به نام خدا

دانشگاه اصفهان
دانشکده علوم و فناوری های زیستی، گروه زیست شناسی سلولی و مولکولی و میکروبیولوژی، آزمایشگاه میکروبیولوژی

آزمایشگاه باکتری شناسی ۲

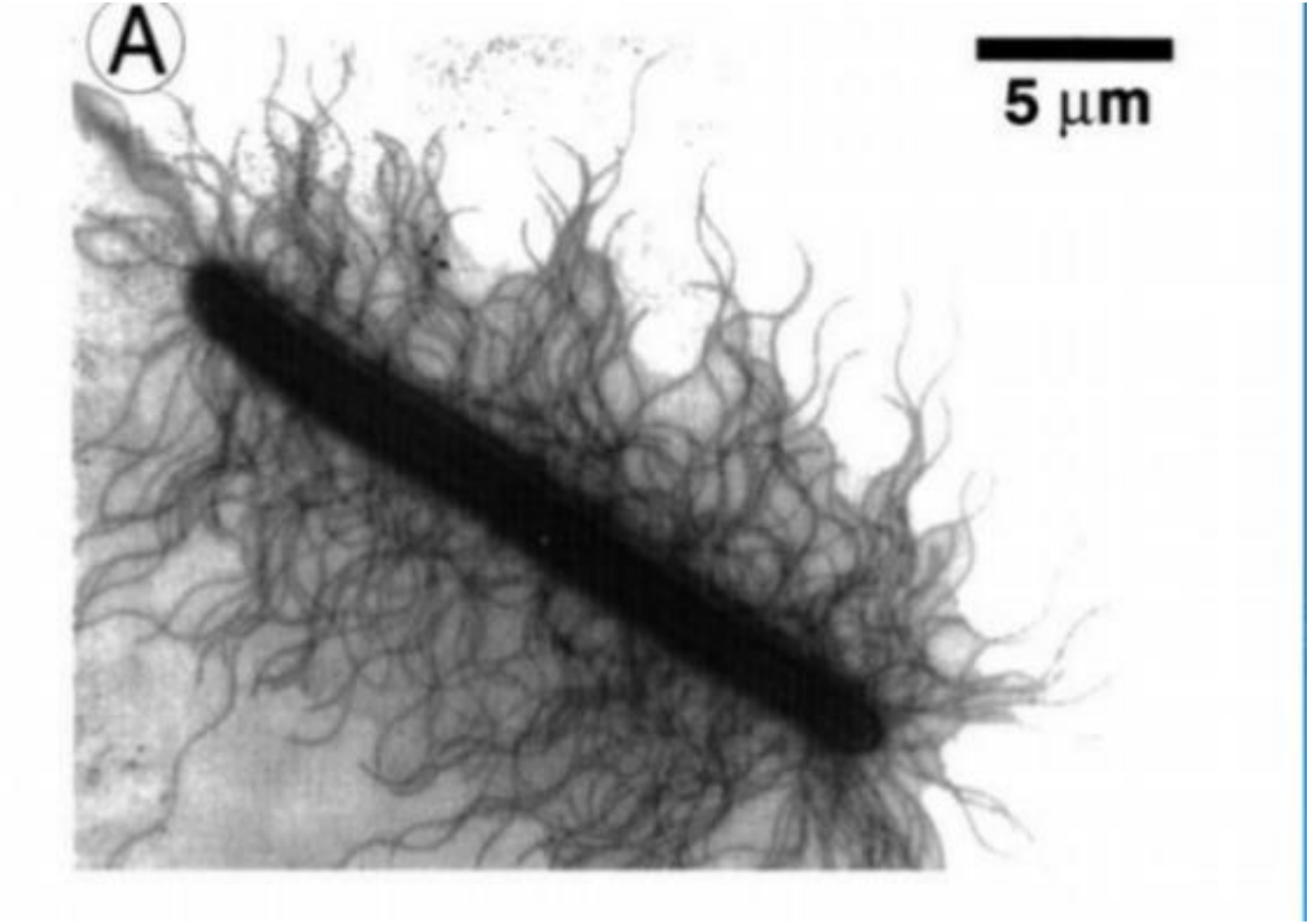
بررسی خصوصیات ماکروسکوپی و میکروسکوپی، انجام آزمون های بیوشیمیایی جهت شناسایی باکتری های خانواده انتروباکتریاسه شامل پروتئوس و سراشیا

تهیه‌کننده: سهیلا عباسی

Proteus

Proteus

- The sub-family include:
  - Proteus.
    - P.mirabilis
    - P.vulgaris
  - Morganella. M.morgani
  - Providencia. P. *rettgeri*


A
5 µm

General characters:

- Member of enterobacteriaceae.
- Saprophytes (life in water & soil).
- Some are commensal in human intestine.
- Non-sporing.
- Non-capsulated.

The main 2 species:

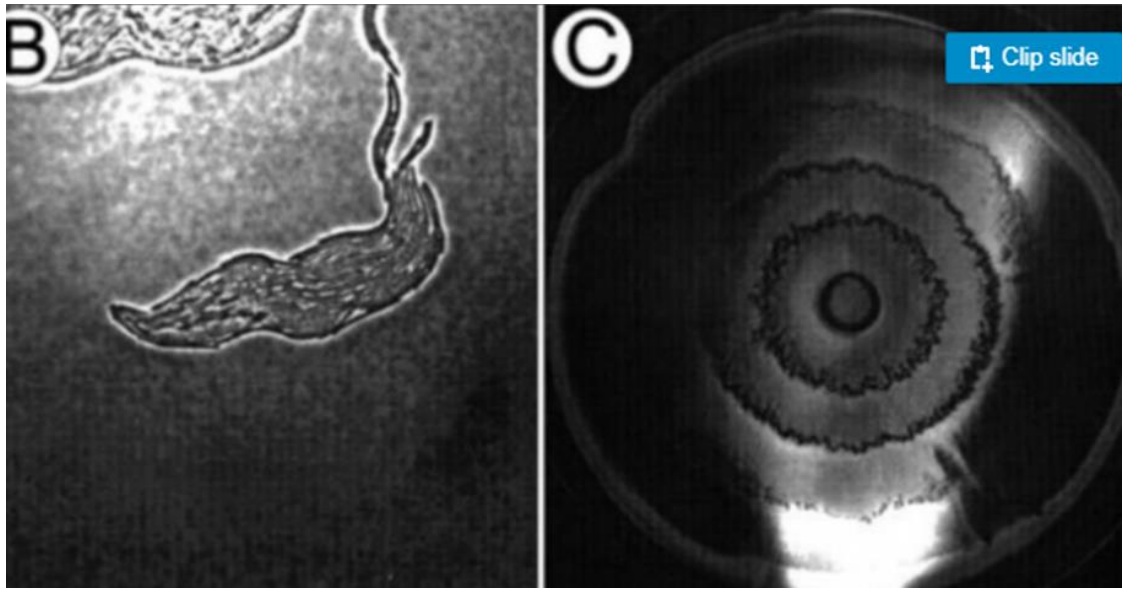
- *P. mirabilis.*
- *P. vulgaris.*

Morphology and stain

Gram negative bacilli, poleomorphic.

Culture characters

- Non-fastidious.
- MacConkey agar: pale yellow colonies (NLF), moderate in size, moist and low convex.
- CLED: pale blue-green colonies (NLF).
- Blood agar & nutrient agar: grow and produced swarming cover all the plate (characteristic).
- Produced fishy smell.


