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Course: MCB 202

1. Biochemical Reactions of Bacteria

There are several biochemical reactions of bacteria and they include;

Carbohydrate Fermentation test

Indole production test

Methyl Red test

Voges- Proskauer test.

Citrate utilization test

Nitrate Reduction test

Urease Test.

Oxidase test

Catalase test

Starch hydrolysis.

A. Carbohydrate Fermentation test

Sugars bare metabolized through different metabolic pathways depending on the types of microbial species and aerobic or anaerobic environment. If fermenting bacteria are grown in a liquid culture medium containing the carbohydrate, they may produce organic acids as by products of the fermentation. These acids are released into the medium and so lower pH of the medium.

B. Indole Production Test.

The aim of this reaction is to determine the ability of microbes to degrade the amino acid tryptophan to produce indole. Tryptophan is broken down by an enzyme tryptophanase to produce indole, pyruvic acid and Ammonia.

C. Methyl Red Test.

The purpose of this test is to differentiate between E.coli and E.aerogen and to determine the ability of microbes to oxidize glucose with production and stabilization of high content of acid

end product.

D. Voges- Proskauer Test.

This test determines the capability of son organisms to produce non-acidic or neutral end products such as acetoin, from the organic acid that results from glucose metabolism. This test identifies bacteria that ferment glucose and producing 2,3-butanediol accumulation in the medium.

E. Citrate Utilization Test.

This test is based on the ability of microbes to ferment citrate as sole carbon source. Presence of citrate permease facilitates transport of citrate into the bacterium. When bacteria oxidize citrate, they remove it from the medium and liberate carbon dioxide. Carbon dioxide combines with sodium(supplied by sodium citrate) and water to form sodium carbonate- an alkaline product.

F. Nitrate Reduction Test.

Certain organisms like chemolitho autotrophic bacteria and many chemoorganoheterotrophs can use nitrate as a terminal electron acceptor during anaerobic respiration. In this process, nitrate is reduced to nitrite by nitrate reductase, it is then further reduced to ammonium ion.

G. Urease Test.

This test is used to determine the ability of microbes to degrade urea by ureas to yield ammonia. The presence of grease is detected, when the organisms are grown in urea broth. It is mainly used for identification or Proteus spp from other genus of lactose non fermenting enteric organisms.

H. Oxidase Test.

Oxidase enzyme plays a key role in electron transport chain during aerobic respiration. Aerobic as well as some facultative anaerobes and microaerophilic bacteria show oxidase activity.

I. Catalase test.

Certain organisms produce hydrogen peroxide during aerobic respiration and sometimes vextremely toxic superoxide radicals. A bacterium must be able to protect itself against such oxygen byproducts or it will be killed. Obligate aerobes and facultative anaerobes usually contain the enzymes superoxide dismutase, which catalyzes the destruction of superoxide. Catalase production and activity can be detected by adding the substrate hydrogen peroxide to an appropriately incubated tryptic soy agar slant culture. If catalse is produced by the bacteria they will liberate free oxygen gas on reaction.

J. Starch Hydrolysis.

Many bacteria produce enzymes called hydolases. Hydrolases catalyze the splitting of organic

molecules into smaller molecules in the presence of water. The starch molecule consists of two constituents.

Amylose- an unbranched glucose polymer

Amylopectin- a large branched polymer.

They are both rapidly hydrolyzed by certain bacteria.

2. Identification/Staining Techniques of Fungi.

Direct microscopic examination without stain lacks sensitivity especially when hyphae are sparse in the specimen. A variety of differential stain are commonly used like Gram, Giemsa, Wright stain, toludine blue O and Weigert's iron hematoxylin to stain fungi. The sensitivity of microscopic examination is improved when fungus enhancing stains like Mayer's mucicarmine, periodic acid Schiff, Gomori's methenamine silver, acridine orange fluorescent, calcoflour white, thiosemicarbazide, Fontana Mass on Fluorescent and Gridley's stains are used.

The method of preparation of smear for staining is as follows.

A. Take a clean grease free glass slide.

B. Place a large drop of Saline solution.

C. Transfer a small quantity of the culture with a loop or the tip of a scalpel into the saline drop.

D. Make a smear over the surface of the slide.

E. Fix by heat, if necessary.

Wright Staining.

It is an alcoholic solution of methylene blue, azure A, thionin and eosin Y. Methyl group are activated and react with charged components of the cell to produce coloration.

Steps

1. Cover the smear with freshly filtered weight stain and leave for 1-3 minute.

2. Without removing the stain,pour on buffer solution (pH 6.4)

3. Gently mix buffer and stain; upon proper mixing,metallic green sheen rises to the surface of the fluid.

4. Leave for 3 minute or longer

5. Wash the slide gently with flowing tap water and wipe bottom of the slide with a clean filter paper.

6. Air-dry the slide and observe under the microscope.

7. Intracellular yeast cells are typically stained blue and specie of pneumocystis stain purple.

Giemsa Staining.

Steps

1. Flood the smear with methyl alcohol and leave for 3-5 minute for fixation.

2. Add prepared Giemsa stain and leave for 45minute

3. Wash slide thoroughly with running tap water

4. Blot dry with absorbent paper.

5. Look fo intracellular budding yeast; fungi stain with purplish-blue.

Reference

J.Sangeetha and D. Thangadurai, staining techniques and biochemical methods for the identification of Fungi.

Prescott's Microbiology.