9. BIOCHEMICAL TESTS

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Introduction

Bacteria do have the biochemical fingerprints that are properties controlled by the cellular enzymatic activity. Biochemical identification characterization of bacteria is based on the extracellular enzyme activity and intracellular enzyme activity. Extracellular enzymes are elaborated out of the bacterium and usually performs the action of hydrolysis to break down complex molecules to simpler building block units which can be further utilized by the bacteria after transporting into the cell. Whereas on the other hand the intracellular enzyme functions inside the cell for the metabolism and the metabolic products are excreted out of the bacterium. This metabolic product accumulated outside of the bacterium is detected in the biochemical test. Biochemical methods involve the identification of activity of both the types of enzymes.

- Tests used to identify the extracellular enzymes activity are starch hydrolysis, lipid hydrolysis, casein hydrolysis, chitin hydrolysis etc.,
- Tests used to identify the intracellular enzyme activity basically identifying the end product of the reaction are carbohydrate fermentation, litmus reaction, H2S production, nitrate reduction, catalase, oxidase, IMVC, TSI etc.,

For the starch, lipid and protein hydrolysis test,starch, tributyrin, skim milk powder are added in the nutrient agar or composition mentioned in appendix section and checked for their respective activity.

Starch hydrolysis

The degradation of starch molecule by amylase to shorter polysaccharides maltose and dextrin. Overnight grown cultures were streaked onto the starch agar and incubated at different temperature according to the optimum growth of the different bacteria for 24 or 48h. Pour potassium iodide solution or gram's iodine solution over the colony and observe it under the light. Observing a zone of clearance against the dark blue background is the positive and no clearance zone around the colony is negative for the starch hydrolysis test.

Lipid hydrolysis

The degradation or hydrolysis of lipid molecule by lipase to shorter fatty acid molecule and glucerol or alcohol. Overnight grown cultures were streaked onto the tributyrin agar and incubated at different temperature according to the optimum growth of the different bacteria

for 24 to 48h. observe it under the light. Observing a zone of clearance around the colony is considered as positive and no clearance zone around the colony is negative for the lipid hydrolysis test.

Protein hydrolysis

The degradation or hydrolysis of high molecular weight protein molecule by protease to shorter peptides. Overnight grown cultures were streaked onto the skim milk or casein agar and incubated at different temperature according to the optimum growth of the different bacteriafor 24 to 48h observe it under the light. Observing a zone of clearance around the colony is considered as positive and no clearance zone around the colony is negative for the lipid hydrolysis test.

Carbohydrate fermentation test

Bacteria obtain their energy through series of enzymatic reactions by majority of cases oxidation of carbohydrate substrates. Some bacteria utilize sugars either in aerobic respiration or through fermentation pathway. Whereas the facultative anaerobes use both pathways. Some of the bacteria do not use sugar also. Bacteria can be differentiated based on the carbohydrate fermentation for many types of sugars. Inoculate overnight grown fresh cultures into the carbohydrate fermentation broth incorporated individually with various sugar. Incubate at various temperature .according to the requirement of bacteria and incubate for 24h to 48h. Observe it for the characteristic colour change.

Oxidase test

During aerobic respiration, oxidase enzymes (intracellular cytochrome) catalyzes the oxidation of reduced cytochrome by molecular oxygen which results in the formation of H20 or H_2O_2 depending on the type of enzyme system they possess. Oxidase activity was found in the aerobic, facultative anaerobes and microaerophiles. Obligate anaerobes were negative for the oxidase activity. In general, Gram positive organism was oxidase negative with exception of Bacillaceae and Gram negative in exception to the Enterobacteriaceae were found in majority of the cases.

Principle

Determination of ability of bacteria to produce cytochrome oxidases. This is confirmed by the oxidization of light pink substrat (p-aminodimethyl alaniline oxalate) as electron donors and the substrate is oxidized to the blackish compound in the presence of free oxygen and oxidase enzyme.

Method

- Prepare for the young culture in TSA slant or plate
- Add directly the substrate containing solution as 1% or 0.5% on the colony or pour the solution on to the Whatman filter paper No.1 and pick a colony of the young culture and streak onto the filter paper loaded with substrate.

Observation

- Dark pink, maroon, finally black or purple colour development denotes positive for oxidase test. No colour change or light pink indicates negative for oxidase test. The result should be read within 10 to 30 seconds.

Catalase test

In aerobic respiration the bacteria produce hydrogen peroxide and toxic superoxide. Accumulation of these toxic compound result in death of cell. In order to avoid this the bacteria, produce catalase to rapidly degrade hydrogen peroxide. Superoxide dismutase is the enzyme used for the degradation of the toxic superoxide. So catalase production can be determined by the addition of 3% H2O2 and observe for the bubbles of free oxygen as gas in the slide. Keep three drops of 3% H2O2 and add a minute quantum of culture picked out from individual isolated colony or drop H2O2 on to the colony and observe for bubbling or foaming.