18/SCI03/008

1a) Starch hydrolysis: Some bacteria are capable of using starch as a source of carbohydrate by hydrolyzing 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 center of a labeled plate.
* Incubate the bacterial inoculated plates for 48 hours at 37◦C.
* After incubation, flood the surface of the plate 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. They can then use these amino acids to synthesize their own proteins and other cellular molecules or to obtain energy. This is a process called PROTEOLYSIS.

Procedures

* Inoculate the organism on the plate either on a straight line or a zigzag pattern.
* Incubate the plate at 25◦C or 37◦C.
* Following incubation, examine the plate for zone of hydrolysis

c) Action on simple sugars: The pattern of utilization by the bacteria is specific and can be used to characterize the bacteria by the production of acid alone or both acid and gas.

Procedure

* Sterilize the media set up.
* Inoculate each indicator sugar broth with each of the bacteria.
* Leave a tube uninoculated as control.
* Incubate the tubes at 37◦C for 2 to 7 days while watching each day.

d) Hydrogen sulfide production: hydrogen sulfide is produced by a certain bacteria following the decomposition of organic sulfur compounds such as cysteine, methionine, or through the reduction of inorganic sulfur compounds.

Procedures

* Stab the medium in the tube with a heavy inoculum.
* Leave a tube uninoculated as control and incubate at 37◦C for 7 days while watching daily.
* A black line along the line of inoculation indicates production of hydrogen sulfide.

e) Catalase production: Most aerobic bacteria are capable of producing the enzyme catalase although to different extents. Others like the obligate anaerobes are catalase negative.

Procedures

* Inoculate the bacteria onto an agar plate and leave an uninoculated 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.

f) Action on litmus milk: To distinguish among the metabolic changes produced in milk, a pH indicator, the oxidation reduction indication indicator litmus is incorporated into the medium. Litmus milk then forms an excellent differential medium in which the microorganisms can metabolize milk substrates depending on their enzymatic compounds.

Procedures

* Inoculate with 4 drops of a 24 hour old broth culture.
* Incubate at 35◦C to 37◦C in ambient air.
* Observe daily for seven days for alkaline reaction, indicator reduction, acid clot, acid reaction, rennet clot and peptonization.

g) Lipid hydrolysis: In order to utilize fats, bacterial cells secrete exoenzymes known as LIPASES outside of the cell that hydrolyze the lipid to fatty acids and glycerol. The bacteria capable of doing this are called LIPOLYTIC bacteria.

Procedures

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

h) Coagulese test: This is used to differentiate *Staphylococcus aureus* which produce the enzyme coagulase (bound and free) from *Staphylococcus epidermis* and *Staphylococcus saprophyticus* which do not produce coagulase.

Procedures

* 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 suspensions.
* Add a human or rabbit plasma to one of the suspensions and mix gently, the other suspension serves as control.
* Look for clumping of the organism within 10 seconds.

i) Urease test: Many organisms especially those that infect the urinary tract have an urease enzyme which is able to split urea in the presence of water to release ammonia and carbon dioxide. The mixture of the products form ammonium carbonate which turns the medium alkaline, turning the indicator phenol red from its original orange yellow color to bright pink.

Procedures

* Using stuart’s urea broth, inoculate with a heavy inoculums from an 18 to 24 hour old pure culture.
* Shake the tube gently to suspend the bacteria.
* Incubate the tubes with loosened caps at 35◦C.
* Observe the broth for a color change at 8, 12, and 24 hours.

j) Mobility test: This is used to detect motility of microorganisms. Motility is apparent by the presence of diffuse growth away from the line of inoculation.

Procedures

* Inoculate with growth from an 18 to 24 hour old culture by stab inoculation with a needle.
* Incubate at a temperature and duration appropriate for the organism being tested.
* Examine tubes for growth and signs of motility.

k) 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 *Enterobacteriaceae* and other genera.

