ENTEROBACTERIA

PSEUDOMONAS

PRACTICAL PART

1. IDENTIFICATIONS OF ENTEROBACTERIA

Cultivation on selective growth media

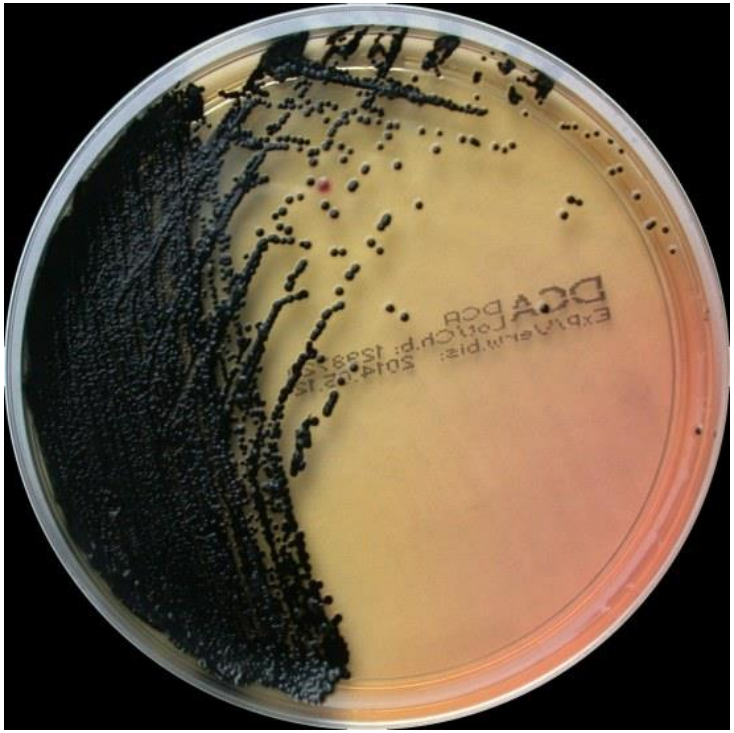
- **Principle:** selective cultivation of enterobacteria used the tolerance of enterobacteria to chemical substances which inhibits the growth of other bacteria (fuchsine, bile), allow the differentiation of species according to ability of lactose utilization (**McConkey agar, Endo agar, Desoxycholate-Citrate agar**), or ability to create hydrogen sulphide (**DC agar**)

Lactose positive
Escherichia coli

Lactose negative
Salmonella
Shigella

Cultivation on selective diagnostic media

DC agar
Hydrogen sulphide creation
Salmonella



Biochemical identification of enterobacteria

HISU – short series of tubes biochemical test

- **Principle:** differentiation of enterobacteria use species specific biochemical activity. Test use detection of specific metabolisms products mostly evaluated by growth media pH changing visualized by color changing

HAJN solid medium: acidification of media by carbohydrates fermentation and medium change color to yellow. Production of H_2S change color to black and production of gas resulted in production of bubble and damage of solid medium

INDOL PRODUCTION + MOTILITY: indole is generated from tryptophan by deamination, the indole production is visualized by Kovac´s reagent which in positive reaction change color in red. The motility is evaluated by spread of bacteria from puncturing area in solid medium.

SIMMONS CITRATE: the utilization of carbon from sodium citrate generate ammonia which alkaline the medium and this lead to change the color in dark blue

UREA: the urea hydrolysis produce ammonia and lead to change color of media in pink

PRACTICAL PART

ENTEROTEST24

ENTEROTEST24

- **Tools**: Enterotest 24, bacterial strain, loops, markers, sachet, paraffin oil, pipette, disinfection
- **Procedure**: Prepare an inoculum of density 1McFarland turbidity scale from a pure culture
- Prepare a strip containing biochemical tests
- Inoculate each well in strips with 100 µl of prepared inoculum
- Drip wells H, G, F, E and D in the first row with paraffin oil (anaerobic conditions)
- Cover plate with lid, place in sachet and incubate at 37°C for 24 hours

ENTEROTEST24

- Additional test for indole formation from tryptophan
- Place a drop of the Indole solution on a square of filter paper
- Spread several isolated colonies into droplets on filter paper
- Incubate for 5 min
- Read the reaction
  - Positive - blue-green color
  - Negative - pink color

