METHOD OF IDENTIFICATION OF MICROORGANISM

A classification scheme provides a list of characteristics and a means for comparison to aid in the identification of an organism. Once organism is identified, it can be placed into a previously devised classification scheme. Microorganisms are identified for practical purposes for example, to determine an appropriate treatment for an infection. They are not necessarily identified by the same techniques by which they are classified. Most identification procedures are easily performed in a laboratory and used as few procedures or tests as possible. Protozoa, parasitic worms, and fungi can usually be identified microscopically. Most prokaryotic organism do not have distinguishing morphological features or even much variation in size and shape. Consequently, microbiologist has developed a variety of methods to test metabolic reaction and other characteristics to identify prokaryotes. Bergey's Manual of Determinative Bacteriology has been a widely used reference. Does not classify bacteria according to evolutionary relatedness but provide identification (determinative) schemes based on such criteria as cell wall composition, morphology, differential staining, oxygen requirements, and biochemical testing.

There are several criteria and methods for the classification of microorganism and the routine identification of some of those organism e.g properties of the organism, the source and habitat of the isolate.

MORPHOLOGICAL CHARACTERISTICS

Morphological (structural) characteristics have helped taxonomist classify organism for 200 years. Higher organisms are frequently classified according to observed anatomical detail. But many microorganisms look too similar to be classified by their structures. Through a microscope, organism that might differ in metabolic or physiological properties may look alike. Literally hundreds of bacterial species are small rods or small cocci. Larger size and the presence of intracellular structures does not always mean easy classification, however, pneumocystis pneumonia is the most common opportunistic infection in immunocompromised individualsand is a significant cause of death in AIDs patients .

Cell morphology tells us little about phylogenic relationships. However, morphological characteristics are still useful in identifying bacteria. For example, differences in such structure as endospores or flagella can be helpful.

The first step in identifying bacteria is **differential staining.** Most bacteria are either gram – positive (G+) or gram-negative (G-). Other differential stains, such as the acid-fast stain, can be useful for more limited group of microorganism. These stains are based on the chemical composition of the cell walls and therefore are not useful in identifying either the wall-less bacteria or the archaea with unusual walls. Microscopic examination of Gram stain or an acid-fast stain is used to obtain information quickly in the clinical environment.

BIOCHEMICAL TEST

Enzymatic activities are widely used to differentiate bacteria. Even closely related bacteria can usually be separated into distinct species by subjecting them to biochemical tests;, such as one to determine their ability to ferment an assortment of selected carbohydrate. Moreover, biochemical tests can provide insight into a species niche in the ecosystem. For example, a bacterium that fix nitrogen gas or oxidized elemental sulfur will provide important nutrients for plan and animals. Enteric, gram-negative (G-) bacteria are a large heterogeneous group of

microbes whose natural habitat is the intestinal tract of humans and other animals. This family contains several pathogens that cause diarrheal illness. A number of test have been developed so that technicians can quickly identify the pathogens, then a clinician can provide appropriate treatment, and epidemiologist can locate the source of an illness. All members of the family Enterobacteriaceae are oxidase-negative. Among the enteric bacteria are member of the genera *Escherichia, Enterobacter* and *Salmonella. Eschericha, Enterobacter,* and *Citrobacter,* which ferment lactose to produce acid and gas can be distinguished from *Salmonella and Shigella,* which do not.

In the clinical diagnosis of a disease, a particular species and even a particular strain must be identified in other to proceed with proper treatment. To this end, specific series of biochemical tests have been developed for fast identification in hospital laboratories. Rapid biochemical systems have been developed for yeast and other fungi, as well as bacteria.

TYPES OF SEROLOGICAL TEST

Serology: Is the science that studies blood serum and immune responses that are evident in serum. Microorganisms are antigenic; that is, microorganisms that enter an animals body stimulate it to form antibodies. Antibodies are proteins that circulate in the blood and combine in a highly specific way with the bacteria that caused their production. For example, the immune system of a rabbit injected with killed typhoid bacteria (antigens) responds by producing antibodies against typhoid bacteria. Solutions of such antibodies used in the identification of many medically important microorganisms are commercially available; such a solution is called an antiserum (**plural:** antisera). If an unknown bacterium is isolated from a patient, it can be tested against known antisera and often identified quickly.

In a procedure called a slide agglutination test, samples of an unknown bacterium are placed in a drop of saline on each of several slides. Then a different known antiserum is added to each sample. The bacteria agglutinate (clump) when mixed with antibodies that were produced in response to that species or strain of bacterium; a positive test is indicated by the presence of agglutination.

Serological testing can differentiate not only among microbial species but also among strain within species. E.g Rebecca Lancefied was able to classify serotypes of streptococci by studying serological reactions. She found that the different antigens in the cell walls of various serotypes of streptococci stimulate the formation of different antibodies. In contrast, because closely related bacteria also produce some of the same antigens, serological testing can be used to screen bacterial isolates for possible similarities. If an antiserum reacts with proteins from different bacterial species or strains, these bacteria can be tested further for relatedness.

MEDIA USED IN MICROBIOLOGY LABORATORY

There are different media used for microbiological isolation and identification of microorganisms. They are generally classified into five:

1. General purpose media.
2. Differential media.
3. Selective media.
4. Enriched media.
5. transport media.

Transport media

MECHANISM OF ACTION OF GENERAL PURPOSE MEDIA

Such a medium normally consists of a mixture of protein digest (peptone, triptone) and inorganic salts, hardened by addition of 1.5% agar. Examples of standard general purpose media that will support the growth of a wide variety of bacteria include nutrient agar are tryptic soy agar.