Procedures

* Inoculate the tryptophan broth with broth culture or emulsify isolated colony of the test organism in tryptophan broth.
* Incubate at 35◦C for 24 to 28 hours in ambient air.
* Add 0.5 ml of Kovac’s reagent to the broth culture.
* Examine for a pink colored rink after the addition of the reagent.

l) Citrate test: This is used to test an organism’s ability to utilize citrate as a source of energy.

Procedures

* Streak the slant back and forth with light inoculums picked from the center of a well isolated colony.
* Incubate aerobically at 35◦C to 37◦C for 4 to 7 days.
* Observe for a color change from green to blue along the slant.

2a) Gram staining: This is a key starting point o identify microbial species. The stain differentiates membrane structures between gram-positive and gram-negative microorganisms. Gram-positive microbes have a thick cell wall made up of peptidoglycans (50-90%) which are stained purple by crystal violet, whereas, gram-negative microbes have a thinner layer (10%) which are stained pin by the counter stain safranin.

Procedures

* Apply two drops of crystal violet on smear for 30 seconds.
* Wash with water.
* Add two drops of gram’s iodine for 60 seconds.
* Wash with water.
* Add 95% ethanol for 10 to 15 seconds.
* Wash with water.
* Leave to dry.
* Observe in microscope under oil immersion.

b) Giemsa staining: Giemsa stain is a member of the Romanowsky group of stains, which are defined as being the black precipitate formed from the addition of methanol. In this stain, eosin ions are negatively charged and stain basic components of cells orange to pink.

Procedures

* Flood the smear with methyl alcohol and leave for 3 to 5 minutes for fixation.
* Add prepared giemsa stain and leave for 45 minutes.
* Wash slide thoroughly with running tap water.
* Blot dry with absorbent paper.
* Observe under oil immersion and look for intracellular budding yeasts; fungi stain with purplish blue.

c) Wright staining: The wright stain is an alcoholic solution of methylene blue, azure A, thionin, eosin Y. methyl groups are activated and react with charged components of the cell to produce coloration. It is used to detect blood parasites, viral and chlamydial inclusion bodies, yeast cells and species of *Pneumocystis*. Eosin ions are negatively charged and stain basic components of cells orange to pink, whereas, other dyes stain acidic cell structures to various shades of blue to purple.

d) Weigert’s iron hematoxylin staining: This stain can be used with fixatives that include poly vinyl alcohol, sodium acetate, and formalin. The staining method involves application of haemalum, which is a complex formed from aluminum ions and oxidized hematoxylin, this stains nuclei of cells blue. Counter stain eosin Y may also be used to color other structures in various shades of red, pink, and orange.

Procedures

* Add staining solution on a smear and leave for 1 to 2 hours, then, rinse with water.
* Add 1% hydrochloric acid.
* Add 70% ethanol.
* Rinse with water.
* Counterstain with eosin Y.
* Dehydrate with ethanol.
* Clear with xylene and observe under microscope.
* Yeast cells stain blue-gray to black.

e) Acridine orange staining: Acridine orange is a fluorochromatic dye that binds to nucleic acids of fungi. Under U.V. light, acridine orange stains R.N.A. and single stranded D.N.A. orange, while double stranded D.N.A. appears green. At neutral pH, fungi and cellular material stain reddish-orange. At acidic pH, fungi remain reddish-orange but background material stains greenish-yellow.

f) Gridley staining: This method is used to identify fungi, based on Bauer chromic acid leucofuchsin stain with the addition of Gomori’s aldehyde fuchsin stain and metanil yellow as counter stains. Against a yellow background, hyphae, conidia, yeast capsules, elastin, and mucin appear in different shades of blue to purple, it can be used to identify *Rhiosparidium seeberi* and *Histoplasma capsulatum.*

