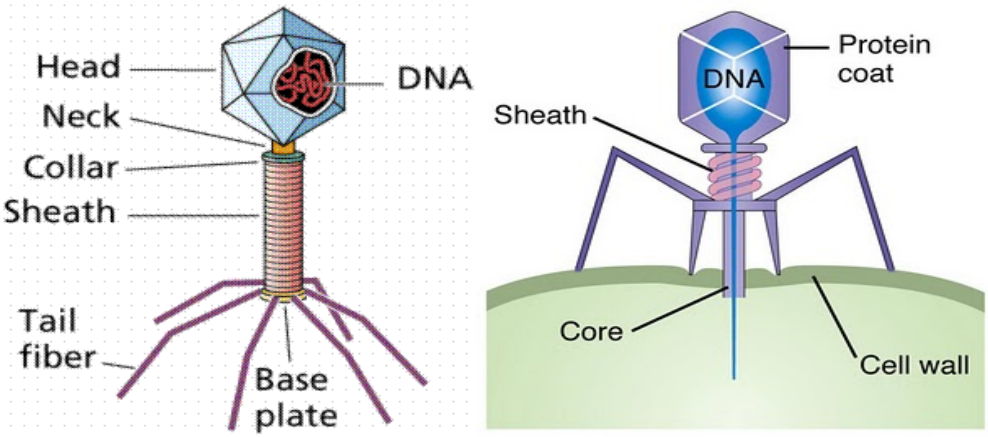
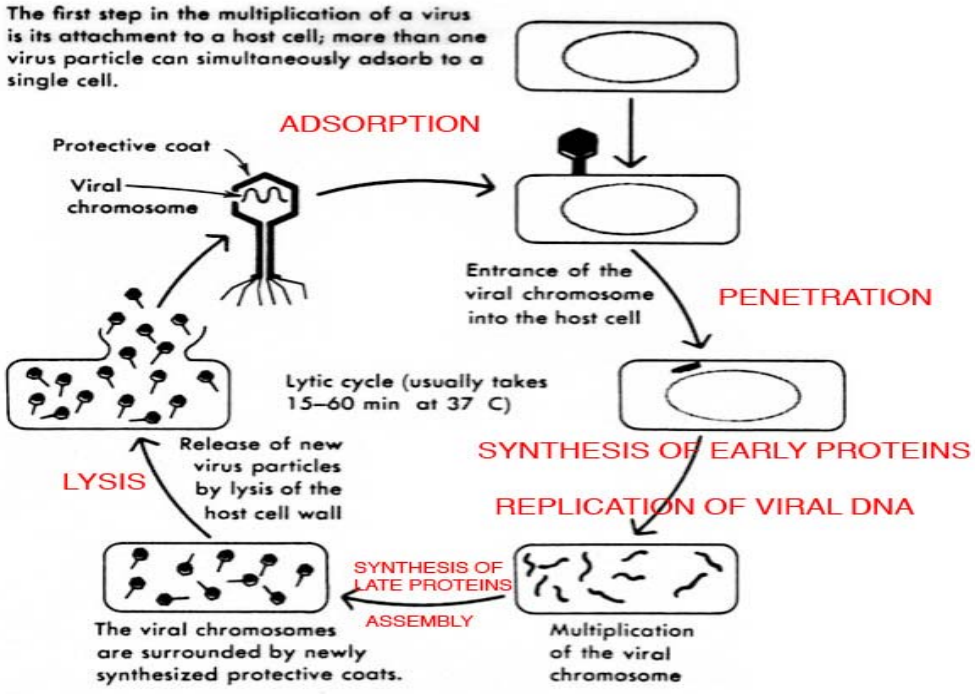
Elimination of Escherichia Coli and other fecal coliform bacteria through Bacteriophages and antagonists Bacteria from River Ganga and Tributaries





CENTRAL POLLUTION CONTROL BOARD
(Ministry of Enviroment & Forests)
Parivesh Bhawan, East Arjun Nagar,
Delhi-110032

[Part -B] : "Elimination of Escherichia Coli and other Fecal Coliform Bacteria through Bacteriophages and Antagonists Bacteria for Maintaining Pristine Water Quality of River Ganga and other Rivers"

1. BACTERIOPHAGES FOR REMOVAL OF FECAL COLIFORM

The viruses are a complex, diverse, and fascinating group, the study of which has done much to advance disciplines such as genetics and molecular biology. Phages are essential components of the ecosystem and are important in both industry and medicine. For example, many phages destroy the gram-positive lactic and acid bacteria that are critical to the production of fermented milk products such as yogurt and cheese. Bacteriophages also can carry a variety of virulence factors that make their bacterial hosts potent pathogens. This seems to be the case for major human pathogens such as *Streptococcus pyogenes, Staphylococcus aureus, Corynebacterium diphtheria, Vibrio cholera, E. coli*, and *Salmonella enteric.* On the positive side, Russian physicians have used bacteriophages for years to treat bacterial diseases. Recent research indicates that phages may indeed be effective in treating bacterial infections, including those caused by antibiotic-resistant bacteria.

Bacteriophages are bacterial viruses that attach to their specific hosts and kill them by internal replication and bacterial lysis involving a complex lytic cycle involving several structural ad regulatory genes. Phages are very specific in that they only attack their targeted bacterial hosts. They cannot infect human or other eukaryotic cells.

In a **one-step growth experiment,** a culture of susceptible bacteria such as *E.coli* is mixed with bacteriophage particless, and the phages are allowed a short interval to attach to their-host cells. The culture is the greatly diluted so that any virus particles released upon host cell lysis will not immediately infect new cells. This strategy works because phages lack a means of seeking out host cells and must contact them during random movement through the solution. Thus phages are less likely to contact host cells in a dilute mixture. The number of infective phage particles released form bacteria is subsequently determined at various intervals by a plaque count.

