

Pseudomonas aeruginosa

Characteristics:

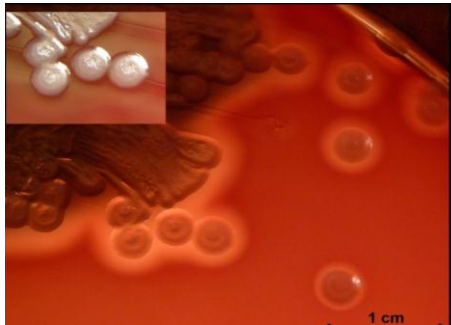
- It is a Gram-negative, aerobic rod belonging to the family *Pseudomonadaceae*.
- These bacteria are common inhabitants of soil and water.
- Motile by means of a single polar flagellum.
- Non spore forming
- Capsulated "Polysaccharide capsule"
- Aerobic
- Oxidase and catalase positive
- It has very simple nutritional requirements (able to grow on commercial distilled water).
- Its optimum temperature for growth is 37 degrees, and it is able to grow at temperatures as high as 42 degrees.
- It is tolerant to a wide variety of physical conditions, including temperature. It is resistant to high concentrations of salts and dyes, weak antiseptics, and many commonly used antibiotics.
- *Pseudomonas aeruginosa* is opportunistic pathogen and associated with a variety of infections including:
 - Urinary tract infections
 - Wound and burn with blue green pus
 - Respiratory system infections
 - Eye infection and may lead to blindness
 - Ear infection (external ear or otitis media)
 - A variety of systemic infections
- **Laboratory diagnosis**
 - **Specimen:** Urine, pus, sputum, or blood
 - **Stain:** Gram-negative rods and motile



Gram stain of *pseudomonas* (gram negative rods)

○ **Culture:**

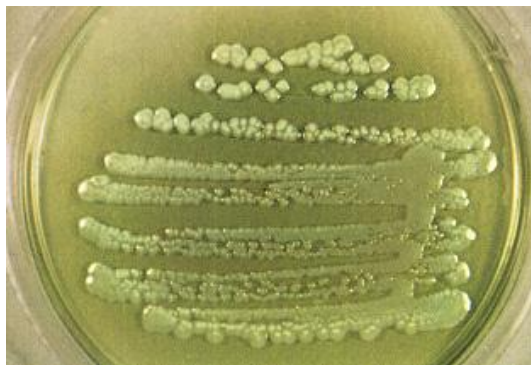
- On Nutrient agar → Colonies are surrounded by bluish green coloration
- On Blood agar → β -hemolytic colonies
- On MacConkey agar → pale yellow colonies i.e. non lactose fermenters
- On selective media "Cetermide" → pigments are more obvious
- *Pseudomonas aeruginosa* able to grow at temperatures as high as 42 degrees.



Pseudomonas aeruginosa on blood agar (beta haemolysis).



On Citramide agar media



Pseudomonas aeruginosa colonies on nutrient agar

○ **Biochemical reactions:**

Oxidase positive

Method: hold a piece of the oxidase test paper with forceps and touch onto an area of heavy growth . Use loop or wood stick

Results : Color change to purple



Gelatin liquefaction test:

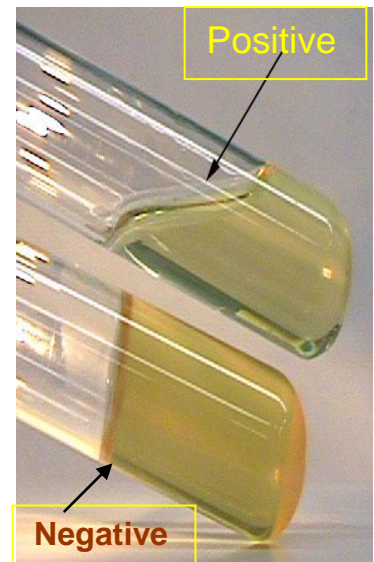
Principle – Upon boiling collagen, a major connective tissue, the product gelatin is formed. Certain bacteria are capable of producing a proteolytic exoenzyme called gelatinase, which hydrolyze the protein to amino acids. At temperature below 25°C,

gelatin will remain a gel, but if the temperature rises about 25°C, the gelatin will be liquid. Gelatin hydrolysis has been correlated with pathogenicity of some microorganisms. It is thought that pathogenic bacteria may breakdown tissue and spread to adjacent tissues.

