Pseudomonas

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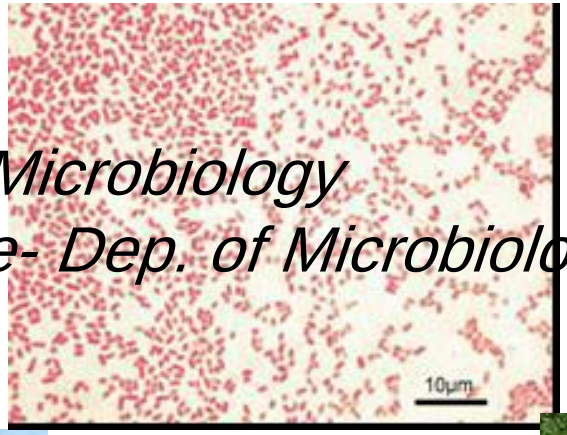
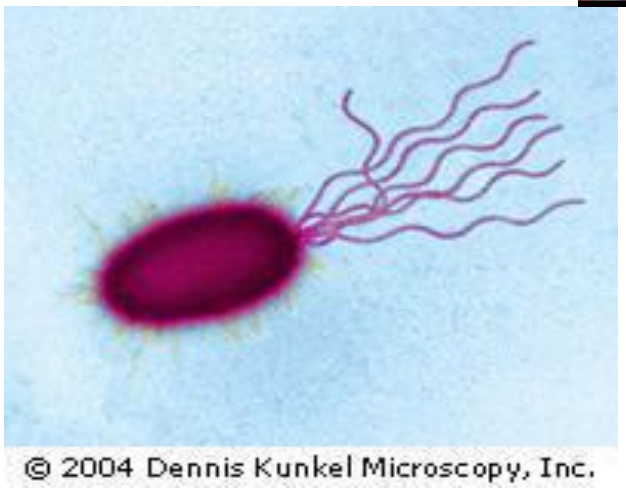
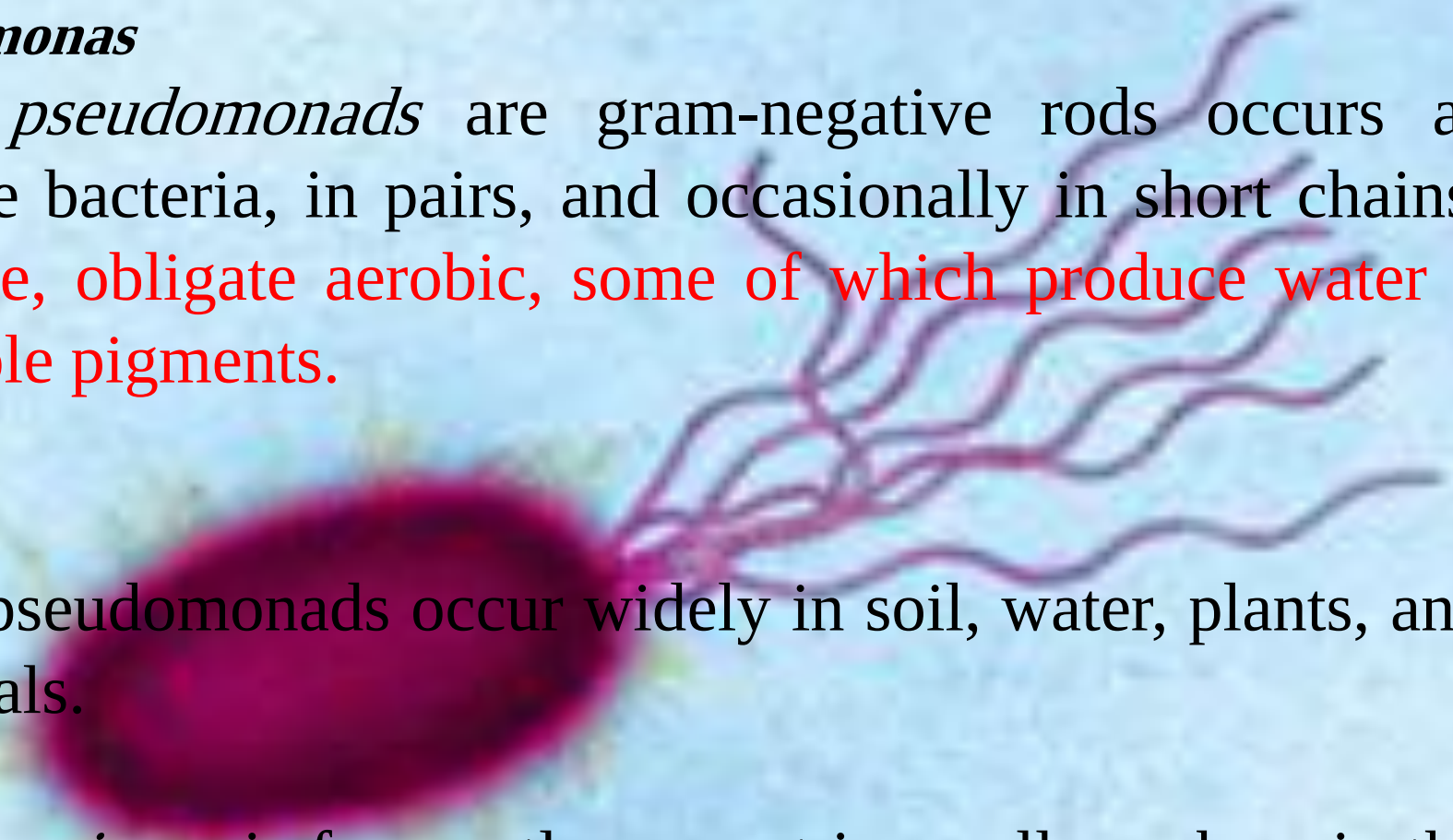