B
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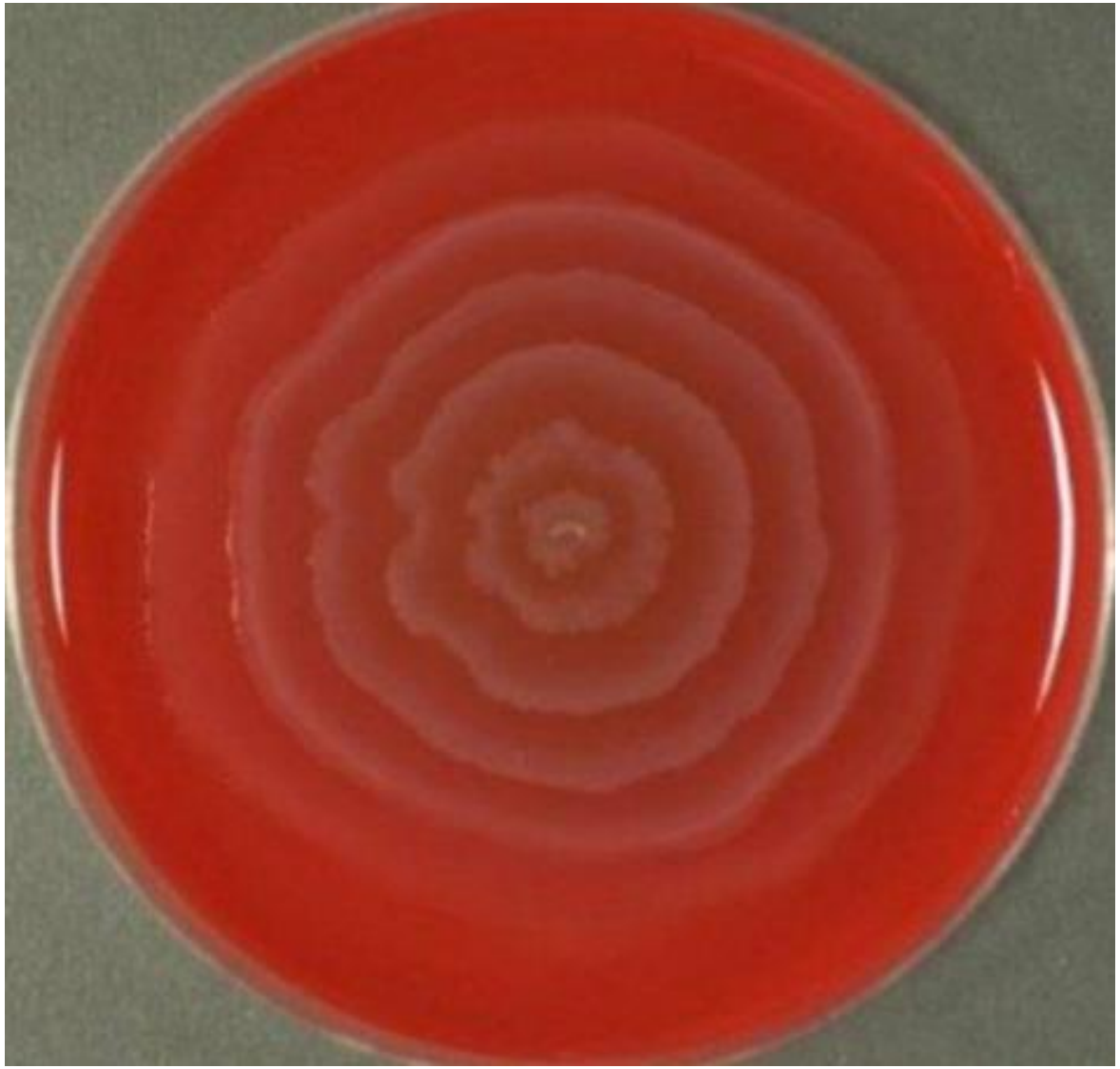


پدیده دینس (Dienes Phenomenon)

در صورتیکه که دوسویه متفاوت پروتئوس برروی یک محیط کشت جامد کشت داده شود حلقه های رشد سوارمینگ آنها بایکدیگر تداخل نمی نمایید وحدفاصل آنها ناحیه ای خالی بین دو گستره پروتئوس مشخص است .درحالی که اگر دو پروتئوس مورد نظر از یک سویه باشند این حلقه ها با یکدیگر ادغام می شوند .

How to prevent swarming

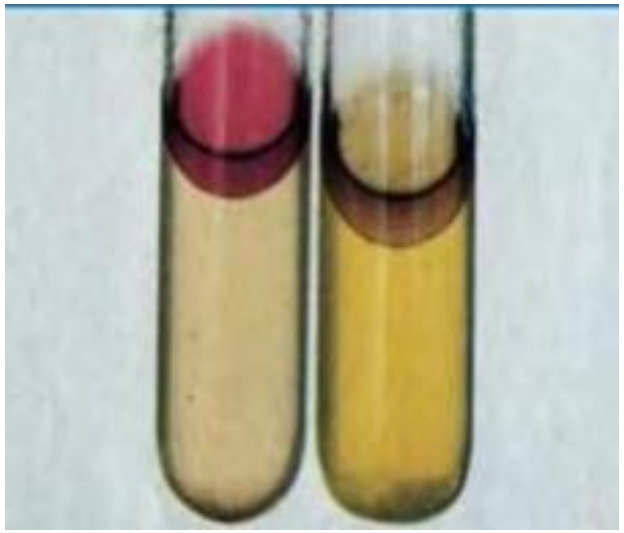
- Increase the agar concentration (from 1.2-1.5% to 6%).
- CLED (electrolytes deficiency).
- To add chemicals: chloral hydrate, Na-azide & paranitroglycerol.
- MacConkey agar (Bile salt).

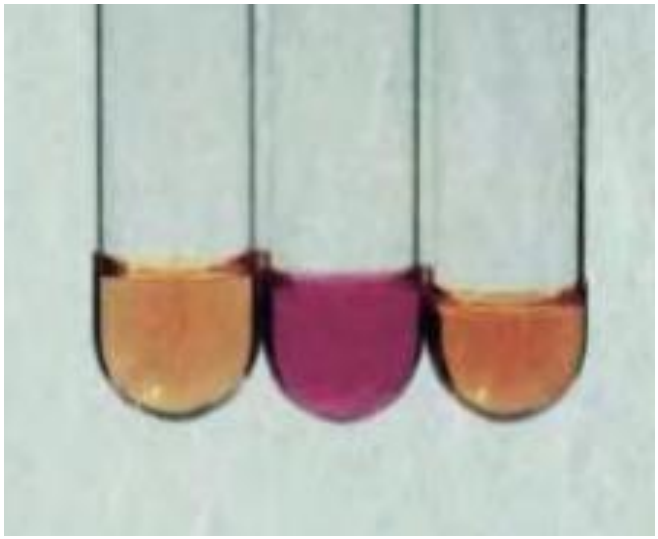
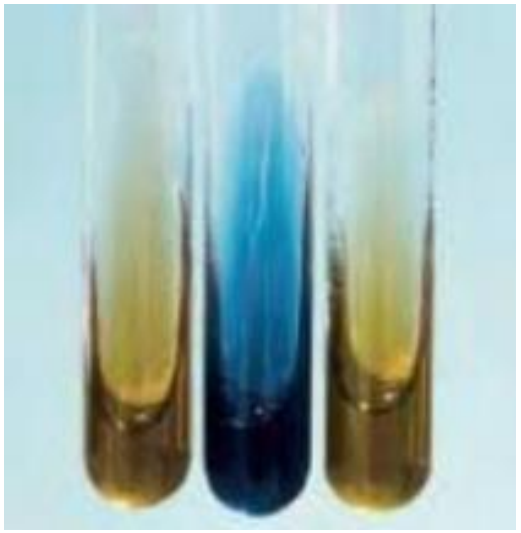
Viability

- Easy to killed by heat.
- Easy to killed by lab-disinfectant.
- Sensitive to most antimicrobial agent used for enterobacteriaceae.