MECHANISM OF ACTION OF DIFFERENTIAL MEDIA

A media which differentiates or distinguishes between different types of microorganism based on differences in appearance of growth or color changes.

G. negative / positive Lactose / non lactose

1. Eosin Methylene Blue (EMB) Agar: Is selective and differential because bacteria that ferment lactose produce dark purple color or a metallic green sheen on the media.

2. Triple sugar ion agar (TSI): Is a differential medium that contains lactose, sucrose, a small amount of glucose (dextrose), ferrous sulfate and the pH indicator (phenol red). It is used to differentiate enterics based on the ability to reduce sulfur and ferment carbohydrates.

3. MacConkey (MAC) Agar: Is both selective and differential, the selective components in MAC are bile salts and crystal violet which inhibit the growth of Gram-Positive (G+) bacteria. The presence of lactose and neutral red, a pH indicator allows the differentiation of gram-negative (G -) bacteria based on the products released when they use lactose as a carbon and energy source. The colours of those that release acidic products are red (e.g *E.coli*)

MECHANISM OF ACTION OF SELECTIVE MEDIA

1. Potato Dextrose agar (PDA): Consists of potato infusion and dextrose. Potato infusion provides a nutrient base for luxuriant growth of most fungi while dextrose serves as a growth stimulant. The incorporation of tartaric acid (TA) in the medium lowers the pH to 3.5 thereby inhibiting bacteria growth. **Chloramphenicol** acts as a selective agents to inhibit bacteria overgrowth of competing microorganism from mixed specimens while permitting the selective isolation of fungi.

2. Sabouraud Dextros Agar (SDA): Is a selective medium primarily used for the isolation of dermatophytes, other fungi and yeast but can also grow filamentous bacteria such as Norcardia.

The acidic pH of this medium (pH about 5.0) inhibits the growth of bacteria but permits the growth of yeast and most filamentous fungi. This medium is also employed to determine mycological evaluation of food, contamination in cosmetic, and clinically to aid the diagnosis of yeast and fungi infections.

The SDA media is comprised of enzymatic digest of casein and animal tissue which provide a nutritious source of amino acids and nitrogenous compounds for the growth of fungi and yeast.

Dextrose is the fermentable carbohydrate incorporated in high concentration as a carbon and energy source. Agar is the solidifying agent. Addition of antibiotics like chloramphenicol and tetracycline acts as broad spectrum antimicrobials to inhibit the growth of a wide range of gram-positive (G+) and gram-negative (G-) bacteria. Gentamycin is added to further inhibit the growth of gram-negative (G-) bacteria. SDA was created by and named after Raymond Sabouraud in 1892.

3. Eosin Methylene Blue (EMB) Agar: Is selective for gram-negative, but inhibit the growth of gram-positive (G+).

4. Mannitol Salt Agar (MSA): Is both selective and differential. It contains 7.5% NaCl and selects for salts tolerant bacteria.

The selective agents for mannitol salt agar (MSA) is sodium chloride. The pH indicator of mannitol salt agar is phenol red.

5. Pheynlethyl Alcohol Agar (PEA): Is selective for gram-positive (G+) but inhibit the growth of gram-negative (G-).

6. Salmonella Shigella (SS) Agar: Is a selective and differential medium. It is used for isolation, cultivation and differentiation of Gram-negative (G-) enteric microorganisms isolated from both clinical and non clinical specimens such as from faeces, urine and clinical suspected food items (fresh and canned foods).

This medium is not recommended for the primary isolation of Shigella. Despite is name, salmonella Shigella (SS) agar is not suitable for isolating Shigella as it is inhibitory to most strains.

COMPOSITION OF SALMONELLA SHIGELLA AGAR AND FUNCTION

1. **Lactose:** Fermentable carbohydrates
2. **Beef extract:** Proteose peptone; Provides the nitrogen, vitamins and amono acids in (SS) agar
3. **Ferric citrate:** Sodium thiosulfate is also sulfur source and acts with ferric citrate as an indicator to detect hydrogen sulfide production.
4. **Sodium Thisosulphate and sodium citrate:** Selective agents, providing an alkaline pH to inhibit gram-positive (G+) organisms and suppress coliforms
5. **Bile salts:** The bile salt inhibits growth of gram-positive microorganisms.
6. **Brilliant Green / Neutral Red pH:** Indicator
7. **Agar:** Solidifying agent

MECHANISM OF ACTION OF ENRICHED MEDIA

Media supplemented by blood or other special nutrients (e.g blood agar) to allow growth of organisms that cannot grow on general purpose media.

Brain Heart infusion agar (BHI): Is a growth medium for growing microorganisms. It is a nutrient rich medium and can therefore be used to culture a variety of fastidious organism. In particular, it has been used to culture streptococci, which can be otherwise challenging to grow.

- (BHI) is made by combining an infusion from boiled bovine or porcine heart and brain with variety of other nutrients.
- (BHI) broth is often used in food safety, water safety and antibiotics sensitivity tests.

Commensality and overgrowth of unwanted bacteria

- Transport media, contains buffers and salts
- Peptone water