Pseudomonas

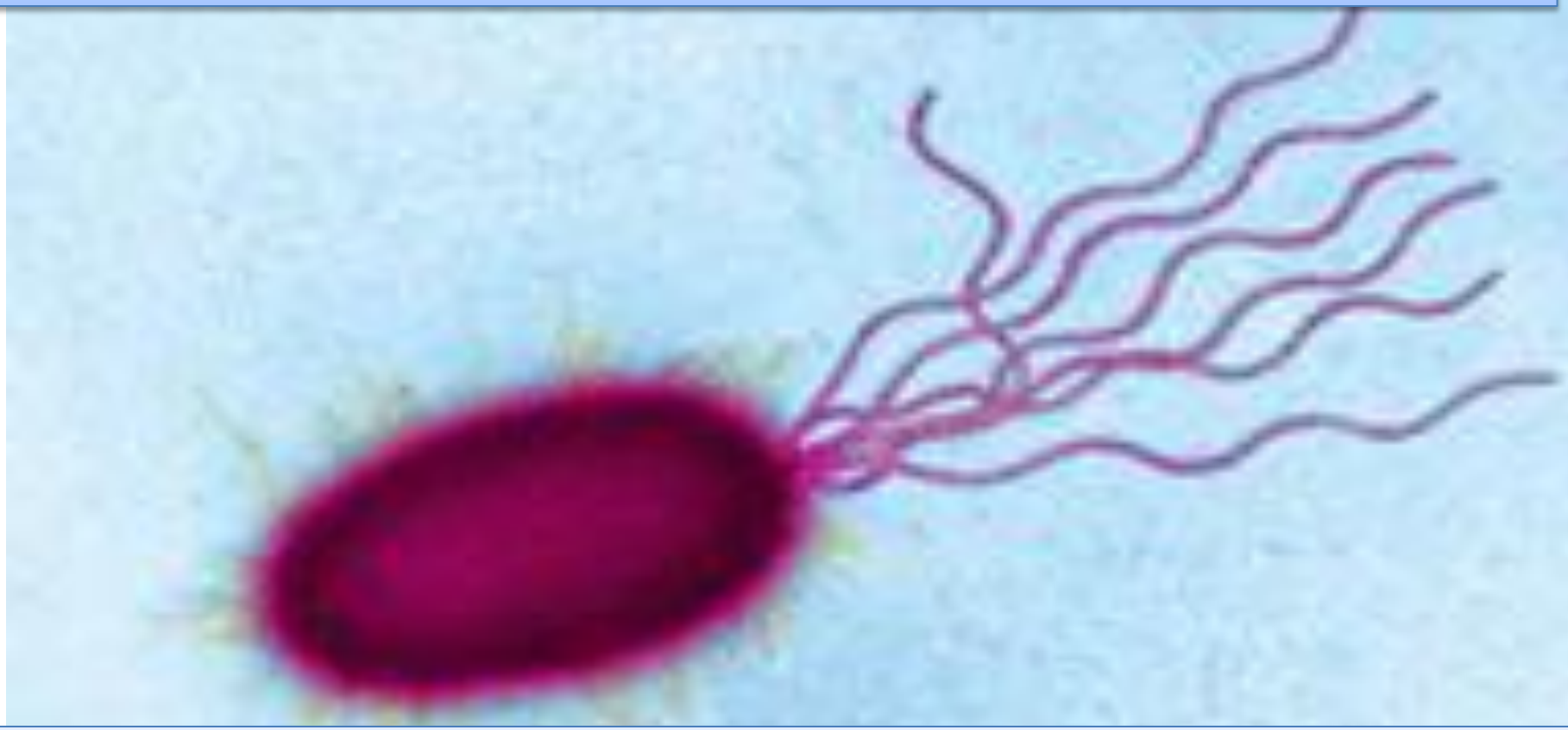
- The *pseudomonads* are gram-negative rods occurs as single bacteria, in pairs, and occasionally in short chains, motile, obligate aerobic, some of which produce water – soluble pigments.