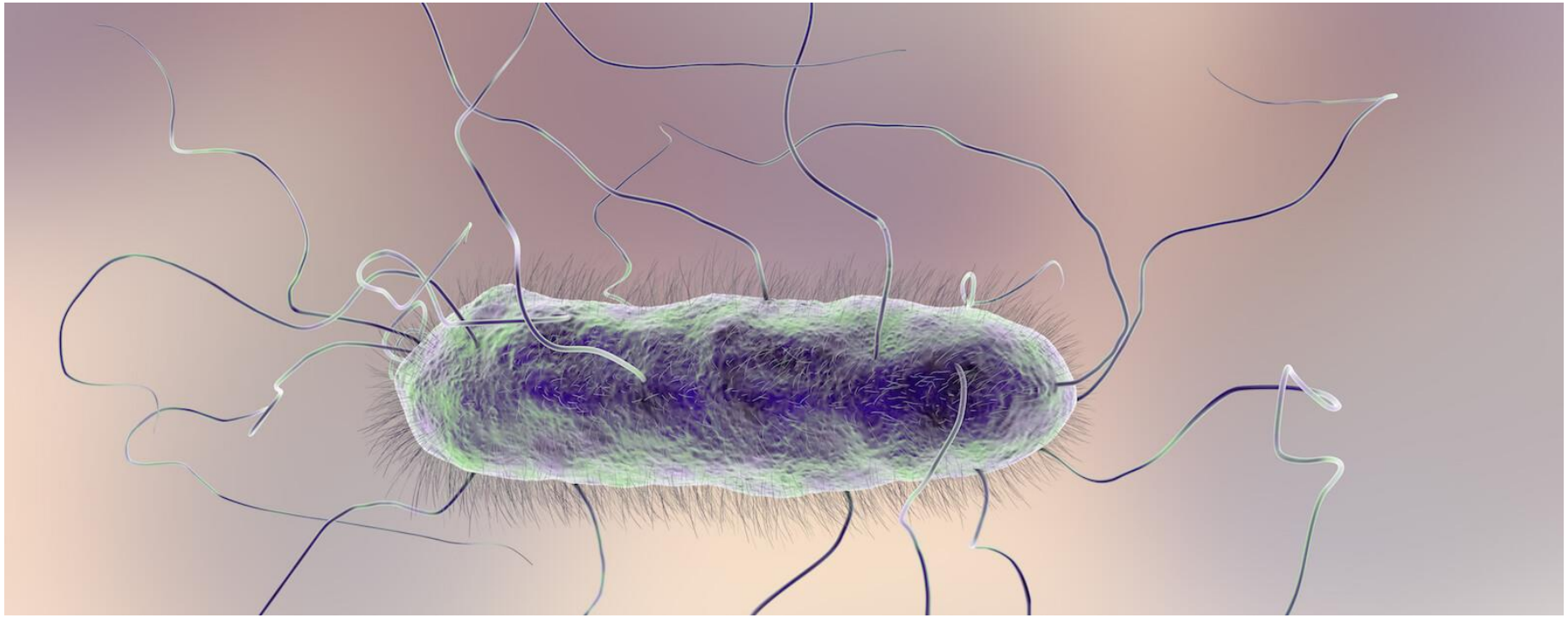
Biochemical reaction

- Urease test: +ve (with 4 hours).
- PPA test: +ve.
- Motility test: +ve.
- H_2S production test: +ve.
- MR: +ve VP: -ve
- Ferment: glucose & maltose.
- Not ferment: lactose.
- Indole & Citrate test: vairable *(P. mirabilis* –ve indole test*)*



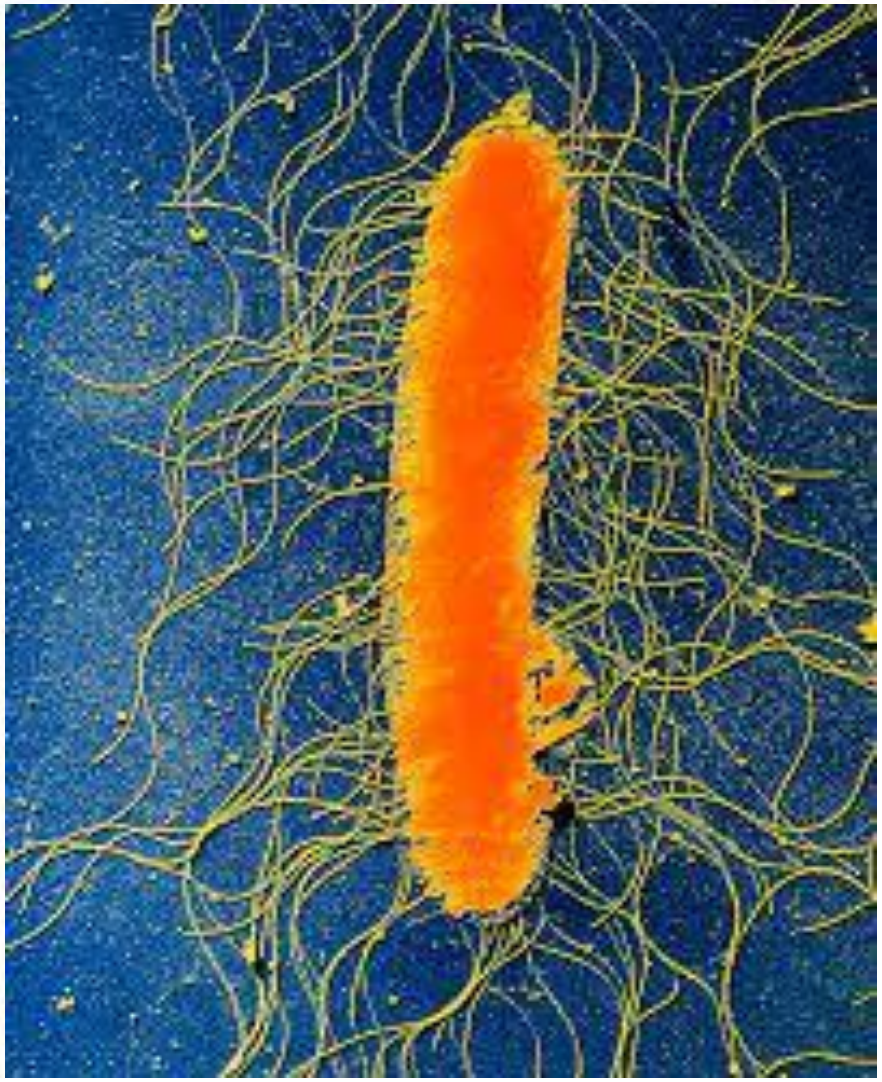



Proteus spp.



General character:

- Rod shape.
- gram-negative.
- **motile.**
- non-capsulated .
- Possessing peritrichous flagella.
- **Non-lactose fermenting.**



-Proteus species are found in the intestinal tract of human.

