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18/MHS06/029
MEDICAL LABORATORY SCIENCE
BIOLOGICAL AND BIOCHEMICAL REACTIONS OF MICROORGANISMS
GENERAL MICROBIOLOGY
MLB 202

QUESTION:
Explain the (step by step) of at least ten (10) biochemical reactions of bacteria.

ANSWER:
BIOCHEMICAL REACTION OF BACTERIA.

(1) UREASE TEST: this test is used to determine the ability of an organism to produce urease which hydrolyses urea; this is done to identify proteus, Morganella, Yersinia enterocolitica and Helicobacter Pylori.

(2) Catalase Test: To differentiate Staphylococci (Catalase positive) from Streptococci (Catalase test negative).

3. DNase Test: This test is used to determine the ability of an organism to hydrolyse DNA. It is primarily used to identify Staphylococcus aureus.

4. Bile solubility test: to differentiate Streptococcus pneumoniae from other alpha hemolytic Streptococci. Bile or a solution of bile salt, such as Sodium deoxycholate rapidly lyses pneumococcal colonies.

5. CAMP Test: Certain organisms such as Streptococcus agalactiae (Group B Streptococci), produce a diffusible extracellular protein (CAMP) factor that acts synergistically with the beta-lysin of Staphylococcus aureus and causes enhanced lysis of RBCs.

6. Indole Test: this test is used to determine the ability of an organism to split tryptophan to form the compound indole.

It is used differentiate gram negative rods particularly E-coli in microbiology laboratory

7. Lysine Decarboxylase test: To assist in the identification of Salmonella and Shigella

8) Bacitracin Sensitivity Test: Bacitracin Sensitivity test differentiate Streptococcus

9. Beta-glucuronidase test (GUS Test): To identify Escherichia coli. Escherichia coli produce the enzyme β-D-glucuronidase, which hydrolyse β-D-glucopyranosid-uronic derivative to aglycons and D-glucuronic acid.

10 Litmus milk decolorization test: To help identify Enterococcus and some Clostridia which have ability to metabolize litmus milk.

9) Explain the identification staining technique of fungi

Morphological studies supplemented with staining techniques and biochemical methods, still plays an important role in the overall identification of fungi in the molecular era

GRAM-STAINING.

this staining technique easily divides bacteria into two groups; Gram positive and gram negative. On the basis of their cell wall and cell membrane permeability.

Gram staining is used to directly examine specimen submitted for microbiologic examination.

GRAM-positive ~~BACTERIA~~ CELL WALL

Gram-positive Bacteria have a thick mesh-like cell wall which is made up of peptidoglycan (90% of cell wall) which stains purple. (peptidoglycan is mainly a polysaccharide composed of two subunit called N-acetyl glucosamine and N-acetyl muramic

acid. As adjacent layers of peptidoglycan are formed, they are cross-linked by short chains of peptides by means of transpeptidase enzyme resulting in the shape and rigidity of the cell wall. The thick peptidoglycan layer of Gram positive organisms allows these organisms to retain the CV-Iodine complex and stains the cells as purple

Gram negative cell wall

Gram-negative have a thinner layer of peptidoglycan (10%) and lose the CV-Iodine complex during decolorization with the alcohol rinse but retain the counter stain safranin thus appearing reddish or pink, they also have an additional outer membrane which contains lipids, which is seperated from the cell wall by means of the periplasmic space

Steps of Gram stain

(1) Application of the primary stain to a heat fixed smear of the fungi culture (CV) dissociate into aqueous solution CV+ and CV- ions. these two ions then penetrate through the cell wall and cell membrane of both gram (+) and gram (-) cells. the CV+ ions later interact with negatively charged bacterial components and stains the bacteria cells purple.

