

EXERCISE NO. 5

Date:

Aim of the Experiment:

TRADITIONAL PROCESS OF IDENTIFICATION BASED ON BIOCHEMICAL METHODS

Principle

Methods of reliable and accurate microbial identification are valuable in a wide range of scientific fields. Biochemical tests are one of the traditional methods for the identification of microorganisms, usually performed with phenotypic identification. They can reveal the vital information necessary for accurately identifying the genera of various bacteria within a sample. The ability of microorganisms to utilize certain biomolecules, resulting in useful organic compounds for themselves forms the basis of various biochemical tests. Biochemical tests are of different types, where the identification or distinction between different microorganisms is made on various bases. Microorganisms can be classified into different groups on the basis of their reaction to such tests. Some tests even allow the distinction of microorganisms to the species level. Biochemical tests are thus, essential as they are inexpensive and relatively simple to perform.

Materials

1. *Lactobacillus* culture
2. MR-VP medium
3. Tryptophan broth/Peptone water
4. Simmon's citrate slant/broth
5. Kovac's reagent
6. Methyl red indicator
7. Barritt's A solution or VP reagent I (5% – α -naphthol solution)
8. Barritt's B solution or VP reagent II (40% KOH solution)

Procedure

IMViC test

This is a battery of test which includes Indole production test, methyl red test, Voges Proskauer test and citrate utilization test.

a. Indole test

Principle: Detects the ability of organisms to produce indole as a metabolic product utilizing amino acid tryptophan. Some bacteria can produce an enzyme called 'tryptophanase' which helps them to metabolize the amino acid 'tryptophan' into 'indole, pyruvic acid, and ammonia'. When indole is combined with Kovac's Reagent (which contains hydrochloric acid and p-dimethylaminobenzaldehyde in amyl alcohol) the solution turns from yellow to cherry red. Because amyl alcohol is not water soluble, the red coloration will form in an oily layer at the top of the broth.

Procedure:

1. Inoculate a loopful of the culture in 5 ml tryptophan broth.
2. Incubate for 24 h at 37 C.
3. Add 0.2 to 0.3 ml of Kovac's reagent in the tube and invert few times.
4. Development of dark red color in the surface layer is a positive test and persistence of yellow color is a negative test.

b. Methyl red test

Principle: Some species of bacteria use the mixed acid fermentation pathway as their glucose metabolism process. When such acid fermenters bacteria are grown in a medium containing glucose (or carbohydrate), they will release the acids, hence; decreasing the pH of the medium to 4.4 or lower. When methyl red indicator is added in a medium containing such acid fermenters, it will turn the medium red." Following the 24-hour incubation on the MR-VP broth, a methyl red indicator is added to the broth. A positive result is indicated by the development of red color while a negative result is indicated by the development of yellowish color.

Procedure:

1. Inoculate a loopful of the culture in 5 ml MR-VP medium.
2. Incubate for 24 h at 37 C.
3. Add 5 drops of methyl red indicator in the tube and mix.
4. A positive test is indicated by red color and negative test by yellow color.

c. Voges-Proskauer test

Principle: Detects the ability of organisms to metabolize the pyruvate into a neutral intermediate product called 'acetylmethylcarbinol' or 'acetoin'. Pyruvate can be metabolized

into a neutral intermediate product called 'acetyl methyl carbinol', commonly called 'acetoin' during the butanediol pathway of 2,3-butanediol production. If acetoin is present in the media, it is oxidized readily to diacetyl in presence of air and KOH. Thus, diacetyl in the presence of α – naphthol, will react with the guanidine component of peptone forming a pink to a red colored product. Following the 48-hour aerobic incubation on MR-VP broth, VP reagents I and II are added and the color change is observed within 30 minutes. A positive result is indicated by the development of pink – red color at the top of the broth immediately or within 30 minutes. No change in color represents a negative VP test.

Procedure:

1. Inoculate MR-VP medium with the loopful of test organism
2. Incubate at 37 C for 24 h.
3. Add 6 drops of 5% alpha-naphthol, and mix well to aerate.
4. Add 2 drops of 40% potassium hydroxide, and mix well to aerate.
5. Allow to stand exposed to the air and observe for development of eosin pink color.

d. Citrate utilization test

Principle: Bacteria are inoculated on a medium containing sodium citrate and a pH indicator such as bromothymol blue. The medium also contains inorganic ammonium salts, which are utilized as sole source of nitrogen. Use of citrate involves the enzyme citrate lyase, which breaks down citrate to oxaloacetate and acetate. Oxaloacetate is further broken down to pyruvate and carbon dioxide (CO₂). Production of sodium bicarbonate (NaHCO₃) as well as ammonia (NH₃) from the use of sodium citrate and ammonium salts results in alkaline pH. This results in a change of the medium's color from green to blue. The alkaline pH changes the pH indicator (bromthymol blue) from green to blue.

Procedure:

1. Streak Simmon's citrate agar slant with the test culture.
2. Incubate at 37 C for 24 to 48 h.
3. If the organism has the ability to use citrate, the medium changes its color from green to blue.

Observations

IMViC test