- Species of medical importance:

- ***P. mirabilis***
- ***P. vulgaris***

P. vulgaris

- Important nosocomial pathogen.
- Isolated in wound infection and urinary tract infection.

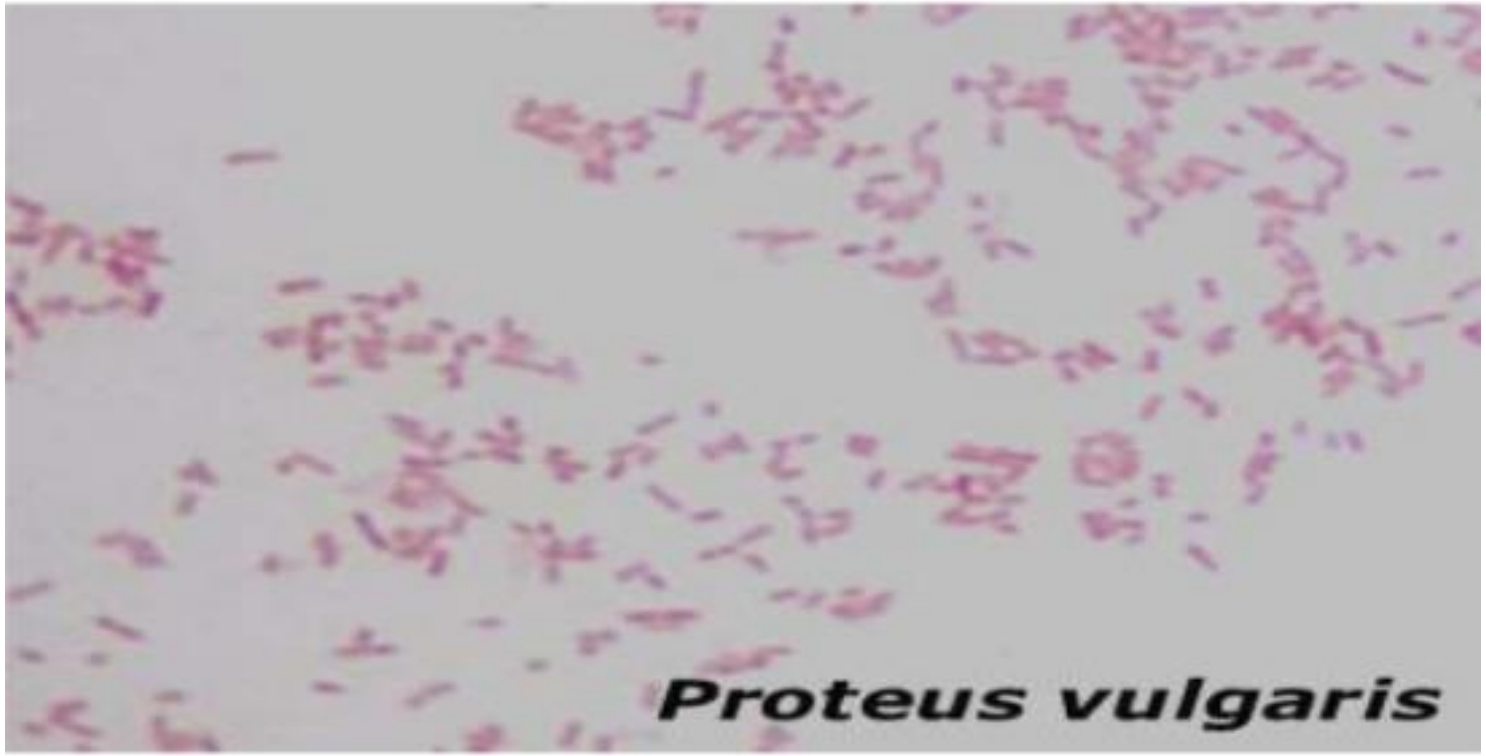
Laboratory diagnosis

Specimen

- Urine.
- Pus.
- blood.
- ear discharge

Gram stain

- Gram-negative rods

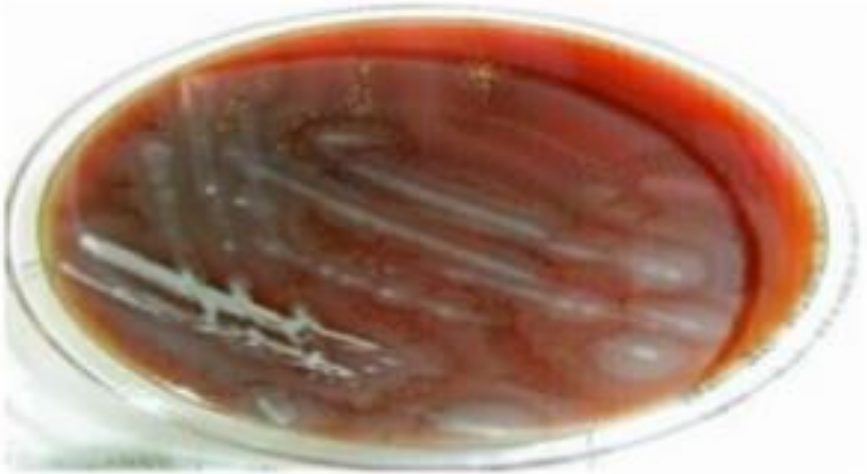


Culture characteristic

On blood agar

- *Proteus mirabilis* does not form distinctive colonies on Blood Agar, instead the bacteria swarm across the surface of the agar.

