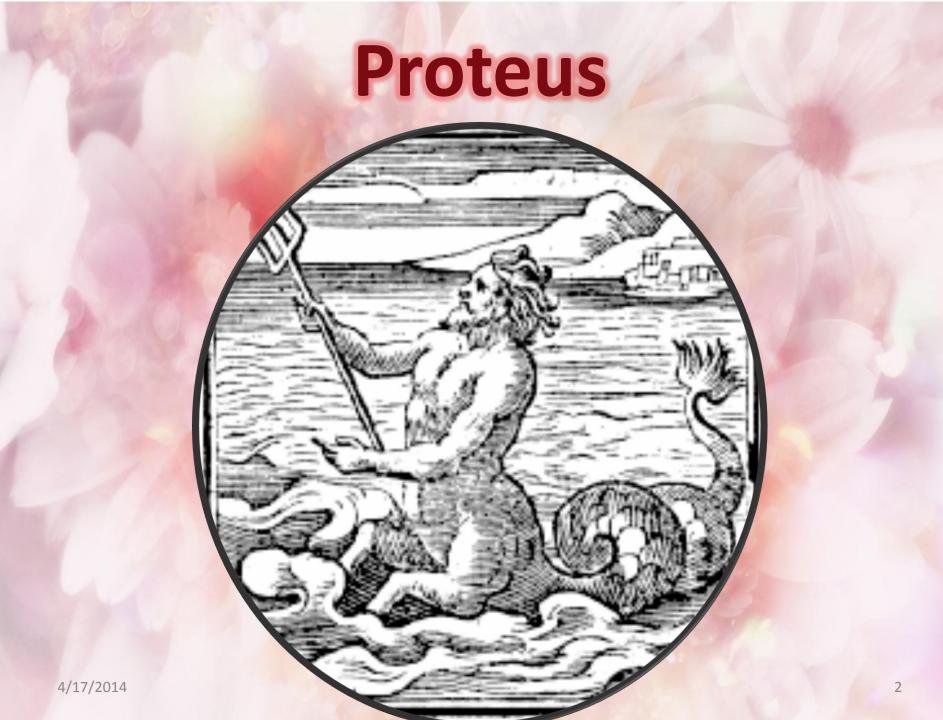
proteus & Pseudomonas proteus & Pseudomonas







**Family:** Enterobacteriace.

**❖Genus:** Proteus.

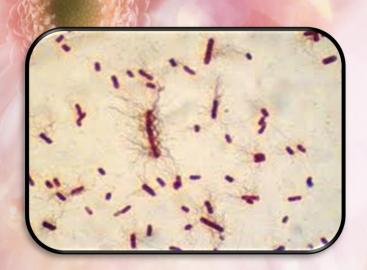
# Species of proteus:

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

# Morphology

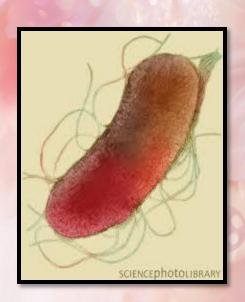
- ✓ Gram negative bacilli, (1–3X 0.5µm).
- ✓ In young cultures, cells are long, curved, and filamentous, arranged concentrically.
- ✓ In older cultures, no characteristic arrangement.
- ✓ Non-capsulated, non-sporing & actively motile.

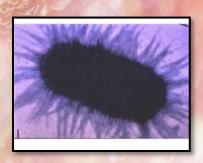




### Cont...

- ✓ Possess peritrichate flagella & many strains posses 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...

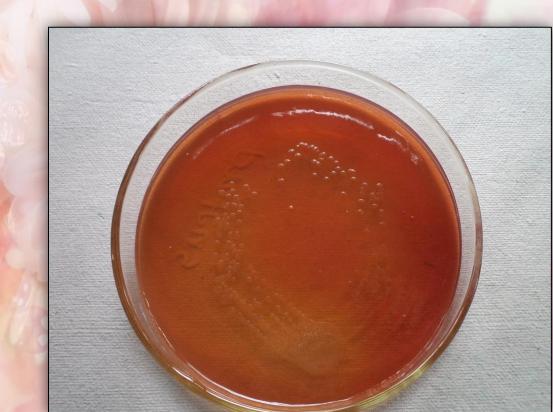
- **➢** Grow on ordinary media.
- ➤ When grown on nutrient agar or blood agar Pr.vulgaris & Pr.mirabilis exhibit swarming (spreading growth).
- Emits a putrefactive (fishy or seminal) odor.





# Culture on MacConkey agar

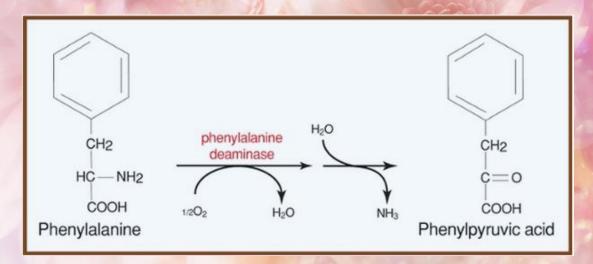
Proteus strains form several sorts of discrete colony on MacConkey agar.



## 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.





### **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).

Sp.	swarming	gas	ure	ind	orn	H <sub>2</sub> S	gel	man	adon	malt	tre	xyl
P.mirabilis	+	+	+	-	+	+	+	-	-	-	+	+
P.Penneri	+	+/-	+	-	-	+/-	+/-	-	-	+	+/-	+
P.vulgaris	+	+	+	+	-	+	+	-	-	+	+/-	+
M.morganii	-	+	+	+	+	+/-	-	+	-	-	-	-
Prov.alcalifaciens	-	+	-	+	-	-	-	+	+	-	-	-
Prov.rettgeri	-	-	+	+	-	-	-	+	+	-	-	-
Prov.rustigianii	-	+/-	-	+	-	-	-	+	-	-	-	-
Prov.stuartii	-	-	+/-	+	-	-	-	+	-	-	+	-

#### cont...

- 5) All species produce an inducible urease; which differntiate 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\A\G+\H2S.
- ✓ 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.

