Biochemical Reaction Tests

Bacteria accomplish their various biochemical activities (growth and multiplication) using raw materials (nutrients) obtained from the environment. The biochemical transformations that occur both inside and outside of bacteria are governed by biological catalysts called enzymes.

This part of the laboratory manual presents the ability of bacteria to use enzymes and degrade carbohydrates, lipids, proteins, and amino acids. The metabolism, or use, of these organic molecules often produces by-products that can be used in the identification and characterization of bacteria.

Sugars Fermentation test

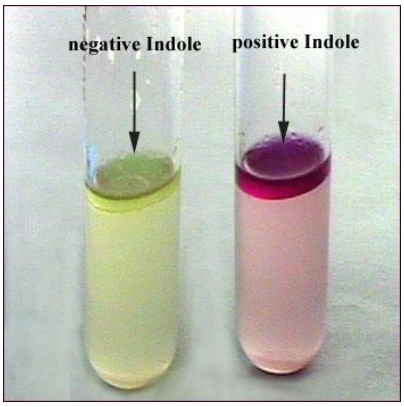
Fermentations are energy-producing biochemical reactions in which organic molecules serve both as electron acceptors and donors. The ability of microorganisms to ferment carbohydrates and the types of products formed are very useful in identification. A given carbohydrate may be fermented to a number of different end products depending upon the microorganism involved. These end products (alcohols, acids, gases, or other organic molecules) are characteristic of the particular microorganisms. For example, if fermenting bacteria are grown in a liquid culture medium containing the carbohydrate glucose, they may produce organic acids as by-products of the fermentation. These acids are released into the medium and lower its pH. If a pH indicator such as phenol red or bromcresol purple is included in the medium, the acid production will change the medium from its original color to yellow (figures 1).

Gases produced during the fermentation process can be detected by using a small, inverted tube, called a Durham tube within the liquid culture medium. After adding the proper amount of broth, Durham tubes are inserted into each culture tube. During autoclaving, the air is expelled from the Durham tubes, and they become filled with the medium. If gas is produced, the liquid medium inside the Durham tube will be displaced, entrapping the gas in the form of a bubble.

Figures 1: Sugars Fermentation test

Indole Production test

The amino acid tryptophan is found in nearly all proteins. Bacteria that contain the enzyme tryptophanase can hydrolyze tryptophan to its metabolic products, mainly, indole, pyruvic acid, and ammonia. The bacteria use the pyruvic acid and ammonia to satisfy nutritional needs; indole is not used and accumulates in the medium. The presence of indole can be detected by the addition of Kovacs' reagent. Kovacs' reagent reacts with the indole, producing a bright red compound on the surface of the medium (figures.2).

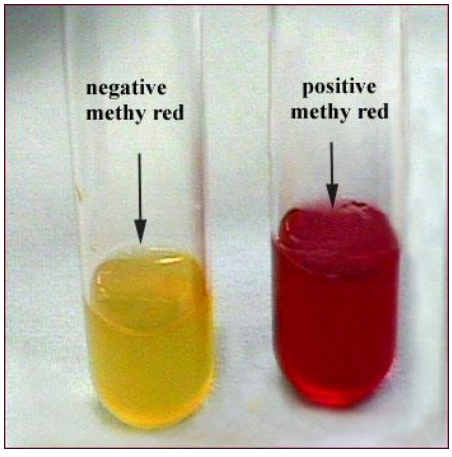


Figures.2: Indole Production test

Methyl Red Test

All enteric bacteria catabolize glucose for their energy needs; however, the end products vary depending on the enzyme pathways present in the bacteria. The pH indicator methyl red detects a pH change to the acid range as a result of acidic end products such as lactic, acetic, and formic acids. This test is of value in distinguishing between E. coli (a mixed acid fermenter) and E. aerogenes (a butanediol fermenter).