A plot of the bacteriophages released from host cells versus time shows several distinct phases. During the **latent period,** which immediately follows phage addition, there is no release of virions. This is followed by the **rise period** or **burst,** when the host cells rapidly lyse and release infective phages. Finally, a plateau is reached and no more viruses are liberated. The total number of phages released can be used to calculate the **burst size,** the number of viruses produced per infected cell.

Many phages lyse their host cells at the end of the intracellular phase. The lysis of E.coli takes place after about 22 minutes at 37^{o}C, and approximately 300 T4 particles are released. Several T4 genes are involved in this process. One directs the synthesis of an endolysin that attacks the cell wall peptidoglycan. Another phage protein called a holing produces a plasma membrane lesion that stops respiration and allows athe endolysin to attack the peptidoglycan. Presumably it forms holes in the membrane.

2. OBJECTIVE OF THE PROPOSAL

The main aim of the project is to demonstrate removal of pathogenic bacteria and fecal coliforms in particular by employing Bacteriophages from sewage. The study would incorporate;

- Isolation of bacteria (different coliforms) from sewage
- Preparation of viral suspension
- Viral isolation/purification and mass multiplication of bacteriophages
- Identification of bacterial host strains
- Identification of phage-host combination
- Potential to use of Phages as a technology to remove these bacteria from sewage without causing harm to natural nonpathogenic bacterial assemblages (normal microflora).

The study will be founded on STPs located on River Ganga, and the sewage drains / using the river Ganga.

The present invention provides novel compositions and cost effective method of using bacteriophages for sewage treatment plant.

(a) to provide novel composition for sewage treatment plant.

(b) to provide an easy and simple method of treating the sewage in the sewage treatment plant by using bacteriophages in different novel composition.

(c) to provide a cost effective method of treating sewage in the sewage treatment plant.

(d) to provide a method of using bacteriophage in controlling the pollution and making the environment clean.

(e) to provide an automated and controlled method for using bacteriophage in sewage treatment plant.

3. PLAN OF ACTION

It is proposed to isolate selective phages for each pathogenic bacteria following, the standards procedures/ methods as per American Public Health Association (APHA). The Isolation of phages shall be amplified to mass cultures for their application for removal of fecal coliform. The phages will be isolated from the sewage samples to be collected from sewage drains sewage treatment plants and river Ganga. The dosage of application of phages will be standardized after obtaining titer values. Thus, the proposed methodology will include following

(i) Isolation of pathogenic bacterial strains

(ii) Isolation of bacteriophages from sewage/ specific culture

(iii) Amplify increase the numbers of phages

(iv) Collection of the phages from the culture by centrifugation and filtration

(v) Detect and *titer* the amplified, isolated phages using a *plaque assay*

(vi) Application of phages in STPs/ sewage drains

Central Pollution Control Board(CPCB) has carried out preliminary experiments on Isolation of bacteriophages and has applied them on pathogenic bacteria and particularly on E.coli. Based on titer values the phages will be loaded in Sewage Treatment Plants and in the drains carrying sewage for removal of fecal coliforms. The experiments will be conducted on STPs located all along the river Ganga and also on the sewage drains joining river Ganga.

For carrying out experimental and demonstrative studies, facilities for mass culture of phages will be developed in each state i.e, Uttarakhand, Uttar Pradesh, Bihar, West Bengal and Jharkhand. Besides, the facilities will also be developed at CPCB's Central laboratory at Delhi.

The present invention provides a methods and a novel composition by which bacteriophage can be very effectively used for treating sewage in the sewage treatment plants. The bacteriophage is administered to the environment. The novel composition then prevents the growth or viability of targeted bacteria by infecting, lysing or inactivating targeted bacteria present in the said environment. The bacteriophage is used with aquous, acidic or with a neutral solvent. It is available in several forms, including, but not limited to, a liquid, a tablet or a powder and may be added directly to the system. To improve contact and adherence of the treatment with the surface to which the treatment is being applied a thickener may be used. The buffering agent is used to control a pH level and/ or other adjuvants.

The bacteriophage treatment may be added either automatically or manually to the waters system or the sewage system. To ensure that an effective concentration of bacteriophage is maintained a control system may be used to monitor it in the water system. The configuration of the control system is done in such a way that a replenishment of the bacteriophage is periodically added to the water system as a function of time or is based on sensed parameters within the water system. Also the pH of the wate system can be monitored by the control system so as to maintain the water system at a pH range that ensures the viability of the bacteriophage.

4. REQUIREMENTS FOR PROJECT EXECUTION

The following requirements would be essential for execution for the project.

4.1 Instrumentation

Orbital shaker, Incubator (large volume), Centrifuge (large capacity), filtration assembly, autoclave, fermentor. The estimated cost will be Rs. 6.0 Crores

4.2 Glassware, Chemicals and Media

For mass culture, the estimated cost for procuring chemical, Media, etc. will be Rs. 3.0 Crore

4.3 Staffing

For each Centre, six staff will be required, Two Research Associates, two Junior Research fellows and two Project Assistants to support field-based activities including preparation of mass cultures. An amount of Rs. 0.80 Crore is required on annual basis.

4.4 Contingencies and Institutional Charges

An amount of Rs. 0.90 Crore will be required for contingency to include transportation, meeting TA/DA expenditures organizing inte30ractive and awareness meetings.

5. EXPERIMENTATION

On the sites of STPs in each state and on the drains, facilities/ minor civil construction/ infrastructure will be required. This would include installation of mixing/ churning devises and designing of Phage embedded filters/ substratum. Rs. 5.0 Crore is required for carrying out such modification on 5 STPs and 5 drains.

Total cost of Project comes to Rs. 15.70 Crores for a period of one year.