- The pseudomonads occur widely in soil, water, plants, and animals.

- *P.aeruginosa* is frequently present in small numbers in the normal intestinal flora and on the skin of humans and is the major pathogen of the group.
- The classification of pseudomonads is based on rRNA/DNA homology and common culture appearances.

Diagnostic Laboratory Tests

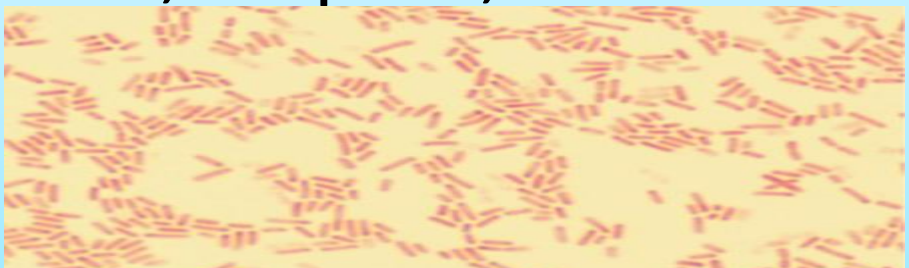


Specimens

- Specimens from skin lesions, pus, urine, blood, spinal fluid, sputum, and other material should be obtained as indicated by the type of infection.

- Smears
- Gram-negative rods are often seen in smears. Although, There are no specific morphologic faces that differentiate *pseudomonads* in specimens from enteric or other gram-negative rods, But, the Morphology & Identification for (Typical Organisms):

- *P aeruginosa* is motile and rod-shaped, measuring about 0.6 x 2 m. It is gram-negative and occurs as single bacteria, in pairs, and occasionally in short chains.



- Culture
- 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.
- Culture is the specific test for diagnosis of *P aeruginosa* infection.

