

Family: Pseudomonadaceae

Phylum: Proteobacteria
Class: Betaproteobacteria
Order: Pseudomonadales
Family: Pseudomonadaceae
Genus: *Pseudomonas*

<i>Pseudomonas</i> Fluorescent group	<i>Pseudomonas aeruginosa</i>
	<i>Pseudomonas fluorescens</i>
	<i>Pseudomonas putida</i>
Nonfluorescent group	<i>Pseudomonas stutzeri</i>
	<i>Pseudomonas mendocina</i>

Pseudomonas aeruginosa

General Characteristics : *P. aeruginosa* is widely distributed in nature and is commonly present in moist environments in hospitals. *P. aeruginosa* is **gram-negative, Motile by one or several polar flagella and rod-shaped**. It occurs as single bacteria, in pairs, and occasionally in short chains. It is **catalase-positive, oxidase-positive, strict aerobic**.

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 **pyoverdine**, 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:

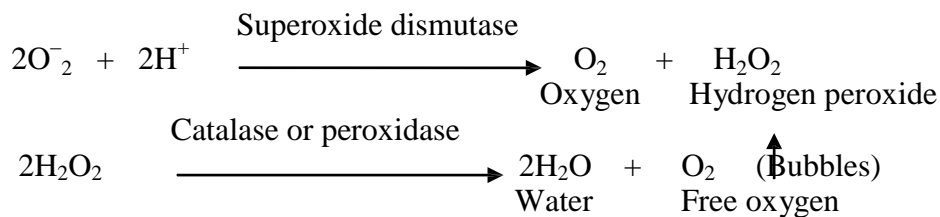
1. *P. aeruginosa* can be typed by lipopolysaccharide immunotype.
2. By pyocin (bacteriocin) susceptibility.
3. rRNA/DNA homology.
4. Serological (H-Ag, O-Ag).
5. Phage typing.

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.

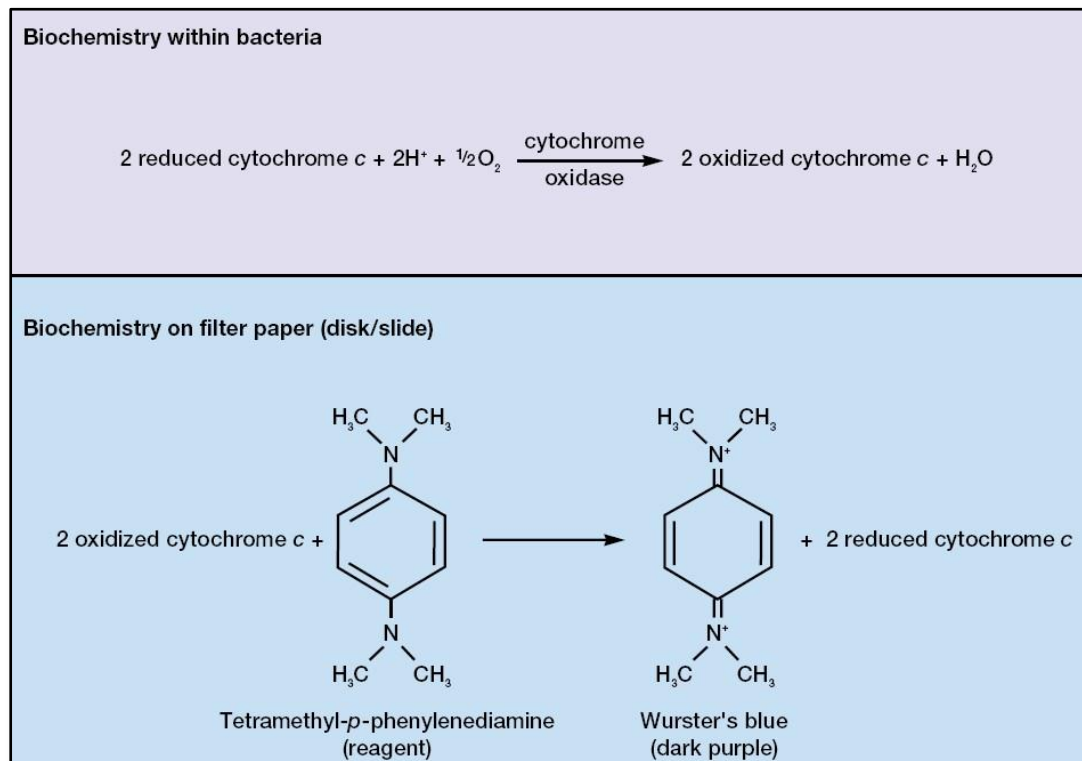
Substrate: H₂O₂ 3%

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:

Biochemistry within bacteria

$$\text{NO}_3^- + 2\text{H}^+ + 2\text{e}^- \xrightarrow[\text{reductase}]{\text{nitrate}} \text{NO}_2^- + \text{H}_2\text{O}$$

Nitrate Hydrogen Electrons Nitrite Water

$$\text{NO}_2^- \xrightarrow[\text{enzymes}]{\text{other}} \text{NH}_3 \longrightarrow \frac{1}{2}\text{N}_2$$

Nitrite Ammonia Molecular nitrogen

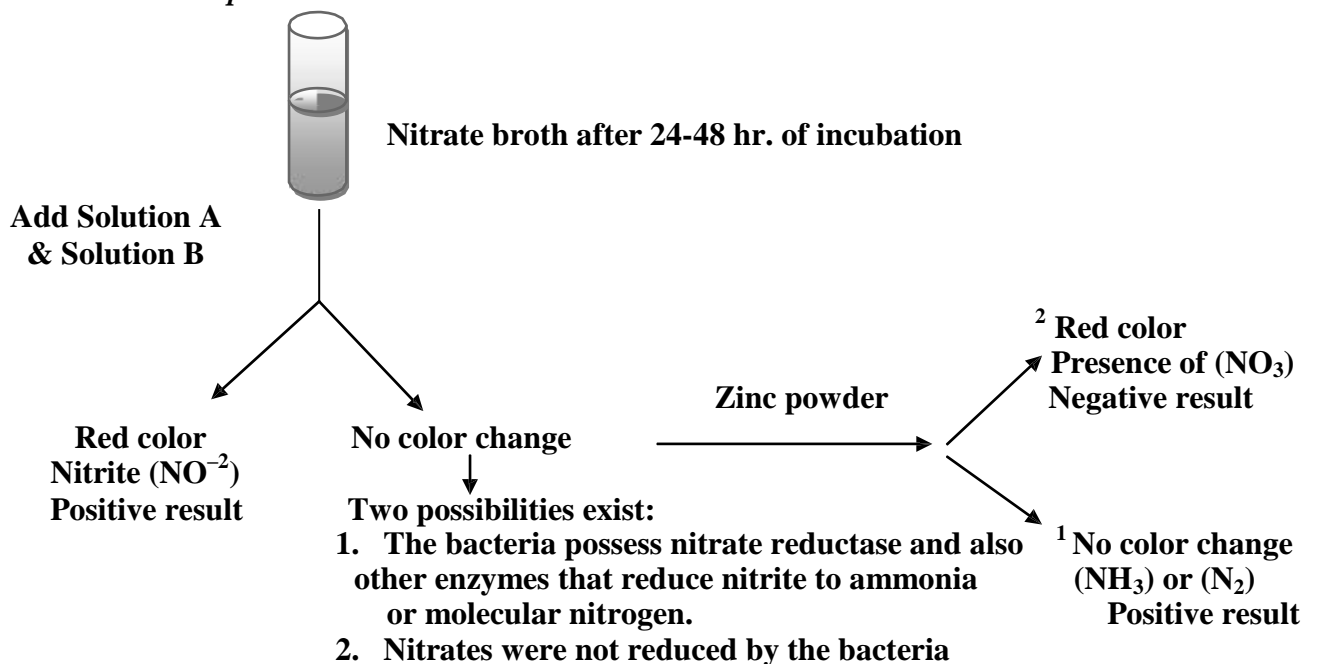
Biochemistry within tubes

Sulfanilic acid + N,N-dimethyl-1-naphthylamine + nitrite ions
(colorless) (colorless)

→ water + sulfobenzene azo-N,N-dimethyl-1-naphthylamine
(red color)

Medium: Nitrate Broth
Substrate: Nitrate (NO₃)
Enzyme: Nitrate reductase
Reagents:

Solution A: sulfanilic acid
Solution B: N,N-dimethyl-1-naphthylamine
 Zinc powder



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 37°C.

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

The results:

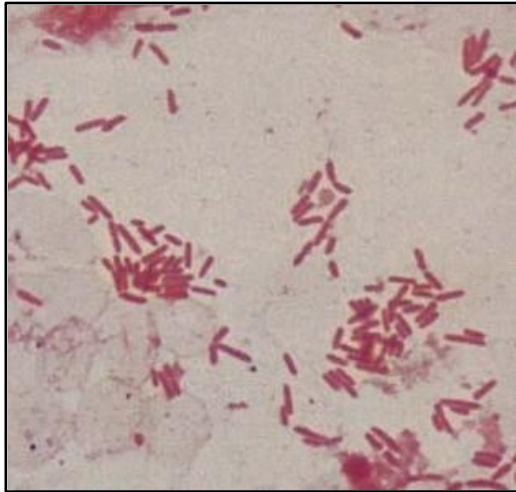
Yellow color in the tube without paraffin and green color in the tube with paraffin mean: oxidation positive and fermentation negative.