g) Calcoflour white staining: This stain is used to detect fungal elements, particularly *Pneumocystis* species. The fluorophore shows a high affinity to chitin forming hydrogen bonds with free hydroxyl groups and stain fungal cell wall blue. The use of calcoflour white staining requires the addition of KOH, which helps dissolve keratinized particles and emulsify solid, viscous material and enhance the visualization of fungal elements in microscopic examination. Positive results are indicated by a bright green to blue fluorescence using fluorescence microscope. A bright yellow-green fluorescence is observed when collagen or elastin is present. This stain can be used to identify *Fusarium solani, Aspergillus fumigatus,* and *Candida albicans.*

h) Meiyer’s mucicarmine staining: Mucicarmine is a red stain that contains aluminum chloride and carmine. Aluminum is believed to form a chelation complex with the carmine and change the molecule to a positive charge, allowing it to bind with the acid substrates of low density, such as mucins. It is used to detect mucin secreting fungi and capsules of *Cryptococcus neoformans* and *Rhinosporandium seeberi.* It will also stain the walls of the spores and the inner surface of the sporangia. However, the cell walls of yeast and *Blastomyces dermatitis* may stain weakly with mucicarmine.

i) Periodic acid Schiff staining: These are effective stains for demonstrating fungal elements of essentially all fungi. Periodic acid attacks some carbohydrates containing 1, 2-glycol or OH group with the conversion of this group to 1, 2-aldehydes, which then reacts with the fuchsin-sulfurous acid to form the magneta color. Identification of fungal elements can be enhanced if a counter stain such as light green is used. Species of *Coccidioides, Cryptococcus, Histoplasma, Candida, Malassezia* and *Aspergillus* can be stained with this stain.

j) Toluidine blue O staining: This stain is primarily used for the detection of *Candida albicans, Rhinosporidium seeberi* and *Pneumocystis carinii.* Background staining is removed by sulfation reagent. Yeast cells get stained differentially and are difficult to distinguish from *Pneumocystis* cell. The stain can be replaced with specific fluorescent stains. Toluidine blue O gives polychromatic staining for all the fungal structures (such as conidia, germ tubes, haustoria, and hyphae) as well as cells.

k) Double oxidation thiosemicarbazide schomrl: The hydrazine group (H2NNH-) combines with any aldehyde generated by periodic acid oxidation. The thiocarbamyl (-CSNH2) is a more powerful reducing agent than aldehydes and rapidly reduces ferricyanide to ferrocyanide, which immediately forms a Prussian blue deposit at the site. The Mallory bleach lightens background staining and improves contrast. It may also produce some aldehyde, which is removed by washing with water. This method is widely used to identify fungal colonies in tissues.

l) Grocott-gomori methenamine silver staining: This method is preferred for screening degenerated and non viable fungi because it provides better contrast. The fungal cell wall contains mucopolysaccharides that are oxidized by GMS to release aldehyde groups, which latter react with silver nitrate. Silver nitrate is converted to metallic silver which becomes visible in the silver stains; this is useful in detecting fungal elements. Fungi stain in black against a pale green background. *Pneumocystis jiroveci, Cryptococcus neoformans, Coccidiodes immitis, Histoplasma capsulatum, Aspergillus fumigatus,* and *candida albicans* can be detected using this staining technique.

m) Fontana-masson staining: The fontana masson stain can be used to detect the presence of melanin in cell walls of dematiaceous fungi such as species of *Bipolaris ,Curvularia*, *Exophiala*, and *Phialophora*. This stain is often believed to be a diagnostic tool to differentiate dematiaceous fungi from *Aspergillus species* and some *Zygomycetes.* Also, it is particularly useful for distinguishing capsule deficient *Cryptococcus neoformans* from *Histoplasma capsulatum* and *Blastomyces dermatitis*. Melanin has the ability to reduce solutions of ammonical silver nitrate to metallic silver without the use of external reducing agents. The intensity and amount of staining may reflect differences in melanin deposition owing to grow rate, age, availability of precursors, or loss of pigment staining associated with hyphal death and destruction. Extent of stain intensity and its distribution in fungal elements in tissues were evaluated by means of intensity; for example, dark brown (strong intensity), medium brown (moderate intensity), and pale brown (weak intensity).