SEROTYPIZATION OF ENTEROBACTERIA reverse agglutination

- **Principle:** indirect method (slide agglutination) used complex antigen structure of enterobacteria (antigens: O – somatic, H – flagellum) for serotype differentiation (combine O and H antigens). Used for identification of pathogenic serotypes
- **Materials:** monovalent sera O55 a O111, tested bacterial culture, physiol. solution, bacteriological loops, disinfections

SEROTYPIZATION OF ENTEROBACTERIA reverse agglutination

Procedure:

- Put a drop of saline solution at the plate into both boxes. Add a colony of tested strain with a sterile loop and make a suspension
- Put a drop of a serum next to the suspension. Mix the drops at each box, use always a new sterile loop.
- **Evaluation:** observe the formation of precipitate

PRACTICAL PART

2. IDENTIFICATION OF PSEUDOMONADS AND OTHER GRAMNEGATIVE NON-FERMENT RODS

O/F TEST

Principle: oxidization/fermentation of glucose is used for differentiation of metabolism type and for differentiation of aerobic (oxidization) and anaerobic (fermentation) bacteria.

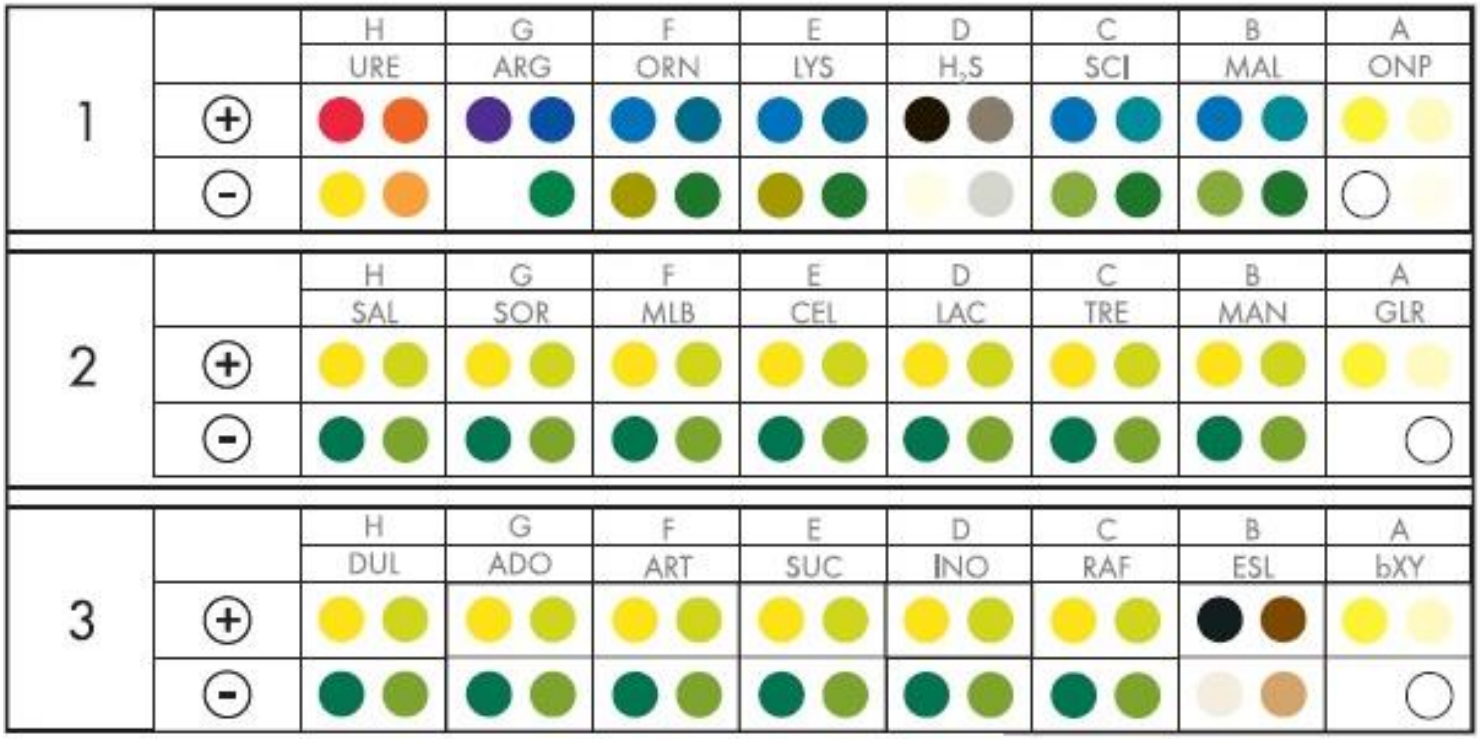
- Test principle – medium with glucose and pH indicators. Tube without paraffin cover – aerobic (oxidization) and tube with paraffin cover – anaerobic (fermentation)
- **Materials:** bacterial culture, tubes with glucose medium, paraffin oil, markers, dropper, bacteriological loops, disinfection
- **Procedure:** mark and sign the tubes, inoculate each tube with the test strain by inserting loop to the bottom of the tubes. Cover one of tube by paraffin and incubate in 37°C for 24hr