Method: Stab a nutrient gelatin tube with inoculums of the tested organism and incubated at 37°C for 24 h

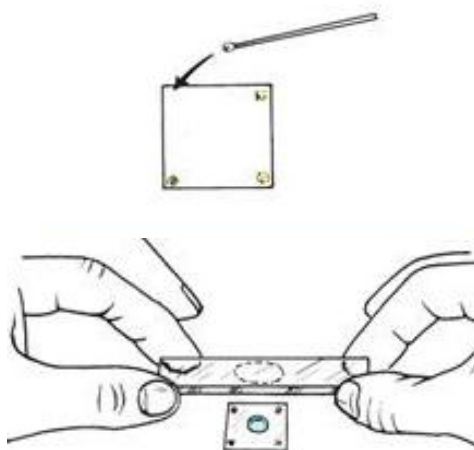
Result *If a tube of gelatin liquefy indicates positive test (*Ps. aeruginosa*) * If a tube of gelatin remains solid indicates negative test (*E. coli*)

Motility test : direct microscopic observation (hanging drop technique)



Procedure:-

1. With a toothpick, spread Vaseline or Petroleum jelly on the four corner of a clean coverslip.
2. After thoroughly mixing one of the cultures, use the inoculating loop to aseptically place a small drop of the bacterial suspension in the center of a coverslip
3. Lower the depression slide, with the concavity facing down, onto the coverslip so that the drop protrudes into the center of the concavity of the slide .Press gently to form a seal.
4. Turn the hanging drop slide over and place on the stage of the microscope so that the drop is over the light hole. Examine the drop by first locating its edge under low power and focusing on the drop. Switch to the high-dry objective (40 x). To increase the contrast and to see the bacteria clearly, close the diaphragm as much as possible.
5. Distinguish between actual motility and Brownian movement
6. Discard the coverslips and any contaminated slides in a container with disinfectant solution.



3 Depression slide is pressed against Vaseline on cover glass and quickly inverted.



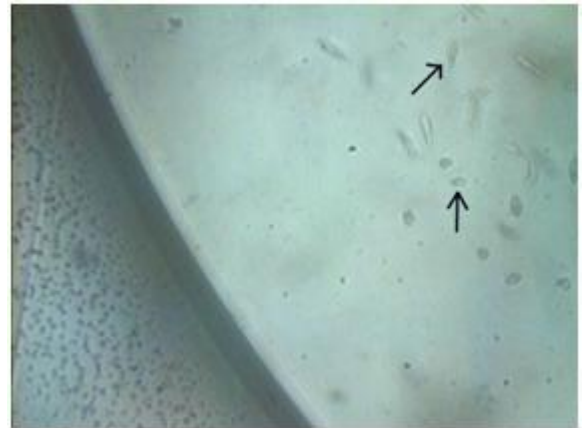
2 Two loopfuls of organisms are placed in center of cover glass.



4 The completed preparation can be examined under oil immersion.

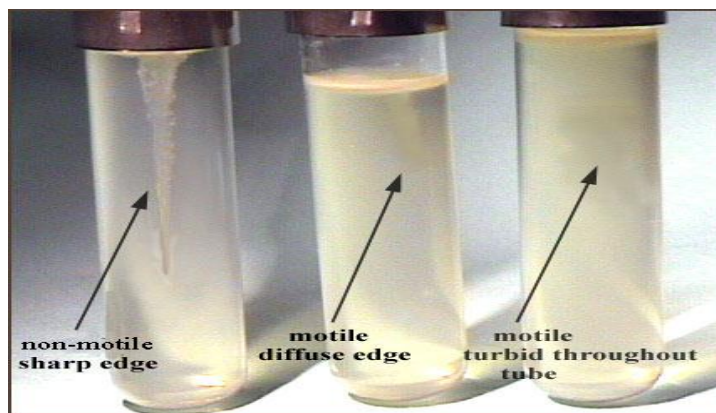
Result :

Motile organisms on hanging drop preparation

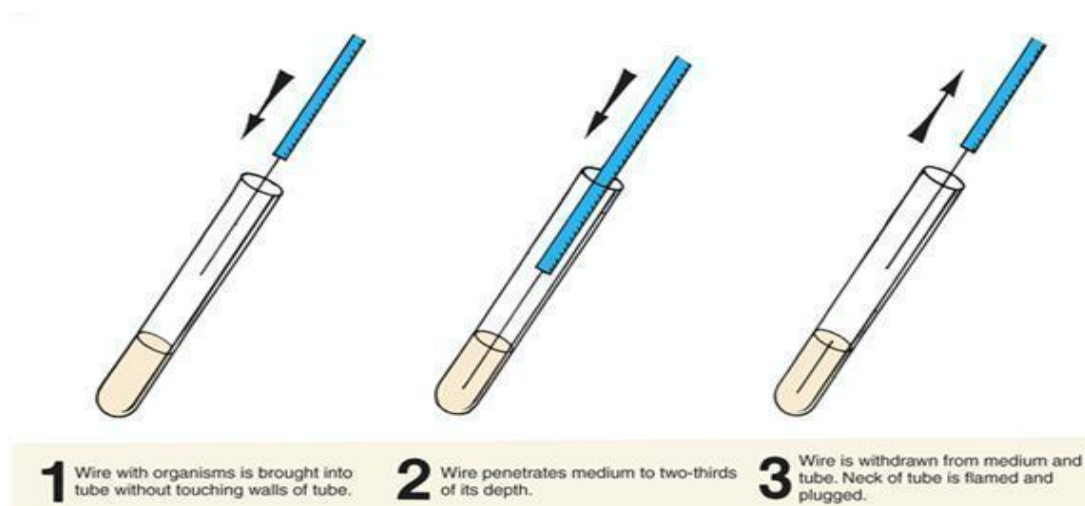


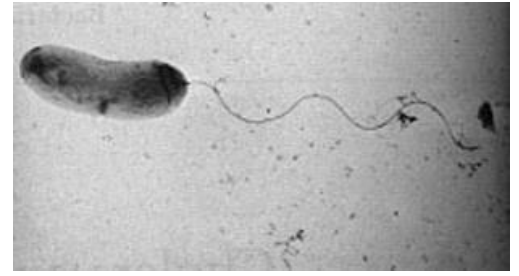
Soft Agar Stabbing (Tube Method) : semi solid agar tube