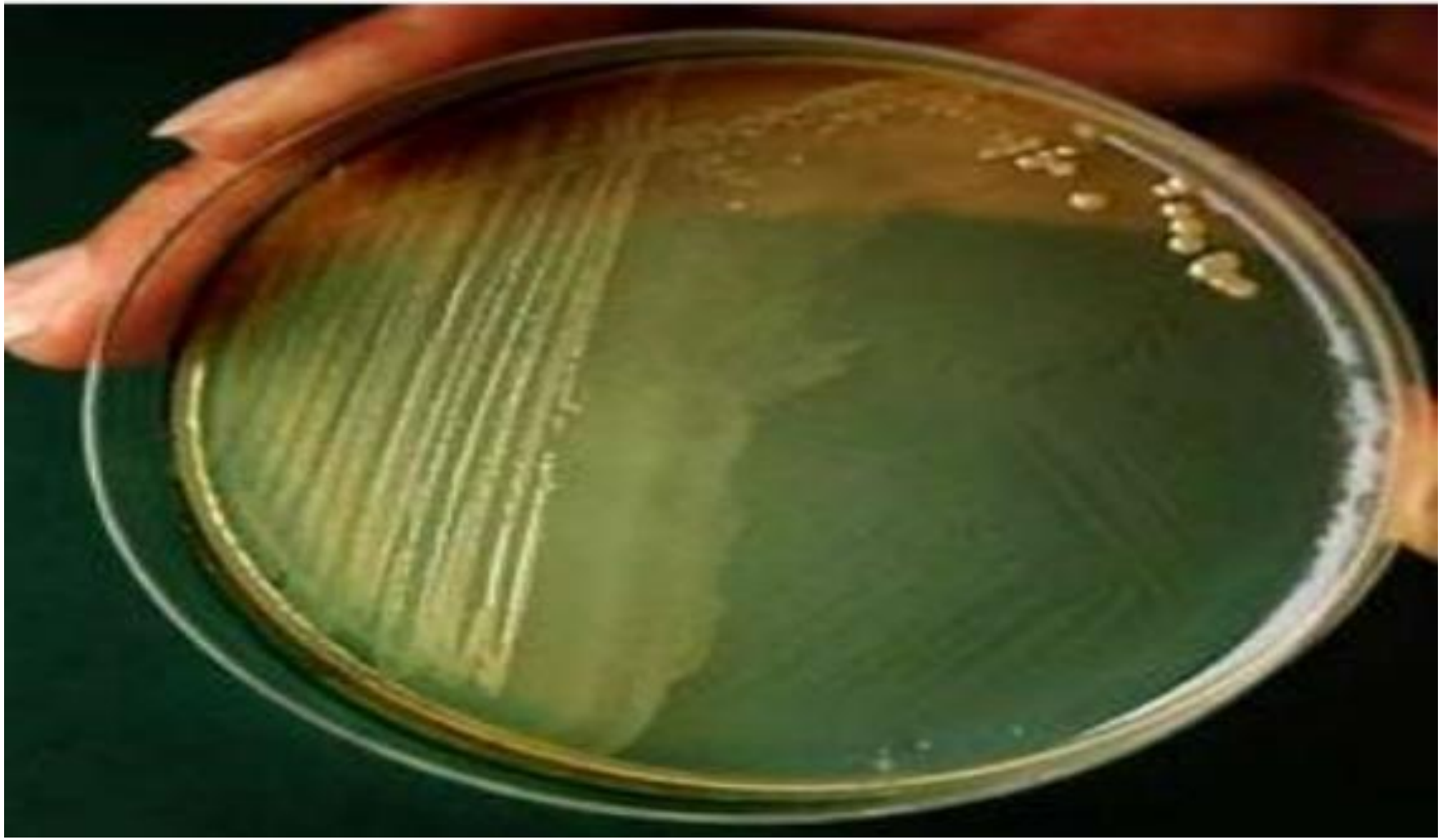
- *P. mirabilis* produces a very distinct fishy odour.



- Discontinuous swarming produces concentric circles around the point of inoculation.

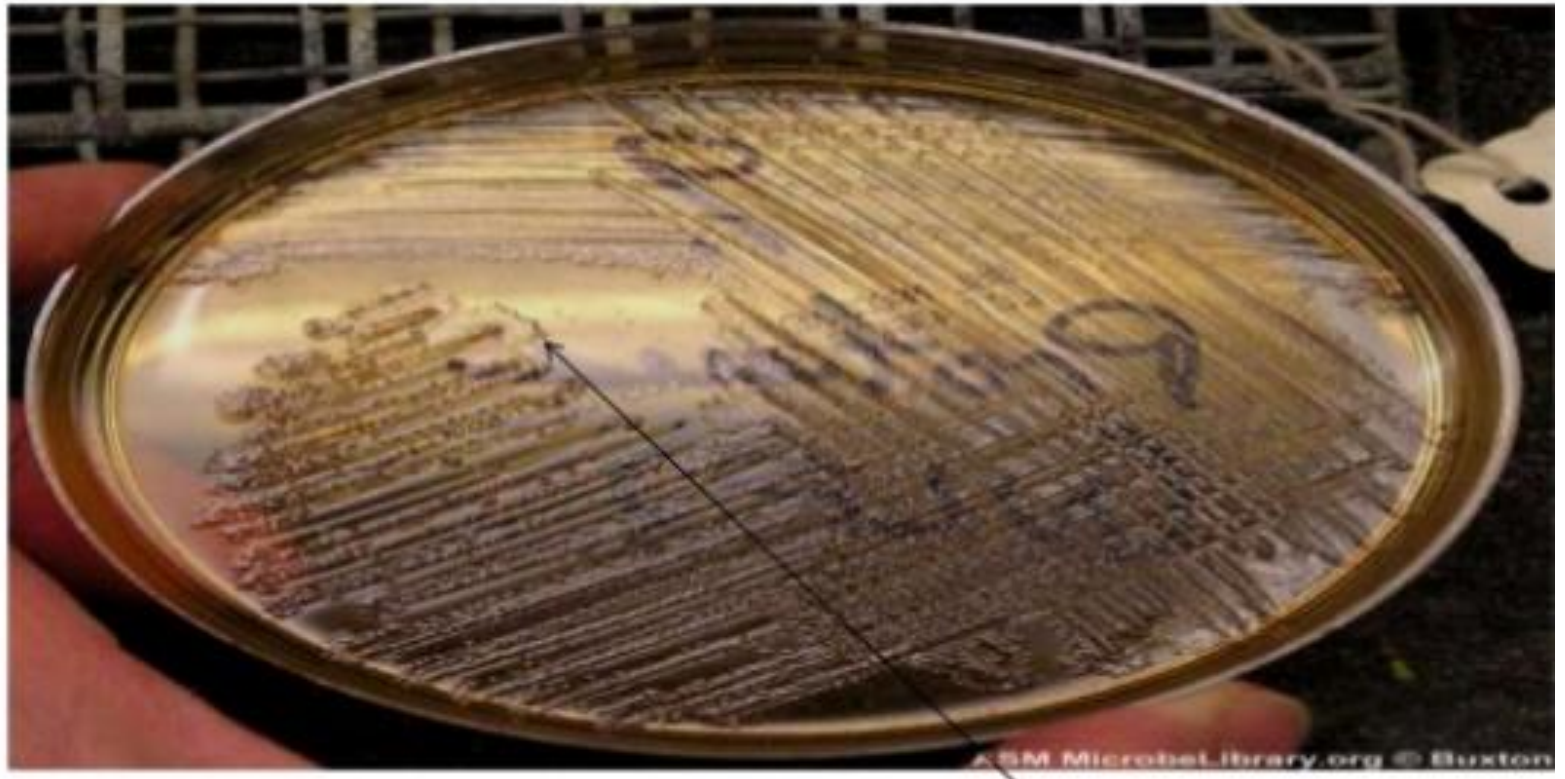
- Continuous swarming produces a uniform film.

On nutrient agar



On MacConkey agar

- Non-lactose fermenters that may exhibit swarming



colorless colonies with slight swarming

P. mirabilis

causes 90% of all *Proteus* infections in humans.

Clinical features:

- Urinary tract infection
- Septicemia
- Abdominal and wound infection
- Secondary invader of ulcer, burn and chronic discharging ear.