- *P aeruginosa* in a culture can produce multiple colony types.
- Sometimes it is not clear if the colony types represent different strains of *P aeruginosa* or are variants of the same strain.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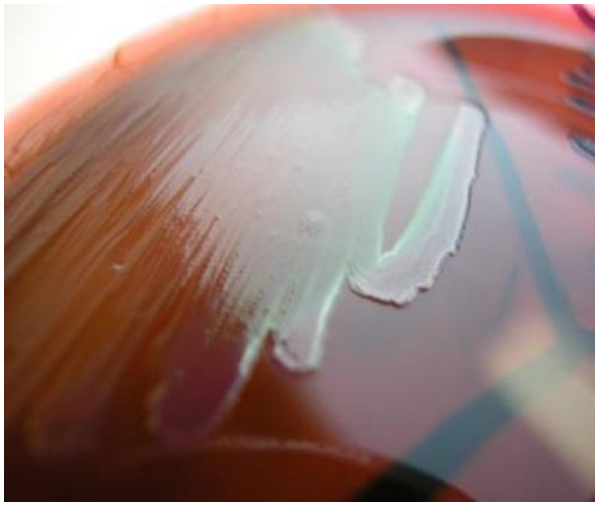
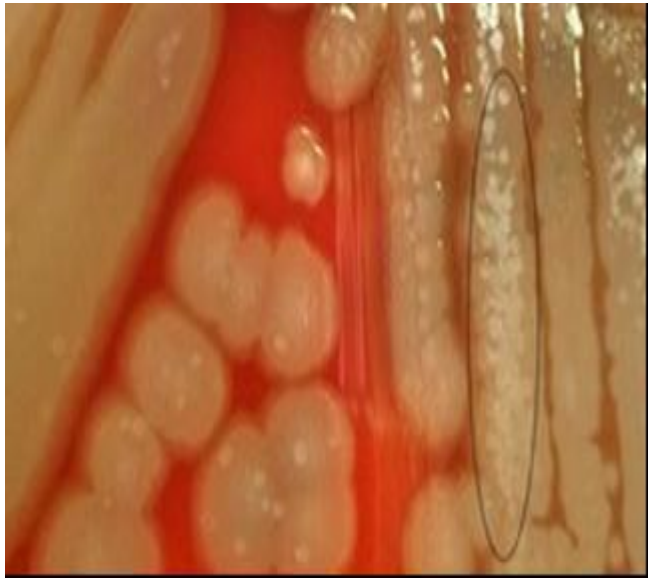
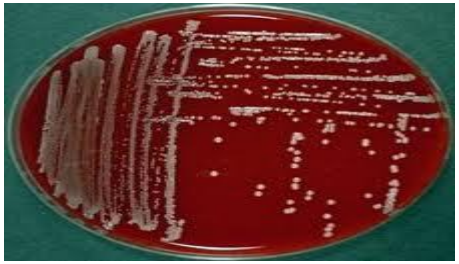


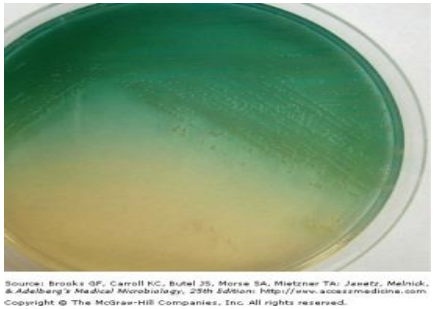


Culture Appearance

- *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**.

Growth Features

- Identification is usually based on:
- growth at 42°C.: *P aeruginosa* grows well at 37–42°C; its growth at 42°C helps differentiate it from other *Pseudomonas* species in the fluorescent group.
- colonial morphology, the presence of specific pigments.
- oxidase positivity, As Biochemical Test

- **Motility test (Sulfide Indole media (SIM)):**

SIM agar may also be used to detect the presence or absence of motility in bacteria as well as indole production. Motility is present when the growth of the culture is not restricted to the stab line of the inoculation. Growth of non motile bacteria is confined to the line of inoculation. In other meaning (Motile cultures will have growth diffusing from the stab line while non motile ones will not)

BIOCHEMICAL TESTS

- The oxidase test distinguishes between groups of bacteria based on cytochrome oxidase activity.
- *Pseudomonas aeruginosa* is a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor and thus is oxidase positive.

Principles:

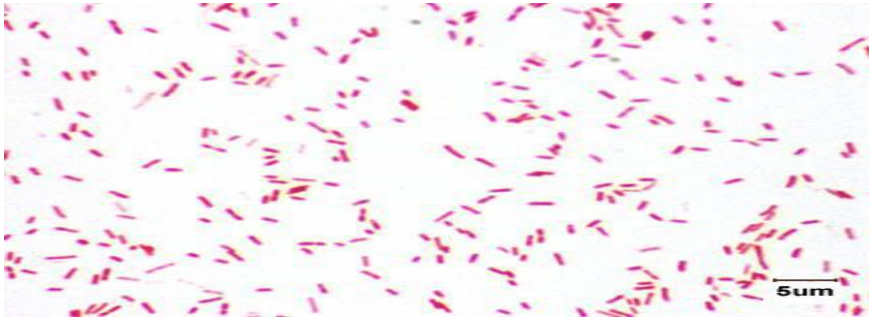
Reaction of Oxidase enzyme with oxidase reagent (tetramethyl-p-phenylene diamine dihydrochloride) → violate color

TSI TEST

- *P. aeruginosa, produce* an acid butt, alkaline slant, H_2S, and gas.