The Motility test medium is a semi-soft medium that is inoculated with a straight needle and incubation . If the bacteria are motile, they will swim away from the line of inoculation in order to find nutrients, causing turbidity or cloudiness throughout the medium. If they are non motile, they will only grow along the line of inoculation



procedure:



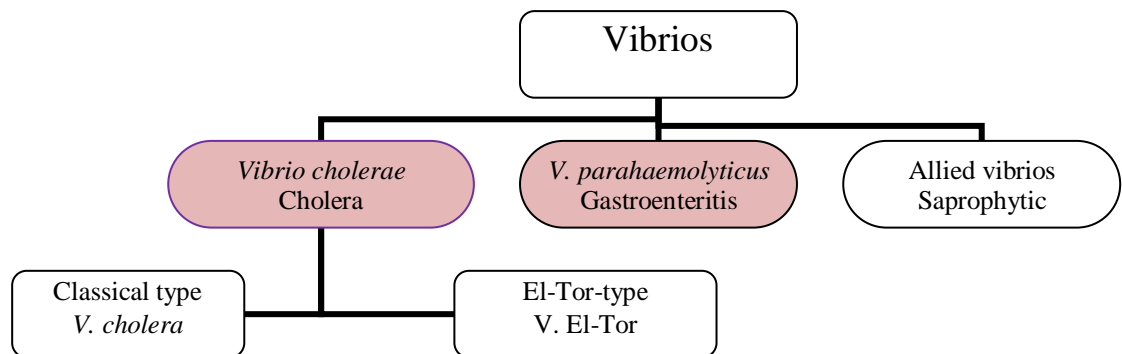


Vibrionaceae: Vibrios

➤ General characters:

1. Gram negative, curved (comma shaped) bacilli
2. Motile by single polar flagella
3. Non spore forming
4. Non capsulated
5. Most vibrios have relatively simple growth factor requirements and grow well in alkaline pH
6. Facultative anaerobes
7. Vibrios are capable of both respiratory .
8. Oxidase and catalase positive
9. Natural inhabitants of aquatic environment

➤ Species of Vibrio:



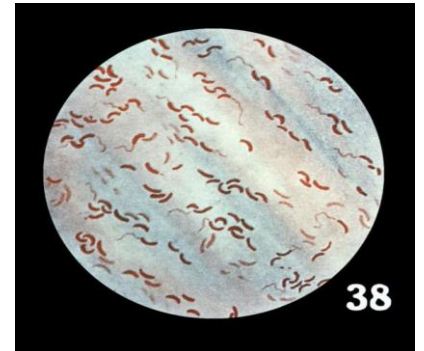
- *V. cholerae* and *V. parahaemolyticus* are pathogens of humans.
- *Vibrio cholerae* is the cause of cholera
- *Vibrio parahaemolyticus* is the cause of acute gastroenteritis following ingestion of contaminated sea-food such as raw fish.
 - *V. parahaemolyticus* is an invasive organism affecting the colon
 - *V. cholerae* is noninvasive, affecting the small intestine through

Cholera:

- **Cholera** is a severe diarrheal disease caused by *Vibrio cholerae*.
- It is endemic in southern Asia (India, Pakistan, and Bangladesh).
- Transmission is by contaminated water or food through oral-fecal routs.
- Incubation period of the disease is 1-4 days.
- It characterized by sudden onset of intense **vomiting** and diarrhea (**rice water stool**) with rapid **dehydration**.
- The disease progresses from the first liquid stool to shock in 4-12 hours, with death following in 18 hours to several days.

- *V. cholerae* produces **cholera toxin** "enterotoxins", whose action on the intestinal mucosal epithelium is responsible for the characteristic diarrhea of the disease cholera through stimulation of cAMP, resulting in active secretion of chloride and secondary loss of sodium and water.
- **Diagnosis of cholera:**
 - **Specimen:** rice watery stool
 - **Gram stain:**

Gram negative curved (comma shape) bacilli



Culture:

Inoculation of rice water stool in alkaline peptone water, pH9 in which the organisms multiply rapidly and tend to form pellicle at the surface of the medium after 6-8 h at 37°C.

Subculture is made into Thiosulphate Citrate Bile Sucrose (TCBS) agar.

TCBS is selective and differential medium for vibrios

This medium is selective because:

It contains bile salt and pH of the medium is alkaline (pH9)



Yellow colonies of *V. cholerae* due sucrose fermentation and green colonies of *V. parahaemolyticus* on TCBS

On mackonkey agar : lactose non fermented

