EXERCISE 14

PREPARATION OF BACTERIOPHAGE STOCKS

Objective

To learn the technique of preparation of phage stocks.

Background

In the plaque assay, each plaque contains a large numbers phages that are progeny of a single phage particle. To obtain high titre of the phage, plaque should be picked up with a sterile needle and suspended in Tryptone broth or dilution fluid. This phage can be allowed to infect a diluted overnight culture of *E. coli* for about 6-10 hrs and that will result the production of high titre of phage.

Materials required

Tryptone broth; Dilution fluid; Overnight culture of *E. coli*; Nutrient agar plate with plaques; sterile needle or tooth pick, sterile pipettes.

Procedure

1. Scoope out a single plaque from the plate with the tooth pick or needle and resuspend them in 1 ml of chloroform saturated tryptone broth dilution fluid.
2. Dilute the overnight *E. coli* culture (1:20 dilution).
3. Add 0.1 ml phage in dilution fluid to the *E. coli* culture.
4. Incubate the culture at 37°C with agitation for 6-10 hrs.
5. Centrifuge at 5000 rpm to remove the bacterial cells.
6. To the supernatant add chloroform to lyse the remaining cells.
7. The solution now contains high titre of bacteriophages (stock).
8. Determine the phage titre as described in next experiment.

EXERCISE 13

ISOLATION OF BACTERIOPHAGES

Objective

To isolate bacteriophages from the sewage samples.

Background

The presence of bacteriophages in the natural environment will be relatively lower and therefore it is essential to use desired host bacteria along with the nutrients as an enrichment technique. After incubation the bacteriophages can be seperated by centrifugation and then by membrane filteration. The filteration by using 0.45um filters has been used to physically remove the bacteria from the liquid. The final step is to produce plaque by seeding a lawn of bacteria with the phage in the filtrate.

Materials required

Nutrient broth; Nutrient soft agar; Nutrient agar plate; Sewage sample; Test tubes; Pipettes; Memberane filters; Filteration appartus.

Procedure:

1. Add 45 ml of the sewage to 5 ml of 10X nutrient broth in a sterile flask.
2. Add 5 ml of *E. coli* or other bacteria to which you are interested in isolating bacteriophage. Mix gently and incubate this for 24 hrs at 37°C.
3. Centrifuge 10 ml of this enrichment culture a 5000 rpm for 10 min. to remove most bacterial cells.
4. Filter the supernatant through membrane filter.
5. Liquify four tubes of soft nutrient agar and cool to 50°C and keep the tube at the same temperature water bath to prevent solidification.
6. Add varying quantities of the filterates (1 to 6 drops) into three tubes and one to be used as control.
7. Transfer 0.3 ml of the *E. coli* culture (or other desirable host) to each of the four tubes of soft agar and mix by rolling the tube between your hands.
8. Pour the contents of the tubes over four nutrient agar plates and label them.
9. Once the agar is cooled, put the plate inverted and incubate at 37°C and incubate for 24 hrs.
10. Observe the plaque formation and the size of the plaque at every two hours.