- **Catalase: positive**
- **Antimicrobial sensitivity test it** is more resistant to antimicrobial drugs. while, it **is more susceptible to anti-pseudomonal groups.**

PROTEUS



Morphology & Identification

- short gram-negative rods , facultative anaerobes .

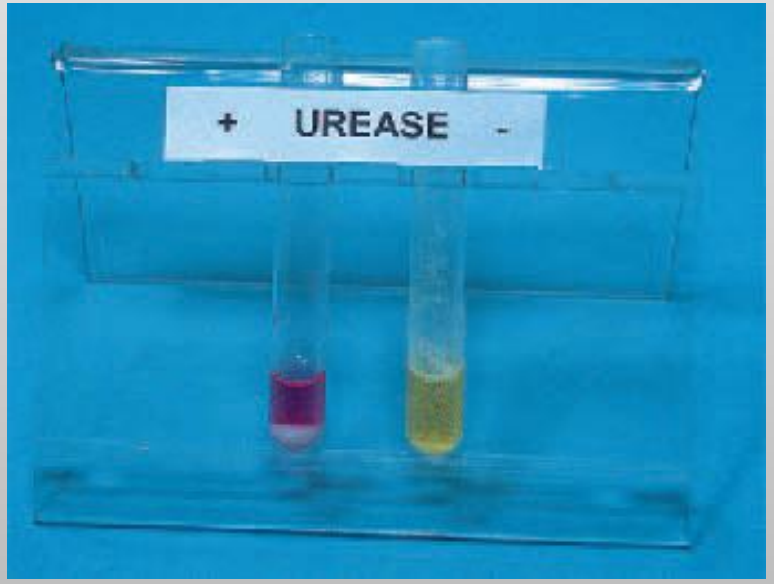
- Culture

- *Proteus* species move very actively by means of peritrichous flagella, resulting in —swarming‖ on solid media.
- the swarming is inhibited by chemicals, such as phenylethyl alcohol or CLED (cystine-lactose-electrolytedeficient) medium.
- The *Proteus* ferments lactose very slowly or not at all.
- *Proteus mirabilis* is more susceptible to antimicrobial drugs, including penicillins, than other members of the group.

BOCHEMICAL TESTS

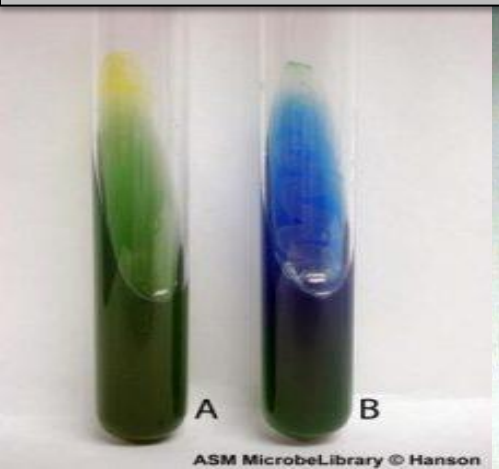
- *Proteus* species are urease positive (smell of ammonia).

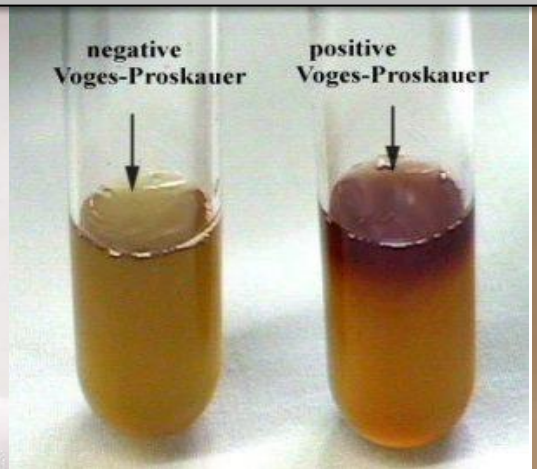
(Urease activity (the **urease test**) is detected by growing bacteria in a medium containing urea and using a pH indicator such as phenol red)