Green color in the tube without paraffin and yellow color in the tube with paraffin mean: oxidation negative and fermentation positive.

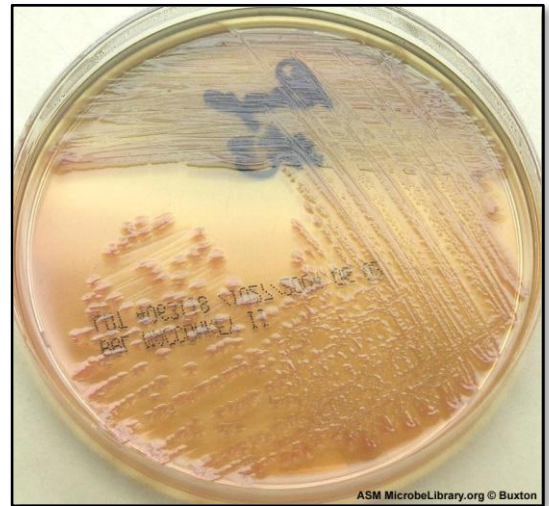
Yellow color in the both tubes mean: oxidation and fermentation positive.

Green color in the both tubes mean: oxidation and fermentation negative (Inert bacteria).

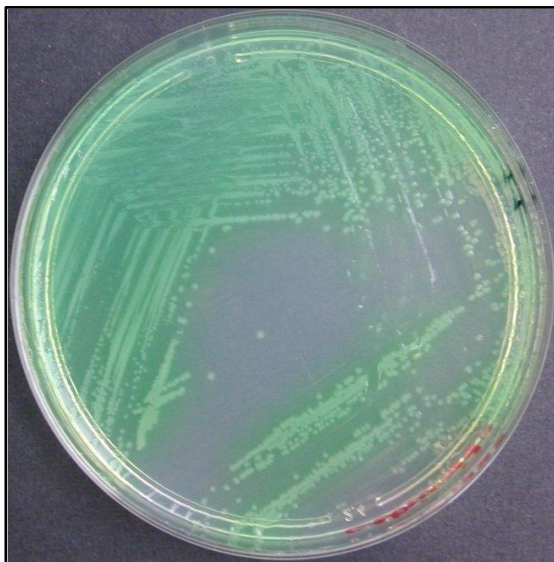
Test	<i>P. aeruginosa</i>	<i>P. fluorescence</i>
Indole	-	-
MR	-	-
VP	-	-
Citrate	+	+
TSI	K/K - -	K/K - -
Nitrate reduction test	+	+
Motility	+	+
Growth at 42°C	+	-
Growth at 4°C	-	+
King A	+ Pyocyanin	± Pyocyanin
King B	+ Flourescen	+ Flourescen
MacConkey agar	L.N.F transparence, irregular	L.N.F transparence,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



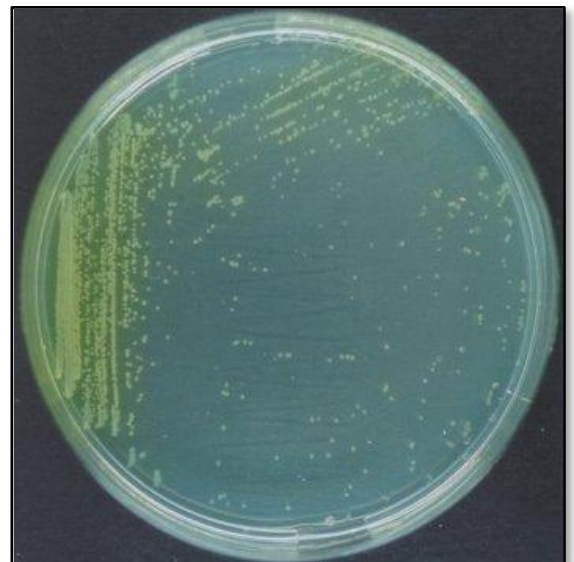
Pseudomonas aeruginosa (Gram-negative bacilli)



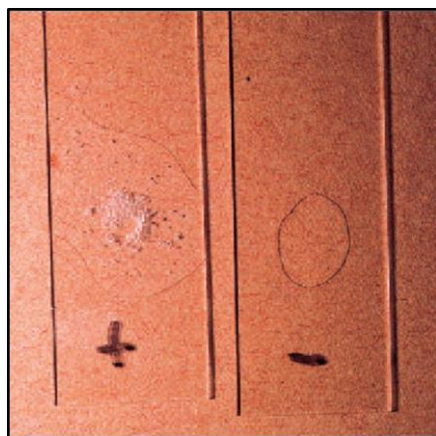
P. aeruginosa on MacConkey agar



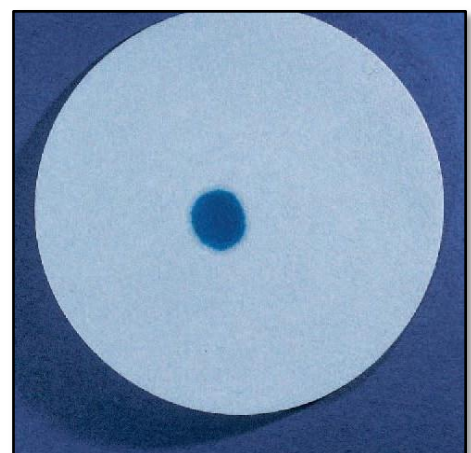
Pseudomonas aeruginosa on Cetrimide agar



