Indian Institute of Technology Delhi
Department of Biochemical Engineering and Biotechnology

I SEMESTER
BBL132 – GENERAL MICROBIOLOGY LABORATORY

EXPERIMENT # 11

Aim

To screen colonies obtained through UV mutagenesis for presence of lactose non-utilizing mutants.

Background

A large variety of microbial mutants have been isolated and studied intensively by microbiologists. Some important categories of mutants include auxotrophic mutants, resistant mutants, metabolic mutants, cryptic mutants, antigenic mutants, conditional mutants etc. Lactose non-utilization is a metabolism-associated mutation.

Escherichia coli belong to the class enteribacteriacae and are characterized as lactose fermenting organisms. Lactose non-fermenting *E. coli* are very important hosts for genetic manipulation and these mutants are called metabolic mutants. Certain differential media are their wherein lactose fermenting bacteria can be differentiated from non-lactose fermentors.

MacConkey agar is one such medium where lactose fermentors will form pink colonies whereas non lactose fermentors will form white colonies. Thus the mutant E. coli can easily be screened. It contains bile salts (to inhibit most Gram-positive bacteria, except *Enterococcus* and some species of *Staphylococcus*), crystal violet dye (which also inhibits certain Gram-positive bacteria), neutral red dye (which stains microbes fermenting lactose), lactose and peptone.

Fig 1: MacConkey Agar plate showing lactose fermenting and lactose non-fermenting bacteria.

Materials required

Mutant colonies of *E. coli* obtained through UV mutagenesis experiment, MacConkey agar plates, tooth picks, incubator etc.

Procedure

1. Assign a number (1-16) to the mutant colonies obtained in the previous experiments by labelling at the back of the plate.
2. Take a plate of Mac Conkey agar and at the back of the plate make 16 squares and label them as 1-16 and inoculate them with these mutants with a correspondingly assigned number using sterile tooth peaks (inoculate by making a small line streak not reaching out of the box boundary).
3. Incubate the plates at 37°C.
4. After overnight incubation, observe for the presence of white colonies.