Identification of bacteria

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Objective

- To study following characteristics of bacteria for their identification:
  - Morphological characters
  - Cultural characters
  - Biochemical characters
  - Serological characters
- Typing of bacteria – Bacteriopahge and bacteriocin typing

Introduction

- Once bacterium is obtained in pure culture. it has to be identified.
- Different characteristics studied and used for identification are:
  1) Morphological characteristics.
  2) Cultural characteristics.
  3) Biochemical characteristics.
  4) Antigenic characteristics.
  5)Bacteriophage and bacteriocin typing.

Morphological characteristics

Morphological characteristics studied are:

1. Shape and arrangement of bacteria.
2. Gram character.
3. Presence of flagella, capsule, endospore.
4. Acidfastness.

Cultural characteristics

- Cultural characteristics are studied on different media.
- On solid media following characters of colony are noted – colony characters

1) Size – In millimetres.
   - If > 1 mm - Pin point
   - large are up to 5-10 mm.
   - limit to diameter.
   - *Proteus, Bacillus* – spreading colonies
2) Shape – circular, irregular, oval, lanceolate

Cultural characteristics

3) Edge / Margin of colony–

- Entire / Smooth
- Undulate - wavy
- Crenated
- Curled
- Erose - spiny projections
- Filamentous
- Rhizoidal – root like projections

FORM

ELEVATION

MARGIN

Cultural characteristics

4) Elevation –
   - Flat
   - Raised
   - Convex (low/high)
   - Umbonate
5) Color - pigment production.
   - Water soluble and water insoluble pigments
   - *S. aureus* – golden yellow
   - *Micrococcus luteus* – lemon yellow
   - *Serratia marcescens* – red

6) Opacity –
   a. Opaque
   b. Translucent
   c. Transparent.
7) Consistency –
   a. Butyrous,
   b. Mucoid,
   c. Dry
      - Brittle,
      - Powdery.

Cultural characteristics

Fluid medium

- Degree of turbidity.
- Presence of deposit.
- Pellicle formation.

Stroke culture

- Degree of growth – scanty, moderate, profuse.
- Nature of growth - discrete /confluent, filiform, spreading

Biochemical characteristics

More important and widely used tests are -

1. Sugar fermentation
2. IMViC tests:
   a. Indole production
   b. Methyl red test
   c. Voges Proskauer test
   d. Citrate utilization
3. Nitrate reduction

Biochemical characteristics

4. Production of ammonia
5. Urease test
6. Hydrogen sulphide production
7. Catalase test
8. Oxidase test
9. Coagulase test

- Biochemical tests help to identify bacteria up to species level.

Sugar fermentation test

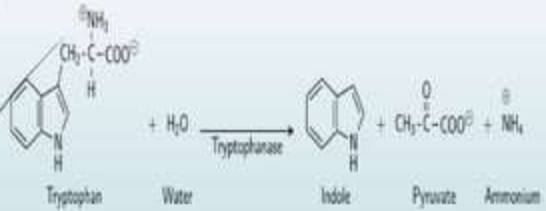
- Peptone water + 1% sugar + Andrade's pH indicator+ Durhams tube
- Sugar fermentation – A or Acid and gas
- Acid – Pink colour
- Gas – Bubble in Durham'stube

Indole production test

- Medium – 1% tryptone water / 2% peptone water
- Some bacteria produce tryptophanase and convert tryptphan to indole
- Reagents: xylene – solvent for indole
- Kovack's ragent – Para-dimethyl aminobenzaldehyde reacts with indole to form pink complex
- Positive test - pink ring

Indole production test

Chemical reaction



Positive indole production test

Methyl red test (MR test)

- Test detects production of acid during fermentation below pH 4.5
- Medium glucose phosphate broth – GPB
- Inoculate tubes and after incubation detect acid production by using pH indicator.
- Use methyl red indicator – 5 drops of 0.04% methyl red
- Red colour - positive test. Bacteria producing only acid form glucose
- Yellow – negative test –bacteria producing acid and some neutral products from glucose fermentation.

