, including **elastases, proteases**, and **haemolysins**: a heat-labile phospholipase C and a heat-stable glycolipid. Many strains of *P. aeruginosa* produce **exotoxin A**, which causes tissue necrosis and is lethal for animals when injected in purified form. The toxin blocks protein synthesis

Classification:

1. *P aeruginosa* can be typed by lipopolysaccharide immunotype.
2. By pyocin (bacteriocin) susceptibility.
3. rRNA/DNA homology.
4. Serological (H-Ag, O-Ag).
5. Phage typing.

***Specimens*:** Skin lesions, pus, urine, blood, spinal fluid, sputum, and other material should be obtained as indicated by the type of infection.

Laboratory diagnostic tests:

1. Gram stain (Gram-negative bacilli).

2. Culture on blood agar (for haemolysis)

3. Culture on milk agar (for pigmentation)

4. Culture on MacConkey agar (lactose-non fermenter)

5. Culture on Selective and Differential media (e.g.; King A, King B, Cetrimide agar)

6. TSI

7. IMViC test

8. Motility test

9. Nitrate reduction test

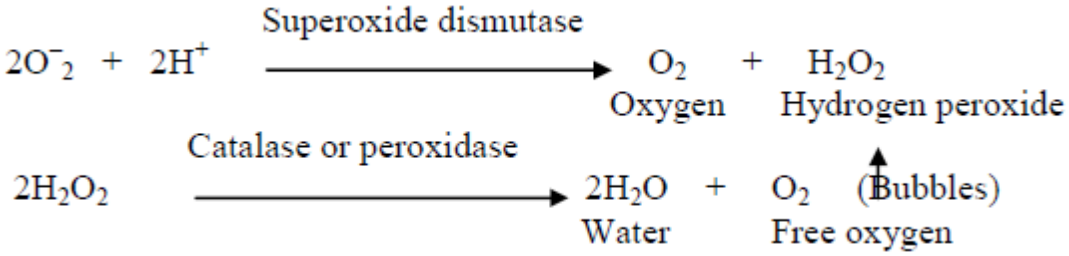
10. OF test (Oxidation-Fermentation) [Hugh & Leifson (HL)]

11. Oxidase test (+ ve)

12. Catalase test (+ ve)

13. Sensitivity test: A penicillin, ticarcillin or piperacillin is used in combination with an aminoglycoside, tobramycin. Other drugs active against *P aeruginosa* include aztreonam, imipenem, and the newer quinolones, including ciprofloxacin. cephalosporins, ceftazidime and cefoperazone.

Catalase test: Some bacteria contain flavoproteins that reduce O2, resulting in the production of hydrogen peroxide (H2O2) or superoxide (O2–). These are extremely toxic because they are powerful oxidizing agents and destroy cellular constituents very rapidly. A bacterium must be able to protect itself against such O2 products or it will be killed. Many bacteria possess enzymes that afford protection against toxic O2 products. Obligate aerobes and facultative anaerobes usually contain the enzymes superoxide dismutase, which catalyzes the destruction of superoxide, and either catalase or peroxidase, which catalyze the destruction of hydrogen peroxide as follows:



Most strict anaerobes lack both enzymes and therefore cannot tolerate O2.

***Substrate:* H2O2 3%**

Enzyme: Catalase

- **Positive catalase test: Bubbles of free O2 gas.**
- **Negative catalase test: The absence of bubble formation.**

Oxidase test: Oxidase enzymes play an important role in the operation of the electron transport system during aerobic respiration. Cytochrome oxidase uses O2 as an electron acceptor during the oxidation of reduced cytochrome c to form water and oxidized cytochrome c.

Substrate: Tetramethyl-p-phenylenediamine dihydrochloride (oxidase test reagent serves as an artificial substrate electrons to cytochrome oxidase)

Enzyme: Cytochrome oxidase

Reagent: (Tetramethyl-p-phenylenediamine dihydrochloride)

- **Positive oxidase test: Dark purple**
- **Negative oxidase test: No color change or a light pink**

Nitrate reduction test: Many chemoorganoheterotrophs (bacteria that require organic compounds for growth; the organic compounds serve as sources of carbon and energy) can use nitrate (NO–3) as a terminal electron acceptor during anaerobic respiration. In this process, nitrate is reduced to nitrite (NO–2) by nitrate reductase. Some of these bacteria possess the enzymes to further reduce the nitrite to either the ammonium ion or molecular nitrogen as illustrated:

Medium: Nitrate Broth

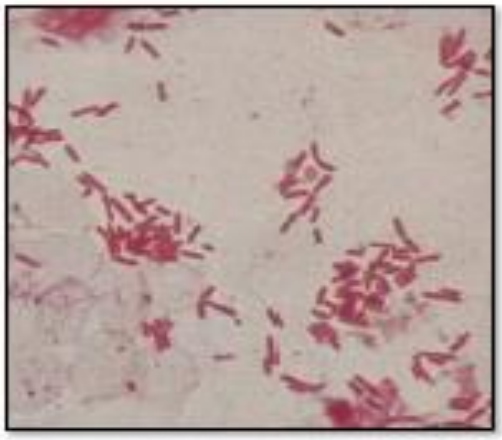
Substrate: Nitrate (NO3)

