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### ASSIGNMENT!!!

- 1.Explain step by step at least 10 biochemical reactions of bacteria.
- 2.Explain the identification/staining technique of fungi

### ANSWERS!!

#### **1.Morphology and Staining:**

Serve as preliminary criteria. The Gram stained smear shows the Gram reaction, size, shape, groupings of the bacteria and intracellular position of the endospore. Special staining reaction can reveal the presence of capsule.

Hanging drop wet preparation can be used to study the motility of bacteria. An unstained wet film is examined under dark ground illumination microscope to observe the exact morphology of delicate spirochaete. A smear is stained by Ziehl Neelsen method to demonstrate the acid fast staining reaction.

#### **2.Cultural Characteristics:**

The growth requirement and the appearance of colonies on media to the naked eye are further criteria to assist the identification of bacteria. A culture is a growth of bacterium on artificial nutrient medium or culture medium prepared in the laboratory.

Attempts are made to grow (to cultivate or culture) the bacteria on media of different compositions (glucose, inorganic salts mixture, meat extract or meat infusion with blood) incubated under a variety of conditions (different temperatures, pH) in the presence of atmospheric oxygen (aerobically).

The ability or inability to grow on medium containing a selective inhibitor (e.g. bile salt, optochin, tellurite, bacitracin, malachite green, low pH, high pH) may also be useful to identify the bacteria.

The growth of bacteria in liquid culture medium (e.g. nutrient broth) may show:

- A uniform turbidity;
- Little deposit at the bottom;
- Surface growth (pellicle formation)

#### **3.Biochemical Reactions:**

E.g. fermentation of various sugars (carbohydrates). Morphology and cultural characters may not be able to distinguish some species of bacteria; but these same species may exhibit distinct differences in their biochemical reactions e.g. typhoid and paratyphoid bacilli (glucose and mannitol are fermented without gas production by typhoid bacilli, whereas paratyphoid bacilli produce acid and gas).

Certain serotypes of the salmonella group may resemble one another in fermentation properties

The growth of the bacteria in liquid medium will ferment particular sugars (glucose, lactose,

mannitol) with the production of acid, which is detected by the changes of colour of Andrade's indicator dye incorporated in the medium; the gas production is detected by the collection of air bubble in a small inverted tube (Durham's tube) immersed in the medium.

Other tests are used to find out the ability of a bacterium to produce particular end products e.g. Indole, hydrogen sulphide, nitrite and certain enzymes (oxidase, catalase, urease, gelatinase, collagenase, lecithinase, lipase) in culture media.

#### **4. Antigenic Characters:**

Species or types of bacteria can be easily and distinctly identified by "specific" antibody reactions observed in serological tests performed on a glass slide. This specific antibody (antiserum) is obtained from the animal (rabbit) immunized against a particular type of microorganisms which agglutinates with the same antiserum.

An unknown bacterium may thus be identified by demonstrating its reaction with one out of a number of standard known antisera.

Similarly, the serum of a person suffering from a bacterial infection may contain specific antibody. The nature of the infection may thus be diagnosed by demonstrating that the patient's serum agglutinates one out of a number of known antigens of laboratory cultures, e.g. Widal test in Typhoid fever.

#### **5. Bacteriophage Sensitivity:**

A single bacterial pathogenic species may include different types of strains which are distinguishable in minor characters. Recognition of the type of a strain isolated from a patient may be of great importance in epidemiological studies related to the source and the spread of the infection in the community.

The typing of stains may be done by special biochemical or serological tests. Another important method of typing is by testing the susceptibility of the culture to lysis by each of a set of type specific, lytic bacteriophages.

#### **6. Animal Pathogen City:**

Final identification of a toxigenic strain of tetanus bacillus may be done by injecting the toxin liberated by tetanus bacillus into the base of the tail of two mice, one of them has already been protected by prior injection of specific antiserum to tetanus toxin (a soluble poisonous protein secreted by the tetanus bacilli).

The unprotected mouse shows the symptoms of tetanus, whereas the protected one without any tetanus symptoms identifies the culture, as an organism producing toxin, as the injected antiserum neutralized toxin liberated by tetanus bacilli. Similarly, diphtheria bacillus is also identified by inflammation and necrosis of the skin of guinea pig brought by diphtheria exotoxin.

#### **7. Antibiotic Sensitivity:**

The organism is tested for its ability to grow on artificial nutrient media containing different antibiotics and chemotherapeutic agents in different concentrations. In disk diffusion test, the culture to be examined is inoculated confluent with swabs over the surface of an agar plate and six to ten paper disks containing different antibiotics are placed in different areas of the plate.

Antibiotic diffuses outwards from each disk into surrounding agar.

# Detection of Fungi using Stains/Staining Reactions

Fungi, yeasts and molds are widespread throughout the environment. For healthy persons they are not a serious problem as long as certain standards of hygiene are maintained. If the immune system is compromised, however, through chronic illness or tumor, they may pose an infection risk or result in manifest illness. They can, for instance, infect the nails, hair, skin, lungs, kidneys or lymph nodes. Detecting them is an essential prelude to instituting targeted remedial measures. Detection of fungi with various stains is quick and simple, and can be optimized through the use of ready-to-use. Depending on the consistency of the specimen material, it may be necessary to perform a simple pre-treatment step with alkali prior to staining.

**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.

**PAS (Periodic Acid Schiff) reaction:** Fungi are very easily visualized in a PAS reaction with Schiff's reagent, this method now being a standard application. Schiff's reagent is available ready-to-use as an individual reagent or as part of the PAS Kit, which also contains the periodic acid required for the oxidation reaction. As Schiff's reagent/PAS Kit is stored at room temperature, no time is required to warm the solution, so the result is obtained more quickly. In the case of specimen materials that need alkali pre-treatment it is important to make sure that they are not allowed to react too long, otherwise they may take on a soup-like or gelatinous consistency. The pre-treated specimen should be neutralized with 10% lactic acid and adjusted to pH 3-5. The PAS reaction involves first oxidizing the specimen and then reacting it with Schiff's reagent until the fungal elements take on a bright red color.

**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.