Indian Institute of Technology Delhi
Department of Biochemical Engineering and Biotechnology

I SEMESTER
BBL132 – GENERAL MICROBIOLOGY LABORATORY

EXPERIMENT # 9

AIM

Biochemical tests for identification of microorganisms (Kit based)

BACKGROUND

All the metabolic activities in microorganisms are mediated by enzymes. The chemical end products of these enzymatic actions could be measured. By making a series of different biochemical and physiological tests, a pattern of activity can be established. On the basis of these, identification of different microorganisms can be made. You will be using performing some of the most common tests.

MATERIALS

(1) Two microbial cultures (*Escherichia coli, Pseudomonas* sp.) (2) Peptone broth with different sugars and phenol red (3) Simmons citrate agar slants (4) Kovacs reagent (5) Methyl red (6) Durham tubes (7) Triple sugar iron agar slants.

PROCEDURE

1. Acid and Gas production from Carbohydrates

The microbes breakdown common carbohydrates to produce various organic acids (like acetic and lactic acids) and gases (like carbon dioxide and hydrogen). The formation of acid can be detected by including a pH indicator in microbial growth media. The gas formation can be detected by placing an inverted Durham tube in the test tube.

a) Prepare three tubes each of the following media: Glucose broth with phenol red and Durham tube, Lactose broth with phenol red and Durham tube.
b) Inoculate the given strains of *E.coli* and others into each tube and incubate at 37 °C overnight. Include an uninoculated control for each set.
c) After 24 hours, observe the cultures for acid and gas formation (Fig-1)

2. IMV_iC
2a) Indole production

Indole, a nitrogen-containing compound formed from the degradatiaon of the amino acid tryptophan, produced only by certain microorganisms.

a) Inoculate two tubes of 1% tryptone broth with *E.coli* and *Pseudomonas.*
b) Incubate at 37 °C for 24 hours.
c) To each tube, add 3 ml of Kovacs solution and mix well. Reddening of alcohol layer indicates the presence of indole (Fig-2)

2b) Methyl-Red/Voges-Proskauer (MR-VP) test

These tests are important in the identification of the gram-negative non-spore forming rods and some species of *Bacillus*. Instead of accumulating mostly acidic products from the fermentation of glucose, some bacteria convert the metabolic intermediate, pyruvic acid, to neutral products. When the Voges-Proskauer test is applied, acetyl methyl carbinol is an easily detected neutral product.

The methyl-red test detects organisms that do not convert acidic products to neutral products and thereby produce a final pH lower than that of organisms producing neutral products. Because of the lower pH, the MR indicator changes to red color, which is a positive test.

Voges-Proskauer (VP) test

a) Inoculate a tube of MR-VP medium (5 ml) with *E.coli* and *Pseudomonas* and incubate for 48 hours at 37 °C.
b) Test for acetyl methyl carbinol.

i. Decant about ¼ volume of culture into a clean test tube
ii. Add 0.5 ml (8-10 drops) of alpha napthol solution (5% in alcohol)
iii. Add 0.5 ml of 40% KOH solution containing 0.3% creatine.
iv. Shake thoroughly and allow to stand for 5-30 minutes.
v. The appearance of a pink to red color indicates the presence of acetyl methyl carbinol. (Fig-2)

Methyl-Red (MR) test

c) Test for acid production by adding few drops of an alcoholic solution of methyl red to the rest of the culture. A distinct red color is considered to be a positive test. Yellow is negative. (Fig-2)

2c) Citrate utilization

The ability to utilize citrate as a sole course of carbon and energy can be used to distinguish between certain gram-negative rods. Growth on Simmons citrate agar is a positive test for citrate utilization. Certain organisms that give a positive test increase the pH, changing the bromothymol blue indicator in the medium from green to blue.

a) Inoculate slants of Simmons citrate agar with *E.coli* and Citrobacter strains.
b) Incubate at 37 °C for 48 hours
c) The presence of growth at the end of incubation is a positive test. (Fig-2)

3. Catalase activity

The catalase enzyme breaks down hydrogen peroxide to give free oxygen

$2H_2O_2 \rightarrow 2H_2O + O_2$

The gas can be readily seen as a white froth when a few drops of 3% H_2O_2 is added to a microbial colony or to a broth culture.

a) Add a few drops of 3% H_2O_2 to the culture growth and observe it closely for the appearance of bubbles of oxygen.
b) Add a few drops of 3% H_2O_2 to broth culture and look for a streaming up of O_2 bubbles. (Fig-3)

RESULTS

Report your result as given below.

