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Proteus
Classification

- **Family:** Enterobacteriaceae.
- **Genus:** *Proteus.*
Species of proteus:

1) **Proteus vulgaris.**
2) **Proteus mirabilis.**
3) **Proteus myxofaciens.**
4) **Proteus penneri.**
5) **Proteus hauseri.**
6) Genomospecies 4, 5, and 6.
Morphology

- Gram negative bacilli, (1–3X 0.5μm).
- In young cultures, cells are long, curved, and filamentous, arranged concentrically.
- In older cultures, no characteristic arrangement.
- Non-capsulated, non-sporing & actively motile.
Cont..

✓ Possess peritrichate flagella & many strains possess fimbriae.
✓ Flagella are more variable in shape.
Cultural characteristics:

- They are aerobic & facultatively anaerobic.
- In nutrient broth uniform turbidity with powdery deposit and a faint ammoniacal odor.
- A thin, fragile pellicle in old cultures.
Cont...

- Grow on ordinary media.
- When grown on nutrient agar or blood agar, *Pr.vulgaris* & *Pr.mirabilis* exhibit swarming (spreading growth).
- Emits a putrefactive (fishy or seminal) odor.
Culture on MacConkey agar

- *Proteus* strains form several sorts of discrete colony on MacConkey agar.
Phenylalanine Agar (PPA)

**Principle:**
- Organism that produce phenylalanine deaminase can be identified by their ability to remove the amine group (NH₂) from the amino acid phenylalanine.
- The reaction requires oxygen & produce ammonia (NH₃) & phenylpyruvic acid.
- Deaminase activity is evidenced by the presence of phenylpyruvic acid.

![Chemical reaction diagram]

Phenylalanine Acid + FeCl₃ → Green Color
Biochemical reactions

All strains are:

1) catalase-positive and oxidase negative.
2) Ferments glucose with small amounts of gas.
3) Lactose fermentation is rare.
4) Sucrose fermented by most strains except *P. mirabilis* (only 15–20% are positive).
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5) All species produce an inducible urease; which differentiate it from shigella and salmonella.

6) All species within the tribe also have the ability to degrade indole on prolonged incubation.

7) Regarding **Triple Sugar Iron** agar, Proteus are: 
   K\(\text{\textbackslash A\textbackslash G}\+\text{\textbackslash H}_2\text{S}.\)

✓ Proteus produce H2S gas during fermentation, this gas will reduce the ferrous sulphate in the medium causing a black precipitate to form in the butt of the tube at 16-24 hours of incubation.
IMVIC phenomena

✓ Growth on Simmons’s citrate medium is variable in some species.

✓ But does not occur with *P. penneri* and *P. hauseri* strains.

✓ Most strains of *Proteus* are MR positive & VP negative.
Among the indole-positive strains of *Proteus*. Those that also acidify salicin and degrade aesculin are strains of *P. vulgaris*. Whereas those that do not acidify salicin or degrade aesculin are either strains of *P. hauseri* or strains of genomospecies 4, 5, and 6.
Disease caused by proteus:

- Strains of *Pr. mirabilis* are a prominent cause of:
  1. Urinary tract infection.
  2. Septicaemia.
  3. Infections, usually of surgical wounds or bedsores.
Sample Collection

Sample from:

1) Urinary tract.
2) Wounds.
3) Blood samples.
1) **Gram staining**: Gram negative bacilli, non-capsulated & non-spore forming.

2) **Hanging drop preparation**.

3) **Biochemical reactions** (PPA & Urease tests).
**Step 1: Preparing Cover Glass:**

- Using a toothpick, place a small dab of Vaseline in each corner of a clean cover glass.
- A septically transfer a loopful of a liquid bacterial culture into the center of the cover glass. Do not spread the drop!
- Alternatively, if you have a solid culture.
Step 2: Attaching Depression Slide:

Carefully lower depression slide with depression facing down.

Vaseline attaching cover glass to depression slide

Step 3: Invert Depression Slide:

Quickly invert depression slide.