On EMB

Proteus vulgaris streaked on Eosin Methylene Blue Agar (EMB)

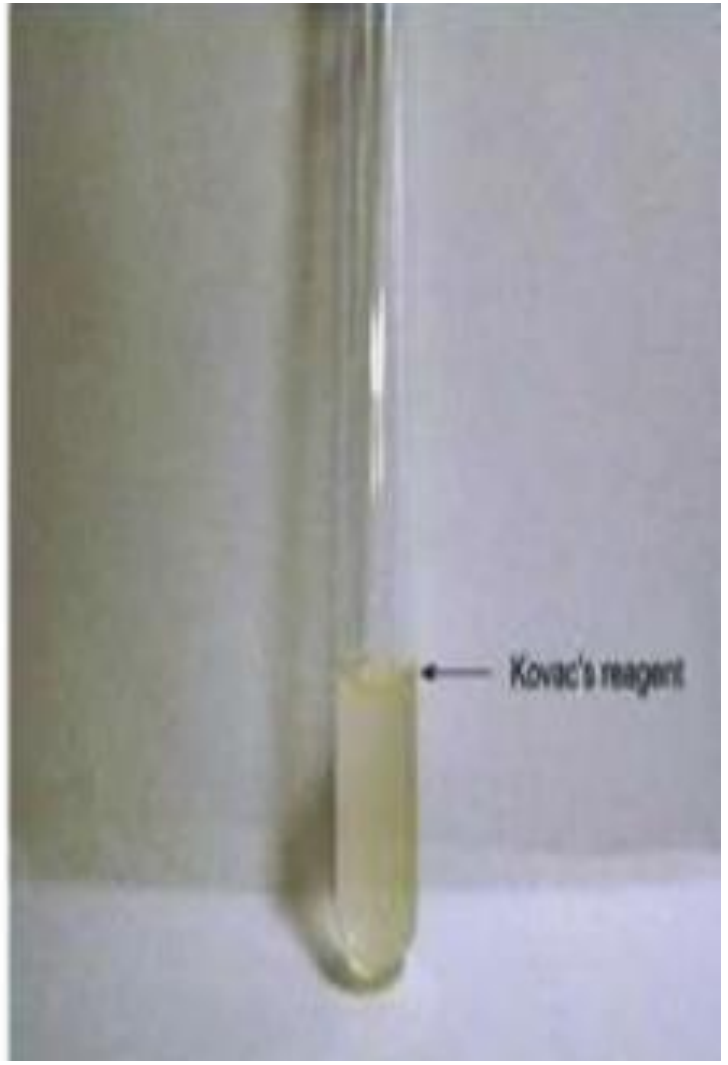
Escherichia coli streaked on Eosin Methylene Blue Agar (EMB)

- **Indole test**

  is used to differentiate

P.mirabilis (-ve) from

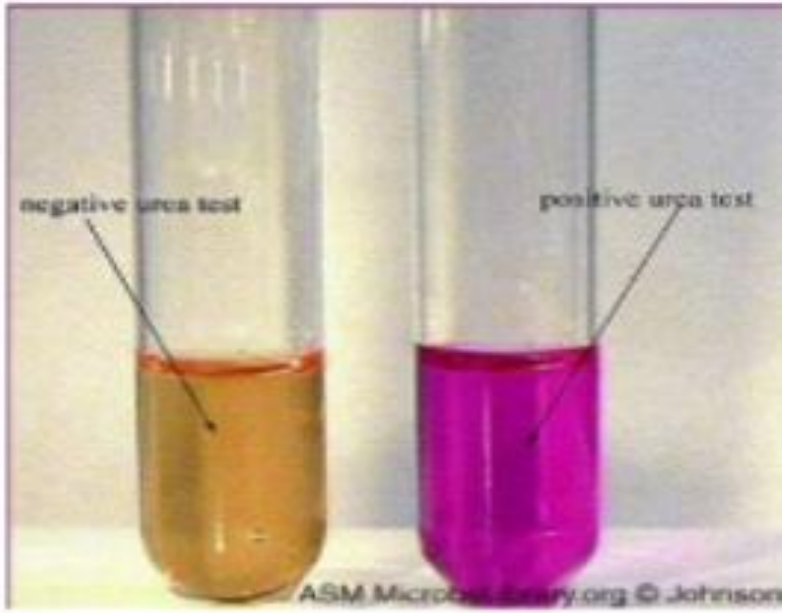
P. vulgares(+ve)



Urease test

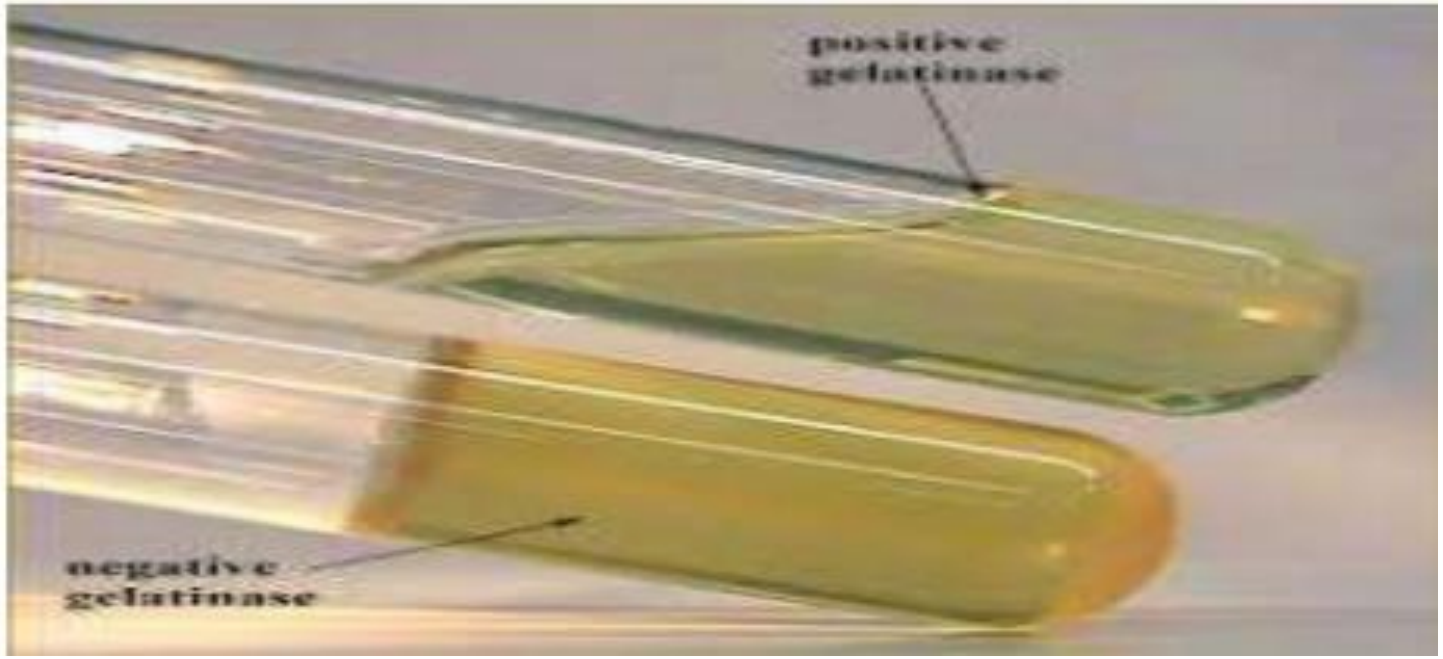
- **positive urease** (which is the fundamental test to differentiate *Proteus* from *Salmonella*).

- Most strains produce a **powerful urease enzyme**, which rapidly hydrolyzes urea to ammonia and carbon monoxide.

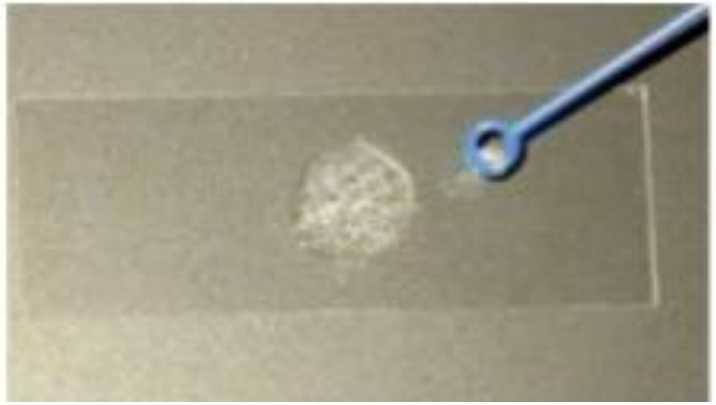


Gelatinase test

- Positive result.
- Liquefaction of gelatin by gelatinase enzyme.

