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Dept: biochemistry

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Assignment on Mcb 202

1. Explain stey by step at least ten biochemical reaction of bacteria
2. Explain the identification/ staining technique of fungi

A. STARCH HYDROLYSIS

Starch is a polysaccharide which appears as a branched polymer of the simple sugar glucose. This means that starch is really a series of glucose molecules hooked together to form a long chain. Additional glucose molecules then branch off of this chain

ORGANISMS: Trypticase Soy broth cultures of Bacillus subtilis and Escherichia coli.

PROCEDURE

1. Using a wax marker, draw a line on the bottom of a Starch agar plate so as to divide the plate in half. Label one half B. subtilis and the other half E. coli.

2. Make a single streak line with the appropriate organism on the corresponding half of the plate

3. Incubate upside down and stacked in the petri plate holder on the shelf of the 37°C incubator corresponding to your lab section until the next lab period.

4. Next period, iodine will be added to see if the starch remains in the agar or has been hydrolyzed by the exoenzyme diastase. Iodine reacts with starch to produce a dark brown or blue/black color. If starch has been hydrolyzed there will be a clear zone around the bacteria growth .

B. PROTEIN HYDROLYSIS

Proteins are made up of various amino acids linked together in long chains by means of peptide bonds. Many bacteria can hydrolyze a variety of proteins into peptides (short chains of amino acids) and eventually into individual amino acids. They can then use these amino acids to synthesize their own proteins and other cellular molecules or to obtain energy.

ORGANISMS: Trypticase Soy broth cultures of Bacillus subtilis and Escherichia coli.

PROCEDURE (to be done in pairs)

1. Divide the Skim Milk agar plate in half and inoculate one half with Bacillus subtilis and

the other half with Escherichia coli as done above with the above starch agar plate

2. Incubate upside down and stacked in the petri plate holder on the shelf of the 37°C incubator corresponding to your lab section until the next lab period. If casein is hydrolyzed, there will be a clear zone around the bacterial growth.If casein is not hydrolyzed, the agar will remain white and opaque

C. FERMENTATION OF CARBOHYDRATES

Carbohydrates are complex chemical substrates which serve as energy sources when broken down by bacteria and other cells. They are composed of carbon, hydrogen, and oxygen (with hydrogen and oxygen being in the same ratio as water; [CH2O]) and are usually classed as either sugars or starches Facultative anaerobic and anaerobic bacteria are capable of fermentation, an anaerobic process during which carbohydrates are broken down for energy production.

ORGANISMS: Trypticase Soy agar cultures of Bacillus subtilis, Escherichia coli, and Staphylococcus aureus.

PROCEDURE

1. Label each tube with the name of the sugar in the tube and the name of the

bacterium you are growing.

2. Inoculate one Phenol Red Lactose broth tube and one Phenol Red Maltose broth tube

with Bacillus subtilis.

3. Inoculate a second Phenol Red Lactose broth tube and a second Phenol Red Maltose

broth tube with Escherichia coli.

4. Inoculate a third Phenol Red Lactose broth tube and a third Phenol Red Maltose broth

tube with Staphylococcus aureus.

5. Incubate the tubes in your test tube rack on your shelf of the 37°C incubator

corresponding to your lab section until the next lab period

D. INDOLE AND HYDROGEN SULFIDE PRODUCTION

Sometimes we look for the production of products produced by only a few bacteria. As an example, some bacteria use the enzyme tryptophanase to convert the amino acid tryptophan into molecules of indole, pyruvic acid and ammonia. Since only a few bacteria contain tryptophanase, the formation of indole from a tryptophan substrate can be another useful diagnostic tool for the identification of an organism. Indole production is a key test for the identification of Escherichia coli.By adding Kovac's reagent to the medium after incubation we can determine if indole was produced. Kovac's reagent will react with the indole and turn red

ORGANISMS: Trypticase Soy agar cultures of Proteus mirabilis, Escherichia coli, and Enterobacter cloacae.

PROCEDURE

1. Stab one SIM medium tube with Proteus mirabilis.

2. Stab a second SIM medium tube with Escherichia coli.

3. Stab a third SIM medium tube with Enterobacter cloacae.

4 . Incubate the tubes in your test tube rack on your shelf of the 37°C incubator

corresponding to your lab section until the next lab period

5. Next lab period add Kovac's reagent to each tube to detect indole production.

E. CATALASE ACTIVITY

Catalase is the name of an enzyme found in most bacteria which initiates the breakdown of hydrogen peroxide (H2O2) into water (H2O) and free oxygen (O2).During the normal process of aerobic respiration, hydrogen ions (H+)are given off and must be removed by the cell. The electron transport chain takes these hydrogen ions and combines them with half a molecule of oxygen (an oxygen atom) to form water (H2O).During the process, energy is given off and is trapped and stored in ATP.

MATERIALS: Trypticase Soy agar cultures of Staphylococcus aureus and Streptococcus lactis, 3% hydrogen peroxide.

PROCEDURE

Add a few drops of 3% hydrogen peroxide to each culture and look for the release of oxygen as a result of hydrogen peroxide breakdown. This appears as foaming.

F ) Catalase test:

1. Nutrient agar medium was preparedThe medium was poured into culture tubes and flasks
2. It was sterilized by autoclaving at 151b pressure for 15 minutes
3. The nutrient agar slants were inoculated with test organisms
4. An inoculated nutrient agar slant was kept as control
5. The cultures were incubated at 35°C and 3-4 drops ofhydrogen peroxide was addeon the growth of each slant culture
6. The culture was observed for the appearance

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1- Lactophenol blue stain

Use of a ready-to-use lactophenol blue solution enables the specimen to be stained in a single step. The fungi are stained dark blue and stand out well

against the light blue background.

2- Fungi are very easily visualized in a PAS reaction with Schiff's reagent, this method now being a standard

3- PAS and methenamine silver staining for histological specimens

When histological material is examined for fungi, the procedure may likewise be a PAS reaction or a silver staining method. In the case of the PAS

reaction the fungi are turned bright red in the tissue. Among the silver staining methods, the Gomori methenamine silver (GMS) stain is the method of

choice. The fungi are seen as brown to black on a light green background that is created by counter¬staining with Light Green SF. The methenamine

silver stain is a tried and tested method that is simply, safely and reliably performed with a kit containing ready-to-use reagents or suitably prepared reagents.