Voges- Proskauer test (VP)

- Test depends on formation of acetyl methyl carbinol from pyruvic acid as an intermediate in its conversion to 2:3 butylene glycol.
- In presence of alkali an atmospheric oxygen AMC is oxidized to diacetyl which reacts with peptone to give red colour.
- Medium GPB
- Chemicals used to detect AMC are 5% solution of α naphthol and 40% KOH
- Positive test – pink colour/ magenta / crimson colour.
- Negative reaction – colourless

Citrate utilization test

- Medium – Koser's citrate , Simmon's citrate agar (citrate, ammonium)
- They contain citrate as sole source of carbon
- Positive test – turbidity in medium/ growth on Simmon's citrate agar
- Bromothimol blue – pH indicator (green below 6.9 pH and blue at pH 7.6 or grater.
- As citrate is used pH of medium increases and medium turns **blue**

- Compare results of IMViC test for *E. coli* and *Enterobacter aerogens*.

Nitrate reduction test

- Some bacteria produce nitrate reductase enzyme which reduces nitrate (NO_3) to (NO_2)
- Medium- peptone water + 1% KNO_3
- Reagent: equal volume mixture of sulphanilic acid and α naphthol in 5N acetic acid.
- Positive test – red colour
- Negative test - Absence of red colour

Production of ammonia

- Some bacteria produce ammonia from proteins of medium
- Medium - Peptone water
- Reagent – Nessler's reagent
- Positive test – brown colour
- Negative test – faint yellow colour

Urease test

- Some bacteria produce urease enzyme
- It hydrolyses urea to ammonia and carbon dioxide.
- Medium – Christensen's urease medium
- pH indicator – phenol red
- Positive test – purple pink colour.
- Ammonia makes medium alkaline and turn medium pink

Hydrogen sulphide production

- Some organisms decompose sulphur containing amino acids producing H2 S
- Medium – peptone water
- Reagent – lead acetate paper hanged in the tube (ferric ammonium citrate or ferrous acetate can be used)
- Browning of paper indicates positive test

Catalase test

- Catalase enzyme hydrolyses H_2O_2 into $H_2O$and O_2
- Pick up growth from solid medium on wire loop
- Insert wire loop in test tube containing H_2O_2
- Prompt effervescence indicate catalase production

Oxidase test

- Reaction is due to cytochrome oxidase which catalyses oxidation of reduced cytochrome by oxygen.
- 1- 1.5 % solution of tetramethyl paraphenylene diamine hydrochloride is poured over colonies
- Oxidase positive colonies become maroon, purple, black
- Kovac's method – filter paper soaked in oxidase reagent and colony is smeared on it. In 10 second growth turns purple / dark

Coagulase test

- Diluted human plasma + *S. aureus* culture
- Incubate at 37 oC for 4 hrs
- Presence of clot in tube indicate positive test
- Test given positive by *S. aureus*
- It produces enzyme coagulase
- Enzyme reacts with coagulase reacting factor in human plasma and forms coagulase- CRF complex
- Complex has action similar to thrombin
- It acts on fibrin and convert it to fibrinogen (clot)

TSI test

- TSI medium – 0.1% glucose, 1% lactose, 1% sucrose, sodium thiosulphate, ferrous sulphate, phenol red.
- Indicates whether bacteria ferments glucose only or lactose and sucrose also with or without gas formation. Medium also indicates production of H_2S
- Medium is distributed in tubes with butt and slant.
- Culture is inoculated in butt and on slant surface.

TSI results -

- Tube 1 – Acid in slope and butt No H_2S
- Tube 2 – Acid in but butt not on slope. No H_2S
- Tube 3 – H_2S production
- Tube 4 – negative test

- What are TSI test results for *E.coli* and *Salmonella*?

- Slant red and butt yellow – only glucose fermented
- Both slant and butt yellow – all sugars fermented
- Bubble in butt – gas production
- Blackening of medium indicate H_2S production - H_2S reacts with ferrous sulphate to form visible black precipitate
- TSI is useful in identification of Gram negative bacteria

Antigenic characters

- By using specific antibodies we can identify bacteria by suitable serological tests.
- Test used are: agglutination test or immunofluorescence test.

Bacteriphage and bacteriocin typing

- Typing is identification of different strains (verities) of bacterial species.
- This is done by using bacteriophage or bacteriocin specificity.
- Bacteriophage typing is based on sensitivity of test strain to the lytic action of standard phages.
- Bacteriocin typing involves ability of test strain to kill standard indicator bacteria.
- This is mainly done for epidemiological purpose.

Thank You