Acid and Gas production from Carbohydrates

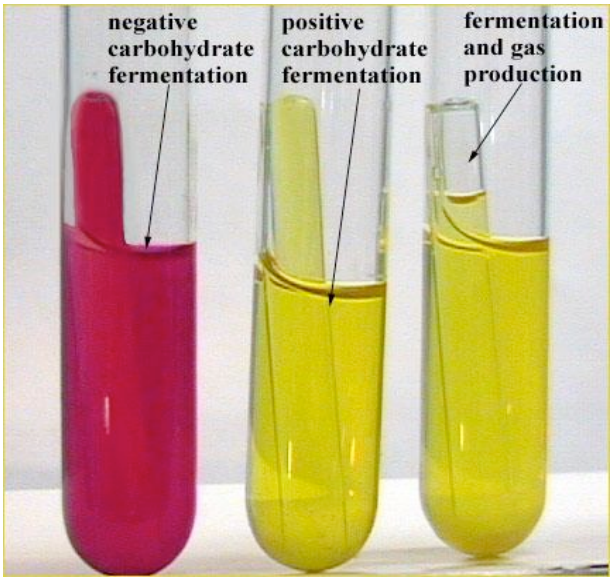

Fig-1

IMV$_i$C

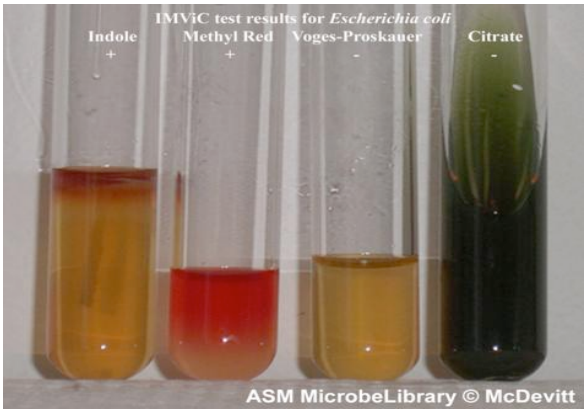

Fig-2

Catalase activity

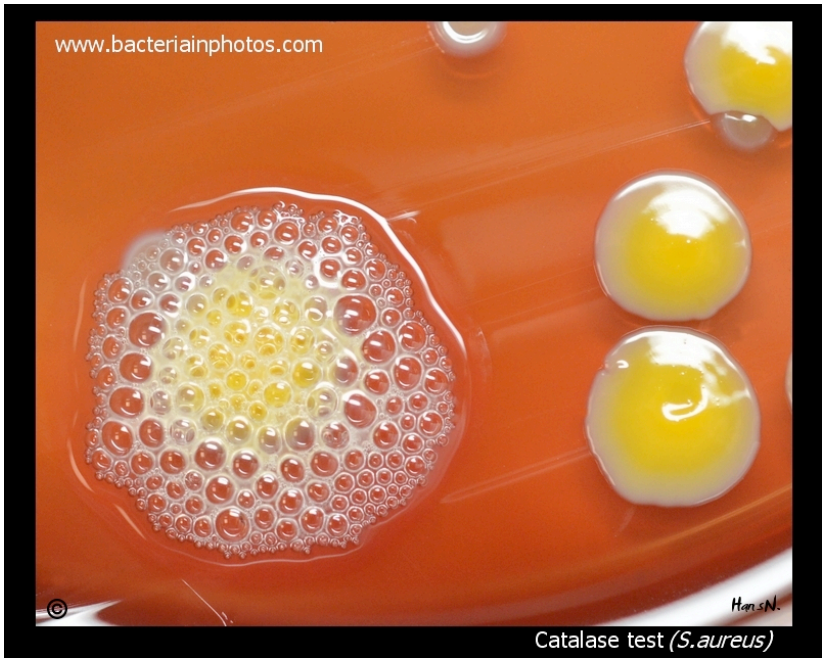

Fig-3