Step 4: Examine under Microscope:

With 10x objective and using course adjustment knob, focus on the edge of the hanging drop.
Urease Test

- The test used particularly to distinguish genus proteus from other enteric bacteria.

- How to Perform Test:
Inoculate urea broth or urea slant agar with inoculating loop.

- Property it tests for:
This test is done to determine a bacteria’s ability to hydrolyze urea to make ammonia using the enzyme urease.

- Media and Reagents Used: Urea Disks or Tablets, Urea broth or Urea slants contains: a yeast extract, monopotassium phosphate, disodium phosphate, urea, and phenol red indicator.
Reading Results: **Urea slant is a yellow color.**

The enzyme urease will be used to hydrolyze urea to make ammonia.

A) If test is positive, ammonia is made & the broth turns a bright **pink color.**

B) If test is negative, broth **has no color** change and no ammonia is made.
It is an important genus in the family pseudomonaceae.

These M.O are:

1) Oxidase ve+ (opposite to Enterobacteriaceae).

2) Non-fermentative for CHO, but oxidative; so the require O2.
Introduction:

- Large group of:
  1. aerobic.
  2. non sporing.
  3. gram negative.
  4. motile by polar flagella.

- Ubiquitous.

- Opportunistic infections like UTI, wound infection and severe infection in hospitalized and compromised persons.
• *P. aeruginosa* (the most important one).
• *P. fluorescenes*.
• *P. putida*.
• *P. stutzeri*. 
**Pseudomonas aeruginosa**

**Morphology:**
- slender, Gram negative bacillus.
- size: 1.5 microns - 3*1.5 microns.
- motile by polar flagella.
- non capsulated though some mucoid strains may sometimes occur.
- some are pilated.
Cultural characteristics

- Obligate aerobe.
- Wide range of temperature \(5^\circ\text{C}-42^\circ\text{C}\)

1) On ordinary media:
Large, opaque, irregular, with distinctive musty, mawkish, earthy smell.
2) On nutrient agar:

- Colonies are smooth, large, translucent, low convex, 2-4mm in diameter.
- Produce sweetish aromatic odor.
- Greenish blue pigment diffuses.
Pigment production on nutrient agar

1) **Pyocyanin:**
- Bluish green phenazine pigment.
- Soluble in chloroform and water.
- Produced by *P. aeruginosa*.

2) **Pyoverdin (fluorescin):**
- It is a greenish yellow pigment.
- Insoluble in chloroform but soluble in water.
- Produced by many other species.
3) **Pyorubin:**
- Reddish brown pigment.
- Insoluble in chloroform but soluble in water.

4) **Pyomelanin:**
- Brown to black pigment.
- Production is uncommon.
3) **On blood agar:**
- Similar to nutrient agar.
- Many are haemolytic.

4) **On MacConkey agar:**
- Colourless, non lactose fermenters.
Biochemical reactions

- O/F test-oxidative.
- Catalase-positive.
- Oxidase-positive.
- Nitrate reduction-positive.
Cont...

- Citrate test-positive.
- Urease test-negative.
Cont...

➤ **Sugar utilization tests:**

Glucose-only acid.
Lactose-negative.
Sucrose-negative.
Mannitol-negative.
**Oxidative-Fermentative Test (OF):**

- Two tubes of semisolid media are used (which have low concentration agar, high CHO content & an indicator called bromothymol blue) They are incubated with the M.O; one is covered by mineral oil and the other is left opened.
- After 24-48 hours the covered tube remains **green**, while the opened becomes **yellow**.
- Also, in this test, the motility could be seen, which have tree-like appearance mainly toward the top of the tube (aerobic M.O).
Disease caused by pseudomonas:

- Important agent in causing nosocomial infections.

- **Most common infections are:**
  1) Urinary tract infections following catheterization.
  2) Acute purulent meningitis following lumbar puncture.
  3) Post-tracheostomy pulmonary infection.
  4) Septicaemia in debilitated patients.
cont..

5) Chronic otitis media and otitis externa.

6) Eye infections.

7) Acute necrotizing vasculitis.

9) Infantile diarrhea.

8) Wound and burn infections.
Thank You