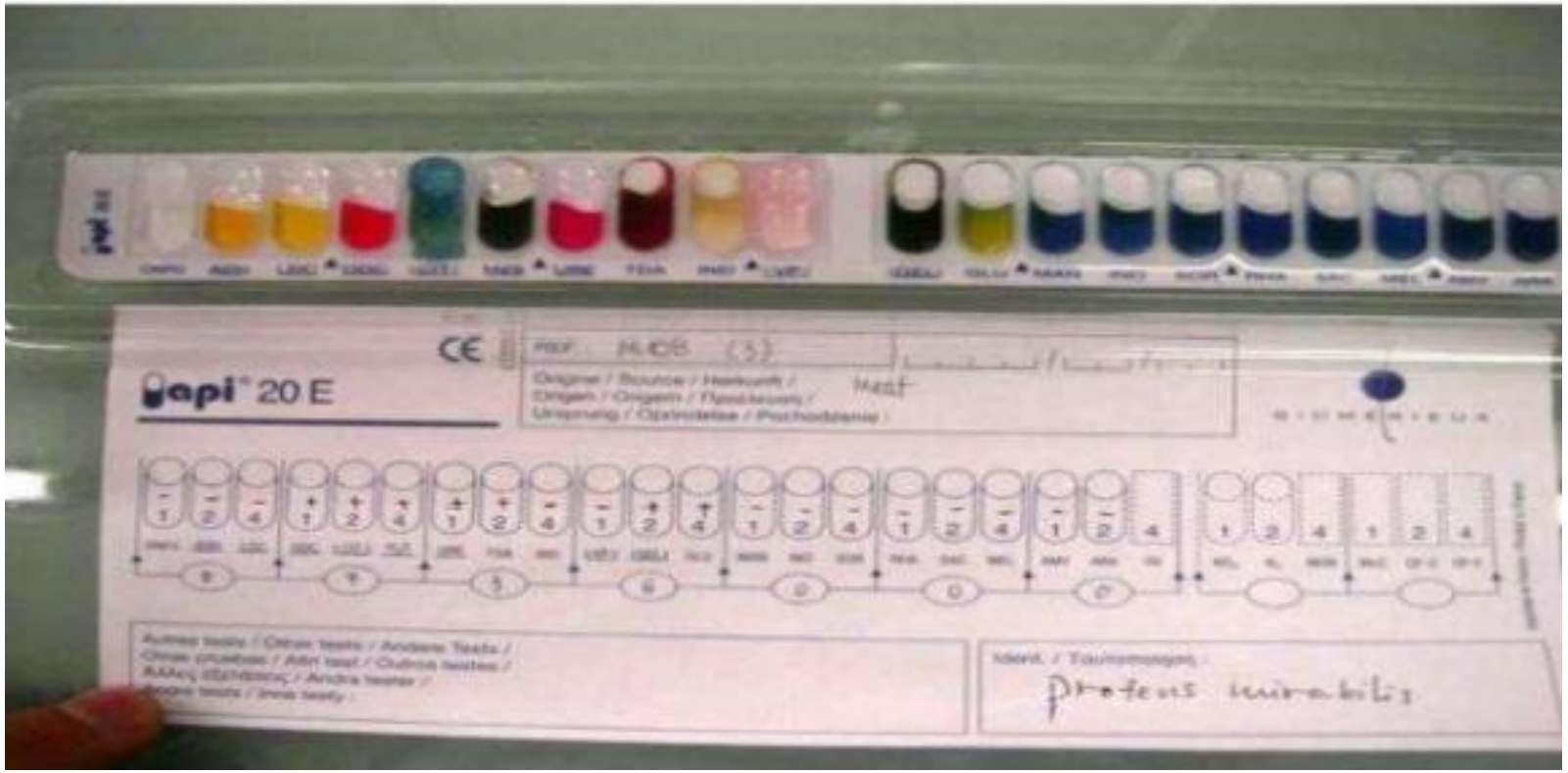


- oxidase-negative
- Catalase-positive.



API20E Test

- *Proteus mirabilis*



Serratia

SERRATIA SPECIES

- Gram negative bacillus
- 7 species
  - ***S. marcescens* most common**
- Motile, often red pigment
- Environmental
  - **water, soil, plants, insects, animals**
- Extracellular enzymes contribute to pathogenicity
  - elastase, lecithinase, caseinase etc.
- May be antibiotic multi-resistant

DR.T.V.RAO MD

SERRATIA MARCESCENS

- Serratia marcescens grows at 37°C, but it can grow in temperatures that range from 5–40°C. They grow in pH levels that range from 5 to 9 . Serratia marcescens is well known for the red pigmentation it produces called prodigiosin.

Prodigiosin is made up of three pyrrole rings and is not produced at 37°C, but at temperatures below 30°C .

- **Many pathogenic strains are non pigment producers**

SERRATIA AND NOSOCOMIAL INFECTIONS

- Over the last 40 years, Serratia marcescens has become an important cause of nosocomial infection. There have been many reports concerning the identification, antibiotic susceptibility, pathogenicity, epidemiological investigations and typing of this organism

S. MARCESCENS CONTAMINATES

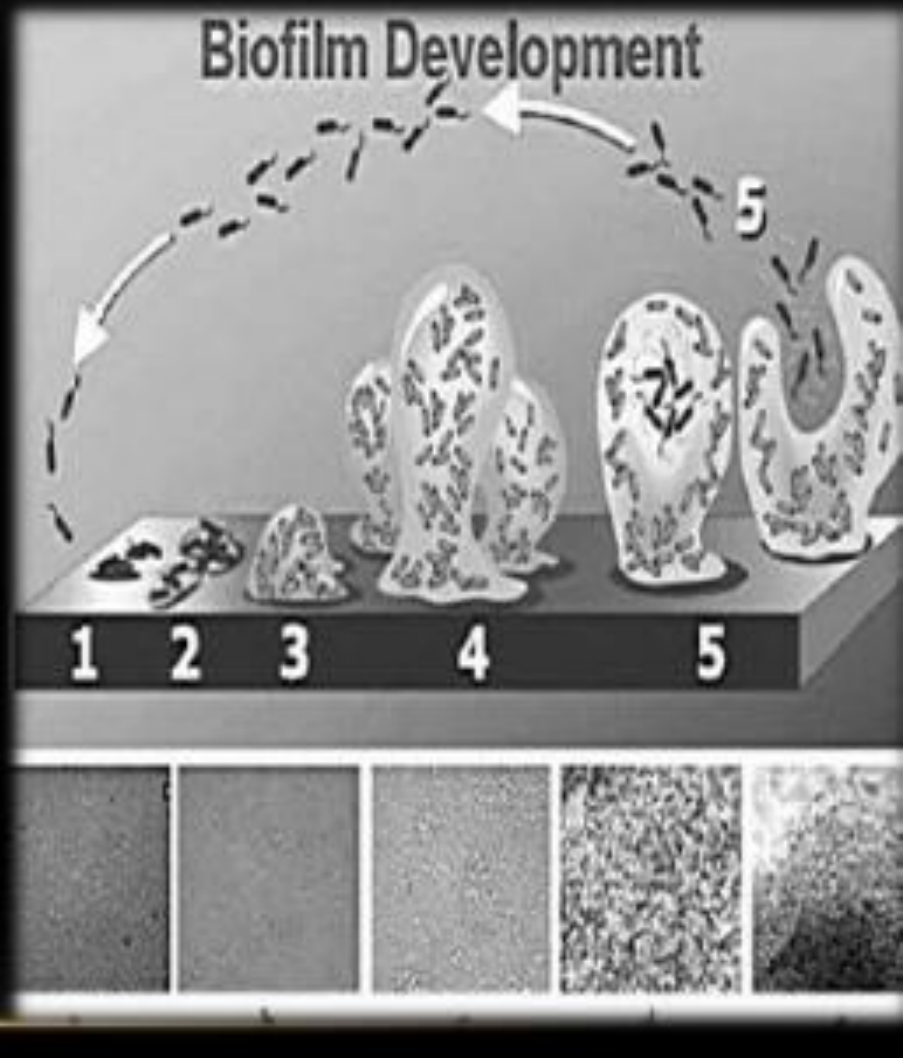
- The growth of S. marcescens in the environment has been investigated in relation to water, disinfectants and plastics such as blood bags. Certain extracellular products are unique to S. marcescens. Pigment (prodigiosin) biosynthesis by S. marcescens has been investigated fully since the emergence of the organism as a cause of infection.