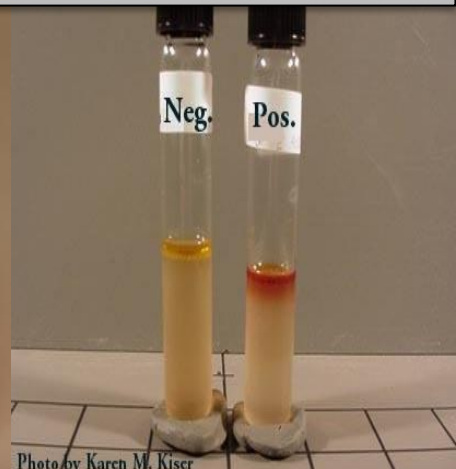


- **Principles**
- Some bacteria are able to produce an enzyme called **urease** that attacks the nitrogen and carbon bond in amide compounds such as urea, forming the end products ammonia, CO2, and water Urease activity (the **urease test**) is detected by growing bacteria in a medium containing urea and using a pH indicator such as phenol red, When urea is hydrolyzed, ammonia accumulates in the medium and makes it alkaline. This increase in Ph causes the indicator to change from orange-red to deep pink or purplish red (cerise) and is a positive test for urea hydrolysis.

- IMViC tests of *Proteus vulgaris*
  - Indole: Positive
  - Methyl-Red: Positive
  - Voges-Proskauer test: Negative
  - Citrate test: Negative
- IMViC tests of *Proteus mirabilis*
  - Indole: negative
  - Methyl-Red: Positive
  - Voges-Proskauer test: Negative
  - Citrate test: positive





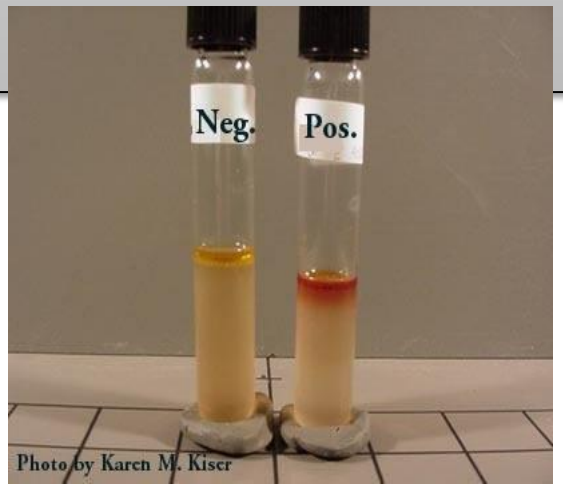


TSI TEST

- *P. vulgaris* produces an acid butt, an acid or alkaline slant, H2S, and gas

- INDOLE TEST

- It is performed on sulfide-indole-motility (SIM) medium or in tryptophan broth, or in motility urease indole (MIU) medium. Result is read after adding Kovac's reagent.
- positive result is indicated by the red layer at the top of the tube after the addition of Kovács reagent.
- A negative result is indicated by the lack of color change at the top of the tube after the addition of Kovács reagent.

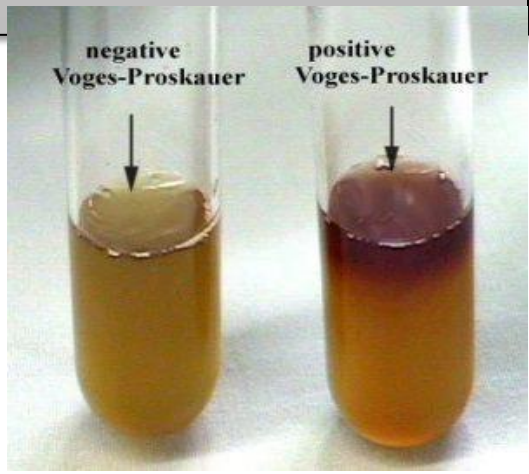


- Methyl Red (MR) Test:

- Positive methyl red test are indicated by the development of red color after the addition of methyl red reagent.
- A negative methyl red test is indicated by no color change after the addition of methyl red reagent

- VOGES-PROSKAUER(VP)TEST

- Negative test is indicated by lack of color change after the addition of Barritt's A and Barritt's B reagents.
- A positive Voges-Proskauer test is indicated by the development of red-brown color after the addition of Barritt's A and Barritt's B reagents.



- Citrate Utilization Test

- The test is performed on Simmons citrate agar:
- Negative citrate utilization test is indicated by the lack of growth and color change in the tube
- A positive citrate result as indicated by growth and a blue color change.