Enzyme: Nitrate reductase

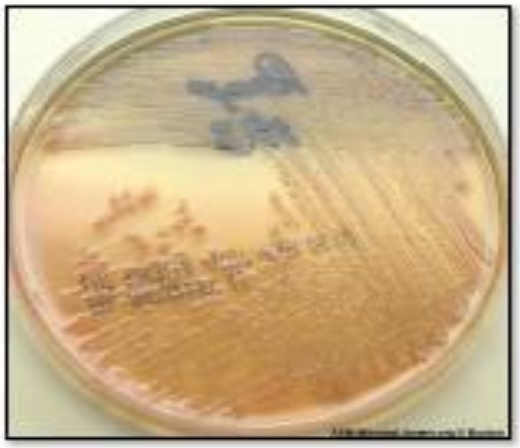
OF test (Oxidation-Fermentation): Two tubes were used, added to one of them paraffin on the surface to produce anaerobic conduction, inoculated by stabbing then incubation at 37ºC.

Medium: Hugh & Leifson (HL) Contains; peptone, glucose, K2HPO4, NaCl, agar-agar and Bromothymol blue as pH indicator.

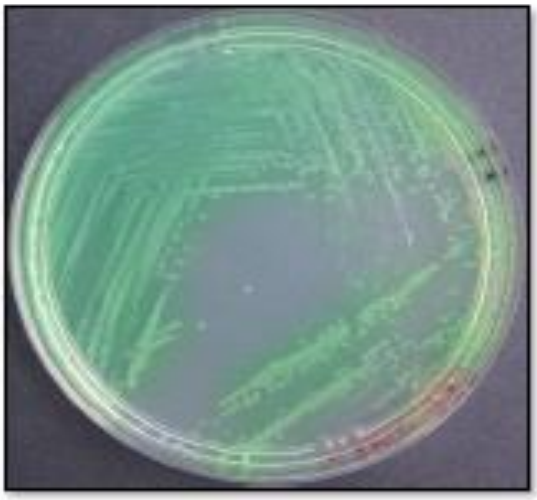
Test	*P. aeruginosa*	*P. fluorescence*
Indole	-	-
MR	-	-
VP	-	-
Citrate	+	+
TSI	K/K - -	K/K - -
Nitrate reduction test	+	+
Motility	+	+
Growth at 42°C	+	-
Growth at 4°C	-	+
King A	+ Pyocyanin	± Pyocyanin
King B	+ Flourescen	+ Flourescen
MacConkey agar	L.N.F transparence, irregular	L.N.F transparence, irregular
Oxidase	+	+
Catalase	+	+
OF medium	Oxidation + Fermentation -	Oxidation + Fermentation -



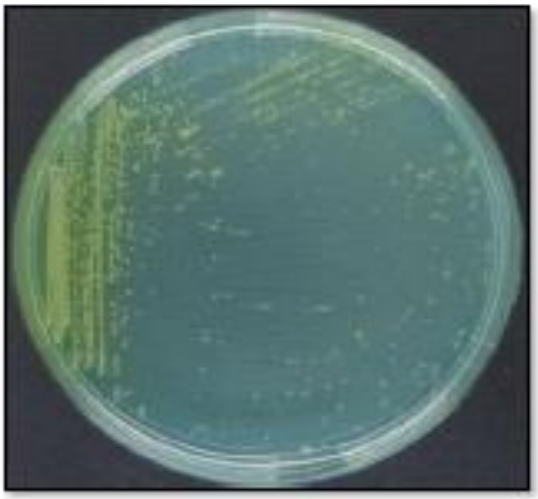
Pseudomonas aeruginosa (Gram-negative bacilli)



P. aeruginosa on MacConkey agar

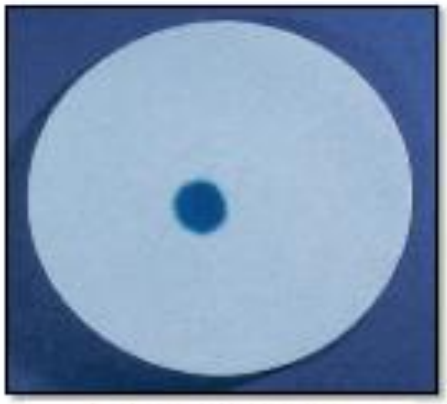


Pseudomonas aeruginosa on Cetrimide agar



Pseudomonas fluorescens on Kings B agar

Catalase test



Positive oxidase test