PRACTICAL PART

1. IDENTIFICATION OF ENTEROBACTERIA - EVALUATION

ENTEROTEST24

- Results
- Use the color chart to evaluate the test results and record them in the enclosed paper form



ENTEROTEST24

- Test evaluation
  - Create a numeric code using the form and use the code book to locate the identified species

OR

  - Enter the evaluated results in the TNW computer program Using TNW Pro Auto 7.0

PRACTICAL PART

2. IDENTIFICATION OF PSEUDOMONADS AND OTHER GRAMNEGATIVE NON-FERMENT RODS - EVALUATION

O/F TEST - evaluation

Evaluation:

•After incubation observe medium color change

- In tube without paraffin oil coverage assess the glucose oxidization
- In tube with paraffin oil coverage assess the glucose fermentation

•**Non-fermenting bacteria** – *Pseudomonas*, *Burkholderia*, *Acinetobacter*…

  – Test of oxidase production for other differentiation

•**Fermenting bacteria** - *Enterobacteriacae*

TEST OF OXIDASE PRODUCTION

- **Test principle**: The test proof the production of cytochrome c oxidase by color reaction of N,N - dimethyl - 1,4 - phenylenediamin with α-naphtol by formation of indolphenol blue.
- **Tools:** unknown bacterial culture, OXI test strips
- **Procedure:** Directly imprint the strip testing zone onto one or several colonies of testing culture
- **Evaluation:** evaluate the color change.

positive: up to 30 s color turned in dark blue

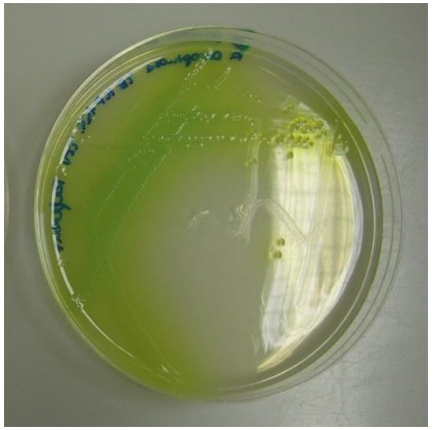
lately positive: up to 2 min color turned in dark blue

negative: without color change, or color change in more than 2 min

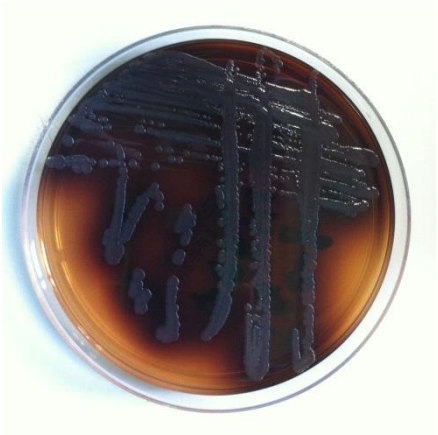
PSEUDOMONAS AERUGINOSA pigmentation

Pseudomonas aeruginosa produce three types of pigments

Pyocyanin



Fluorescein



Pyorubrin

ISOLATION of *PSEUDOMONAS AERUGINOSA* PIGMENT(pyocyanin)

- **Tools:** broth culture of *Pseudomonas aeruginosa,* chloroform, rubber cap, pipette
- **Procedure:** Add 1 ml of chloroform into the tube with 2ml of inactivated *Pseudomonas aeruginosa* broth culture. Tightly cover the tube with cape and carefully shake.

Be carefully in manipulation with chloroform!!

- **Evaluation:** describe color change of chloroform layer