Mixed acid fermenters such as E. coli produce a mixture of fermentation acids and thus acidify the medium. Butanediol fermenters such as E. aerogenes form butanediol (acetoin), and fewer organic acids. The pH of the medium does not fall as low as during mixed acid fermentation. As illustrated in figure.3, at a



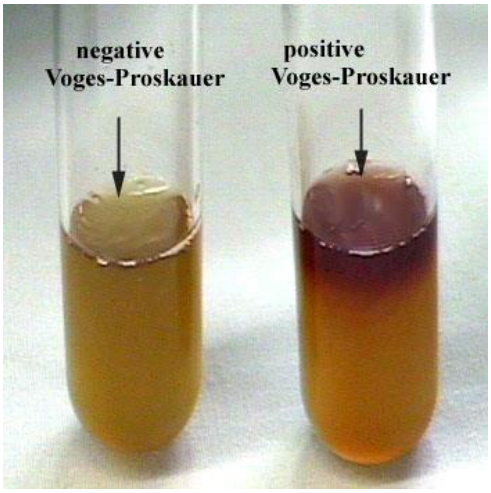
pH of 4, the methyl red indicator turns Red (a positive methyl red test). At a pH of 6, the indicator turns yellow (a negative methyl red test).

Voges-Proskauer Test

The Voges-Proskauer test identifies bacteria that ferment glucose, leading to [illegible] edium. The addition of 40% KOH and a 5% [illegible] ute ethanol (Barritt's reagent) will detect the [illegible] e synthesis of 2,3-butanediol.

Figures.3: Methyl Red Test

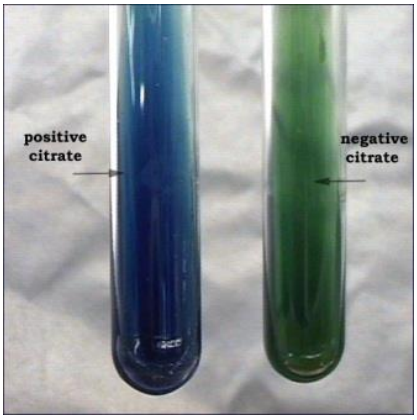
In the presence of the reagents and acetoin, a cherry-red color develops. Development of a red color in the culture medium with 15 minutes following the addition of Barritt's reagent represents a positive VP test; absence of a red color is a negative VP test (figure.4).



Figures.4: Voges-Proskauer test

Citrate Utilization Test

The citrate utilization test determines the ability of bacteria to use citrate as a sole carbon source for their energy needs. This ability depends on the presence of a citrate permease that facilitates transport of citrate into the bacterium. Inside the bacterium, citrate is converted to pyruvic acid and CO2. Simmons citrate agar slants contain sodium citrate as the carbon source, NH4+ as a nitrogen source, and the pH indicator bromothymol blue. This test is done on slants since O2 is



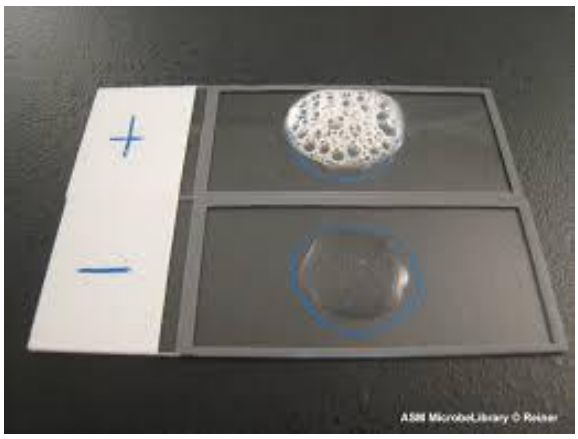
Figures.5: citrate utilization test

necessary for citrate utilization. When bacteria oxidize citrate, they remove it from the medium and liberate CO2. CO2 combines with sodium (supplied by sodium citrate) and water to form sodium Carbonate (an alkaline product). This raises the pH, turns the pH indicator to a blue color, and represents a positive citrate test; absence of a color change is a negative citrate test (figure.5). Citrate-negative cultures will also show no growth in the medium.