#### cont...

✓ 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.

## Laboratory diagnosis

1) Gram staining: Gram negative bacilli, non-capsulated & non-spore forming.

2) Hanging drop preparation.

3) Biochemical reactions (PPA & Urease tests).

### Hanging drop preparation

### **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:**

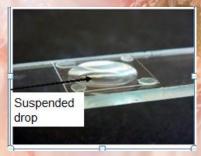


Carefully lower depression slide with depression facing down.

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.

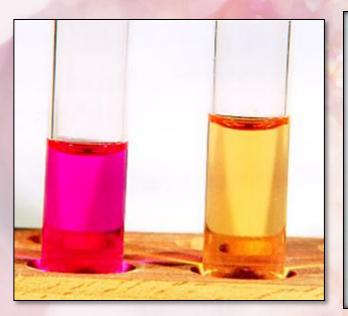
**❖ Media and Reagents Used:** Urea Disks or Tablets, Urea broth or Urea slants contains: a yeast extract, monopotassium phosphate, disodium phosphate, urea, and phenol red indicator. 

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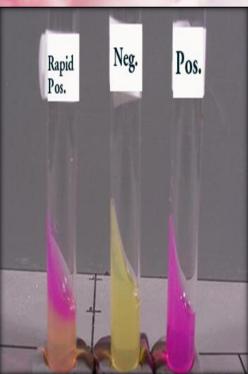
- ☐ Reading Results: Urea slant is a yellow color.

  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.

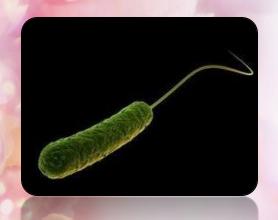
3.gram negative.

4.motile by polar flagella.

> Ubiquitous.

oppurtunistic 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\*1.5microns.
- > 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°c-42°c

## 1) On ordinary media:

Large, opaque, irregular, with distinctive musty, mawkish, earthy smell.

### Cont...

### 2) On nutrient agar:

✓ Colonies are smooth, large, translucent, low convex, 2-4mm in diameter.

- ✓ Produce sweetish aromatic odor.
- ✓ Greenish blue pigment diffuses.

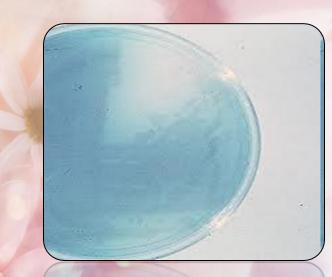
# Pigment production on nutrient agar

### 1) Pyocyanín:

- ✓ Bluish green phenazine pigment.
- ✓ Soluble in chloroform and water.
- ✓ Produced by P.aeruginosa.

## 2) Pyoverdín (fluorescín):

- ✓ It is a greenish yellow pigment.
- ✓ Insoluble in chloroform but soluble in water.
- ✓ Produced by many other species.





### Cont..

# 3) Pyorubín:

- ✓ Reddish brown pigment.
- ✓ Insoluble in chloroform but soluble in water.

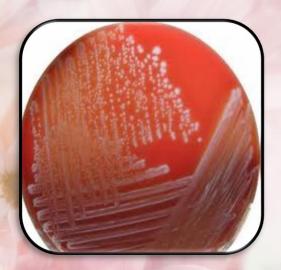
### 4) Pyomelanin:

- ✓ Brown to black pigment.
- ✓ Production is uncommon.

### Cont..

## 3) On blood agar:

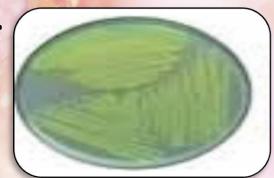
- Similar to nutrient agar.
- > Many are haemolytic.





### 4) On MacConkey agar:

Colourless, non lactose fermenters.



# Biochemical reactions

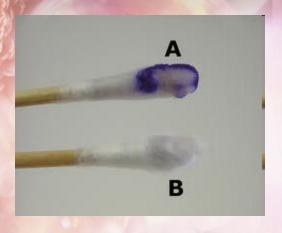
➤ O/F test-oxidative.

> Catalase-positive.

➤ Oxidase-positive.

➤ Nitrate reduction-positive.

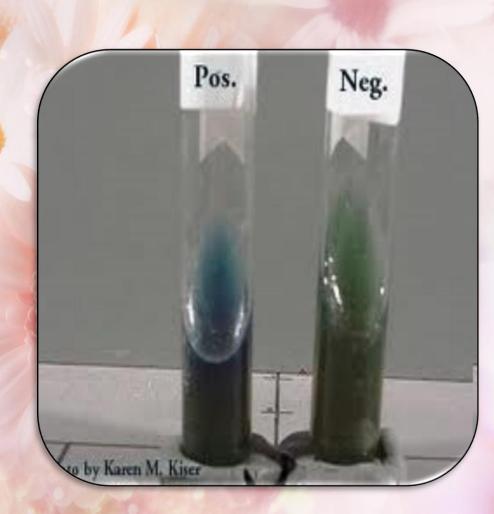




### Cont...

> Citrate test-positive.

Urease test-negative.



### Cont...

## > Sugar utilization tests:

Glucose-only acid.

Lactose-negative.

Sucrose-negative.

Mannitol-negative.

# Oxiditive-Fermantative Test (OF):

- Two tubes of semisolid media are used (which have low concentration agar, high CHO content & an indicator called bromothymol blue) They are inculated 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.