IDENTIFICATION OF S. MARCESCENS

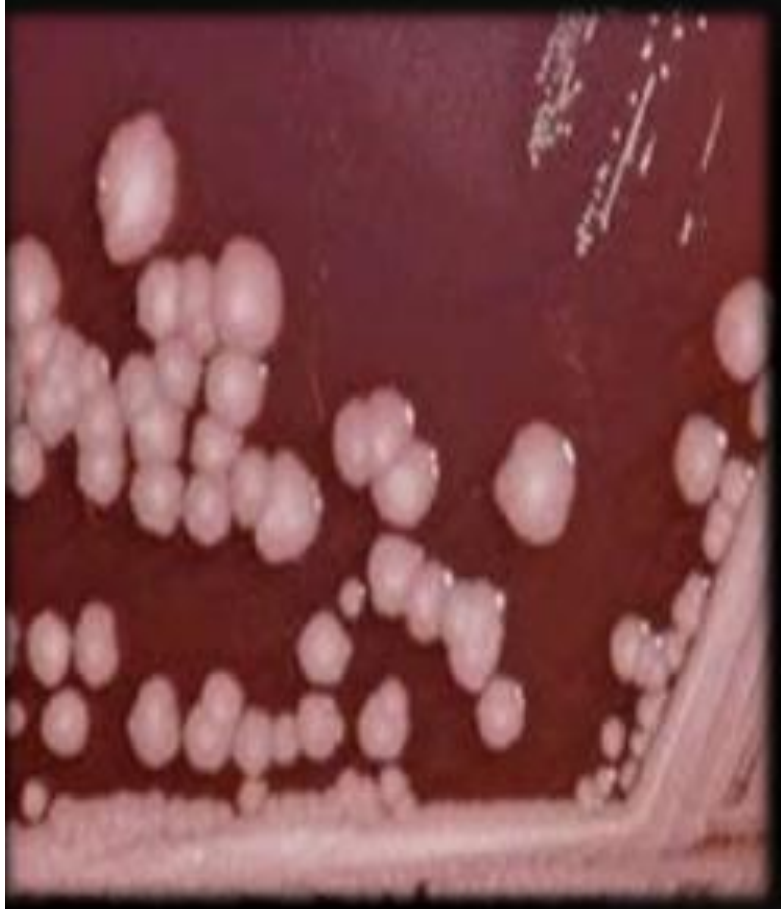
- S. marcescens was defined by Grimont and Grimont as an oxidase-negative gram-negative bacillus producing DNAase. . S. marcescens is unable to ferment arabinose in peptone water, whereas all S. liquefaciens strains are arabinose-positive.. To confirm the identity of S. marcescens, a short series of sugars, including arabinose and raffinose, should be tested in tubes,

S. MARCESCENS AND BIOFILMS

- S. marcescens possessing type 1 fimbriae use biofilms to regulate quorum sensing . Biofilms are formed when S. marcescens aggregate together and attach to a surface . When these microbes aggregate together they can communicate with one another via quorum sensing. Serratia can benefit from biofilms because they are proposed to provide protection against external .



SOME SERRATIA ARE NOT PIGMENT PRODUCERS

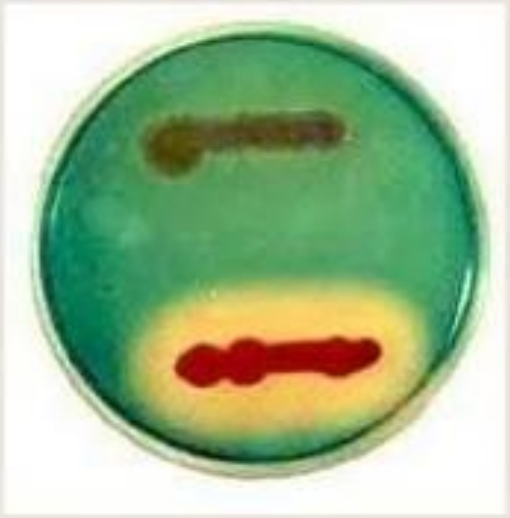


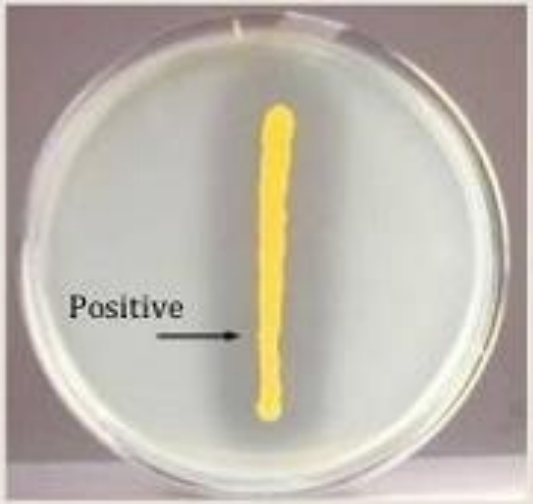
- The red pigment production is not present in all strains but in those that it is present, it can resemble red pigment.

Differences of the Group

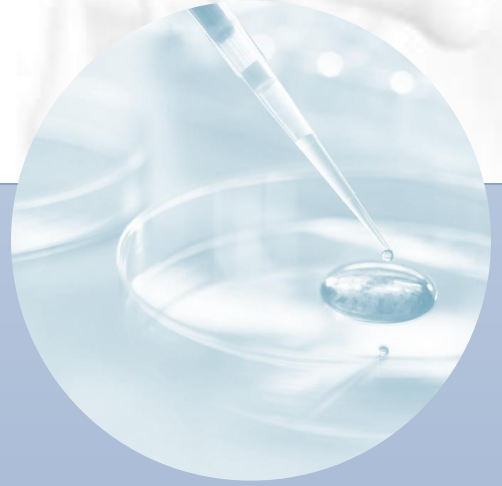
Character	Klebsiella	Enterobacter	Serratia
Motility	-	+	+
Indole	+	-	-
Red pigment	-	-	+
Quellung reaction	+	-	-

DNASE





- *Escherichia coli* (top): negative-no clearing
- *Serratia marcescens* (bottom): positive-clearing



با تشکر از توجه شما