Catalase test

The catalase enzyme serves to neutralize the bactericidal effects of hydrogen peroxide. Catalase expedites the breakdown of hydrogen peroxide (H2O2) into water and oxygen (2H2O2 + Catalase → 2H2O + O2). This reaction is evident by the rapid formation of bubbles. The catalase test facilitates the detection of the enzyme catalase in bacteria. It is essential for differentiating catalase-positive Micrococcaceae from catalase-negative Streptococcaceae. The catalase test is also valuable in differentiating aerobic and obligate anaerobic bacteria, as anaerobes are generally known to lack the enzyme. In this context, the catalase test is valuable in differentiating aerotolerant strains of Clostridium, which are catalase negative, from Bacillus, which are catalase positive.

Figures 6: Catalase test



Oxidase test

Oxidase enzymes play an important role in the operation of the electron transport system during aerobic respiration. Cytochrome oxidase uses O2 as an electron acceptor during the oxidation of reduced cytochrome C to form water and oxidized cytochrome C.

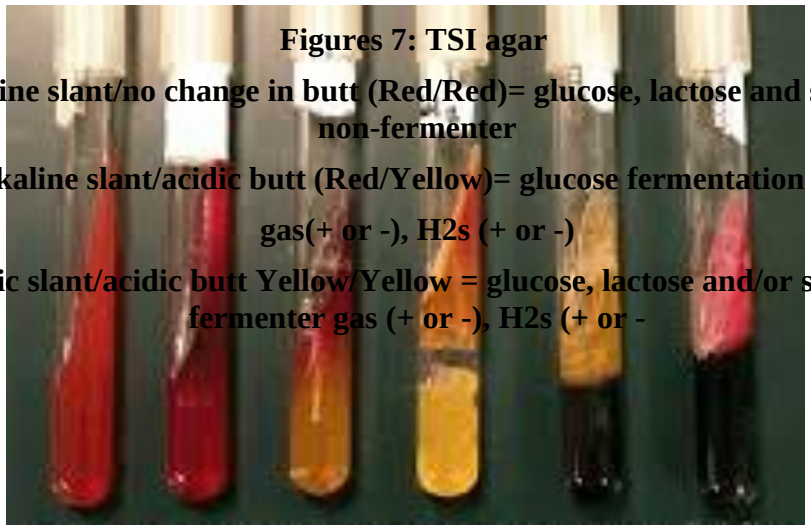
This test should be done on all gram negative bacilli. It is depend on the production of cytochrome oxidase by certain bacteria. This test required chemical reagent named (tetramethyl para-phenylene diamine dihydrochloride)

A colony of tested microorganism transferred to filter paper, with addition few drops of (1 % oxidase reagent). Development purple color in 10-30 sec. indicate a positive result.

Figures 6: Oxidase test

Triple Sugar Iron (TSI) medium

As originally described in 1911 by F. F. Russell, the triple sugar iron (TSI) agar test is generally used for the identification of enteric bacteria (Enterobacteriaceae). It is also used to distinguish the Enterobacteriaceae from other gram-negative intestinal bacilli by their ability to catabolize glucose, lactose, or sucrose, and to liberate sulfides from ferrous ammonium sulfate or sodium thiosulfate. TSI agar slants contain a 1% concentration of lactose 1% sucrose, and a 0.1% glucose concentration. The pH indicator, phenol red, is also incorporated into the medium to detect acid production from carbohydrate fermentation. Often Kligler Iron Agar (named after I. J. Kligler in 1917), a differential medium similar to TSI, is used to obtain approximately the same information.



Figures 7: TSI agar

Alkaline slant/no change in butt (Red/Red)= glucose, lactose and sucrose non-fermenter

Alkaline slant/acidic butt (Red/Yellow)= glucose fermentation only

gas(+ or -), H2s (+ or -)

Acidic slant/acidic butt Yellow/Yellow = glucose, lactose and/or sucrose fermenter gas (+ or -), H2s (+ or -