(4) Addition of Gram iodine: Acts as a mordant and as a trapping agent (this mordant increase affinity of the cell walls for a stain by binding to the primary stain thus forming an insoluble complex which get trapped in the wall

(5) Decolorization with 95% ethyl Alcohol: this dissolves the lipid outer membrane of Gram (-) bacteria which increases the porosity of the cell then leaving the peptidoglycan layer exposed. this step must be performed carefully otherwise decolorization may occur. If decolorization agent is applied on the cell for a long time, the Gram (+) organism to appear Gram (-)

Counter Stain with Safranin

the decolorized Gram (-) cells can be rendered visible with a suitable counter-stain which is usually positively charged Safranin which stains them pink. pink colour which stain the Gram (+) is masked by the purple of the G+.

Wet Mount Techniques. is a technique used on identification of fungal specimen through suspension of culture in either water or saline mixed with alkali to dissolve background material or mixed with a combination of alkali and contrasting dye (eg lactophenol cotton blue or India ink) the dyes nonspecifically stain the fungal material which increases contrast with the background and permits examination of the detailed structure.

- Potassium Hydroxide Wet mount
- Lactophenol cotton Blue Wet mount
- India Ink Wet mount

Staining Techniques

- Giemsa staining this is a member of the Romanowsky Group of stains which are defined as being the blue precipitate formed from the addition of methanol
- this stain is widely used to examine Pneumocystis Jirovecii, Rhinosporidium Seeberi and Histoplasma capsulatum
- flood the smear with methyl alcohol and leave for 3-5 min for fixation
- Add prepared Giemsa stain and leave for 45 min
- wash slide thoroughly with running tap water
- Blot dry with absorbent paper
- Observe under oil immersion
- Look for Intracellular budding yeast, Fungi stain with purplish blue

- WRIGHT STAINING: It is used to detect blood parasites, viral and chlamydial inclusion bodies, yeast cells and spores of

pneumocystis

- GROCOTT STAINING: It is used to identify fungi based on Schiff's chromic acid leucofuchsin stain with the addition of Gomori's aldehyde fuchsin stain and metanil yellow as counterstain.

- KLIGERIS MODIFIED HEMATOXYLIN STAINING: this stain can be used with fixatives that include polyvinyl alcohol, sodium acetate and formalin. this staining method involves application of heamalum which is a complex formed from aluminium ions and oxidized hematoxylin.

- CALCOFLUOR WHITE STAINING: this is used to detect fungal elements particularly pneumocystis species

- ACRIDINE ORANGE STAINING: it's a fluorochromatic dye that binds to nucleic acids of fungi. under UV light, acridine orange stains RNA and single stranded DNA orange while double stranded DNA appears green.

- PERIODIC ACID SCHIFF STAINING: PAS reactions are effective stains for demonstrating fungal elements of essentially all fungi

- MAYER'S MUCICARMINE STAINING: Mucicarmine is a red stain that contains aluminium chloride and carmine. Aluminium 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 substrate of low density such as mucins

- GROCOTT-GOMORI METHENAMINE SILVER STAINING: (GMS) staining is preferred for screening degenerated and nonviable fungi because it provides better contrast.

- Double oxidation Thiosemicarbazide: ($CH_2N_4H_4$) combines with any aldehyde generated by periodic acid oxidation. thiocarbonyl group ($-CSNH_2$) is a more powerful reducing agent than aldehydes and rapidly reduces ferricyanide to ferrocyanide which

immediately forms a prussian blue deposit at the site.

Fontana-masson staining: This can be used to detect the presence of melanin in cell walls of dematiaceous fungi such as species of Bipolaris, Curvularia, Exophiala and phialophora. It's often believed to be a diagnostic tool to differentiate dematiaceous fungi from Aspergillus sp. and some Zygomycetes

Toluidine Blue O staining: Its primarily used for the detection of Candida albicans, Rhinosporidium Seeberi and Pneumocystis Carinii.

Manual Biochemical method for identify groups of fungi are.

1) utilization of Carbon sources
2. Liquid auxanographic method (pour plate auxanographic)
3. utilization of nitrogen source
4. Carbohydrate fermentation
5 Casein Hydrolysis
6. Cycloheximide Resistance