MCB 202

(a) Starch hydrolysis: Some bacteria are capable of using starch as a source of carbohydrate by hydrolysing or breaking down the starch so it may enter the cell

Procedure

=> Using a sterile technique make a single streak inoculation of the organism to be tested into the centre of labelled plate.

=> Incubate the material inoculated plates for 48 hours at

=> After incubation flood the surface of the plates with iodine solution with a dropper for 30 seconds

=> Pour off the excess iodine

=> Examine for the clear zone around the line of bacterial growth

(b) Protein hydrolysis: Many bacteria can hydrolyze a variety of proteins into peptides and eventually into individual amino acids.

Procedure

=> Inoculate the organism on the plate either a straight line or zigzag

=> Inoculate the plate at 25°C or 37°C

=> Following incubation, examine the plate for zone of hydrolysis

(c) Action on simple carbohydrate: The pattern of utilization by bacteria is specific and can be used

and gas.

Procedure

=> Sterlize the media setup

=> Innoculate each indicator sugar broth with each of the bacteria

=> leave a tube in innoculate as control

=> Incubate tubes at 35° C for 2 or 7 days and watch dai

d Catalase Productim: Most aerobic bacteria are capable of producing the enzyme. catalase acting to different extents. others like the obligate anaerobes are catalase negatives

Procedure:

=> innoculate the bacteria onto agar plate and leave an innoculated agar to serve as control

=> Incubate for 24 hours then add several drops of hydrogen peroxide

=> Presence of effervescence indicates the production of catalase by the bacterium

e Action on litmus milk: To distinguish among the metabolic changes in milk, a ph indicator the oxidation reduction indicator litmus is incorporated into the medium

Procedures

=> Inoculate with 4 drops of 24 hour old broth culture

=> Incubate at 35°C to 37°C in ambient air

=> Observe daily for seven days for alkaline reaction indicated reduction in acid dof.

f Hydrogen Sulphite production: H_2S is produced by a certain bacterium following the decomposition of organic Sulphur compounds such as Cysteine, methionine through the reduction of organic Sulphur compounds

Procedure

=) Stab the medium in the tube with a heavy innoculum
=) leave a tube uninoculated as control and incubate at 37°C for 7 days and examine daily
=) A black line along the line of innoculation indicates production of H_2S

G: Lipid Hydrolysis: In order to utilise fats, bacterial cells secret exoenzymes known as lipases outside of the cell that hydrolyse the lipid to fatty acid and glycerol. the bacteria capable of these are called lipolytic bacteria

Procedure

=) Inoculate the tributyrin agar medium with single streaking of the organism.
=) Incubate anaerobically in a gas pack jar immediately after streaking and transfer into the incubator maintained at 35°C - 37°C for 24 to 48 hours
=) Observe for the clear zone around the bacterial growth

th Coagulase Test

H Citrate Test: This is used to test an organism ability to utilize citrate as a source of energy

Procedure

f Streak the slant back and forth with a light inoculum

=> Inoculate with growth from the 18-24 culture by starch inoculation with a needle
=> Incubate at a temperature and duration appropriate for the organism being tested.
=> Examine tubes for growth and signs of motility.

J Indole test: This test is used to determine the ability of an organism to split amino acid tryptophan to form the compound indole. It helps to differentiate ~~intestinal~~ bacteria and other gonora
Procedure
=> Inoculate the tryptophan broth with broth culture or emulsify isolated colony of the test organism in tryptophan broth.
=> Incubate at 37°C for 24-28 hours in ambient air
=> Add 0.5ml of Kovac's reagent to the broth culture
=> Examine for a pink coloured ring after addition of the reagent

K Coagulase test
Procedure:
=> Place a drop of physiological saline on two separate slides
=> With the loop, straight wire or wooden stick, emulsify a portion of the isolated colony in each drop to make two thick suspension
=> Add a human or rabbit plasma to one of the suspension and mix
=> Look for clumping of the organisms within 10 seconds

② Explain the identification/staining techniques of fungi

1) Gram staining: Gram staining is a key starting point to identify microbial species. Gram staining differentiates membrane structure between gram positive and gram-negative microorganism. Gram-positive microbes have a thick cell wall made up of peptidoglycan (50-90%) which are stained purple by crystal violet dye as gram negative microbes have a thin peptidoglycan layer (10%) which are stained pink by the counter stain safranin

Procedure

=> Apply two drops of crystal violet on smear for 30 second
=> Wash with distilled water
=> Add two drops of Gram's iodine for 30 second
=> wash with distilled water
=> Add 95% ethanol
=> wash with distilled water.
=> Add two drops of safranin
=> wash with distilled water.

ii) India ink WET MOUNT: India ink can be added to form background that will highlight unstained yeast cells and capsular interval. This method is used to detect microbes surrounds by capsule

iii) LACTOPHENOL COTTON BLUE WET MOUNT: (LCB) is a mounting medium commonly used in microbiology laboratories for preparing mounts of fungal cultures LCB used as both mounting fluid and stain.

Gram-negative microorganism: Gram-positive mic... have a thick cell wall made up of peptidoglycan (G... which are stained purple by crystal violet whereas gram negative microbes have a thin layer (10%) wh... are stained pink by the counter = Stain safranin

Procedure

=> Apply two drops of crystal violet on smear for 30 second
=> Wash with distilled water
=> Add two drops of Grams iodine for 30 second
=> wash with distilled water
=> Add 95% ethanol
=> wash with distilled water.
=> Add two drops of safranin
=> wash with distilled water.

(ii) India INK WET MOUNT: India ink can be added to fixed to provide dark background that will highlight negative yeast cells and capsular interval. This method is used to detect microbes surrounds by capsule

iii) LACTOPHENOL COTTON BLUE WET MOUNT: (LCB) is a mounting medium commonly used in microbiology laboratories for preparing mounts of fungal cultures LCB used as both mounting fluid and stain.

iv) GIEMSA STAINING: Giemsa stain is a mixture of the Romanowsky group of stains, which are defined as being the blade precipitate formed from the addition of methanol

V WRIGHT STAINING: The wright stain is an alcoholic solution of methylene blue, azure A, throm and contains. It is used to detect blood parasites, viral and chlymydial inclusion bodies, Yeast cells and species of Pneumocystis

VI GROCOTT-GOMORI METHENAMINE SILVER STAINING: The fungal cellwall contains mucopolysaccharides that are oxidised by GMS to release aldehyde groups which later react with silver nitrate.