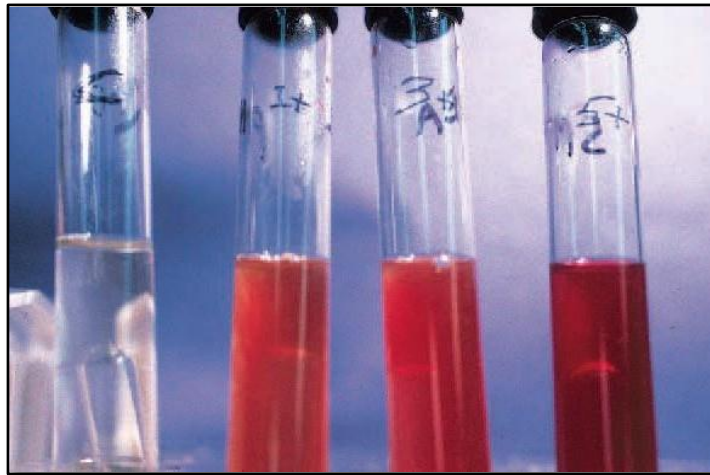
Pseudomonas fluorescens on Kings B agar



Catalase test



Positive oxidase test

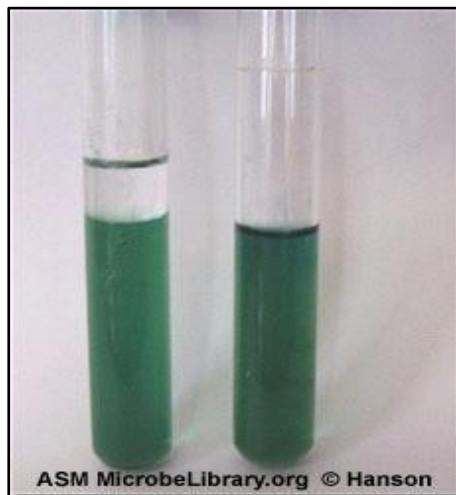


Nitrate reduction test : Control

+1

+3

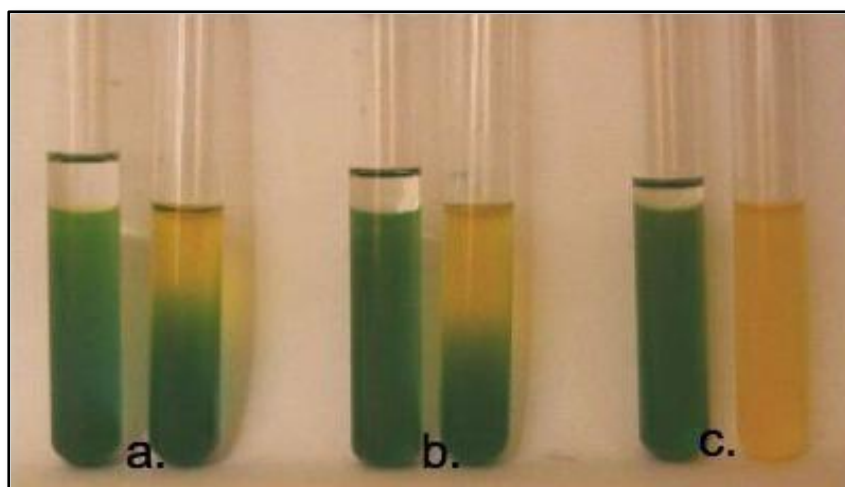
+5



Oxidative-fermentative (OF) test (Control)



Oxidative-fermentative test inoculated with *E. coli*. Acid production in both the open and oil-covered tubes



Oxidative - fermentative test inoculated with *P. aeruginosa*. Acid production in the open tube and not the oil-covered tube indicates an oxidative result. (a) *P. aeruginosa* incubated for 24 hours. Note pH change in the top of the open tube only. (b) *P. aeruginosa* incubated for 48 hours. Note the diffusion

of the acid down the tube. (c) *P. aeruginosa* incubated for 5 days. Note the diffusion of the acid throughout the tube. The result is best if read within 24 to 48 hours

