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Evaluation of Bacteriophage Therapy against Bacterial Pathogens *(Pseudomonas, Bacillus*)

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ABSTRACT: *The use of bacteriophage to treat bacterial infection is a novel approach and is being seriously considered. Bacteriphage had the potential to kill the bacteria that causes infection in humans as well as agriculturally important plants and animals. Phage therapy is a form of biological control, the use of one organism to suppress another and reduction in the usage of chemical agents against pest species, which in the case of phage, means a reduction in the usage of chemical antibiotics. Brinjal plant is used for this phage therapy experiment; phage is isolated from the sewage and was confirmed by plaque assay method. Various concentrations of phages were treated with brinjal plant. Plant protected with phage injection exhibited no symptoms when compared to unprotected one. This protection could be attributed to the marked reduction of the pathogen load in stem of the brinjal plant in response to the presence of persistence of phage.The aim of present study is to treat the bacterial infections using specific bacteriophages. Since bacteria developed resistant to majority of antibiotics, phages are extensively used.*

Keywords*: Bacteriophage therapy, Bacillus sp, Pseudomonas sp, Brinjal*.

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I. Introduction

Bacteriophage were discovered a little over 80 years ago in 1915 by the Englishman Frederick Twort and in 1917 by the French Canadian Felix D' Herelle independently. The idea of Bacteriophage (Phage) application in the treatment of infectious disease of bacterial origin is not new. Bacteriophage therapy was initiated in 1921 by Bruynoghe and Maisin in the treatment of ***Staphyloccocal*** infections.

The degree of interest with phage therapy for bacterial infections showed substantial fall during 20 inter-war years with the introduction of sulphanamides and then antibiotics like penicillin and chloramphenicol. Renewed interest in this problem emerged again after 40 years and connected with the appearance of more and frequent infections induced by antibiotics and chemotherapeutic resistant bacteria.

Bacteriphage had the potential to kill the bacteria that causes infection in humans as well as agriculturally important plants and animals. This idea formed the basis for much research as well as for the Pulitzer prize-winning 1924 novel Arrow smith by Sinclair Lewis. Phage have been proposed as plant pathogen control agents in a process known as phage therapy; the application of specific phages to specific ecosystem in order to reduce that population size of specific bacteria

Brinjal plant protected with lower dose of phage had initially exhibited symptoms such as blight, leaf spot, necrosis, yellowing etc. hence this study emphasis the need for proper optimization of phage dose for effective control of bacterial infection without the risk of onset of the disease.

Basit *et.al*, for example has isolated phage that effective against naturally occurring competitors. By coating seeds with phage are effective only against these potential competitors. Johnson proposed a general biological control model which suggests that the success of a particular treatment will be influenced by agent and target densities. The modern era of bacteriophage research is usually dated from 1938 when the expatriate German physicist, Max Delbruck began his work on phages at the California Institute of Technology.

In 1942 Tom Anderson was obtained the first electron micrographs of phages showing a tadpole like shape. Recombinant DNA and other `modern' techniques have made it easier to study the molecular biology of fruit flies, sea shegs they have also greatly increased the sophistication of the experiments that can be done with phages. Thus for these scientific problems where phages provide advantageous experimental systems, bacteriophage research is still vigorous and in many cases leading the field.

D. Harelle gave the report about the application of specific phage on bacterial infected plants such as brinjal, tomato, cotton etc and who coined the bacteriophage, which means "Bacteria eater". Phages are particularly effective when sprayed or poured directly into lesions, leaf spot infection on plants, since they can first multiply in bacteria and finally kill the bacteria.

Special mixtures of phage suspension were developed for dealing with strains giving problems of bacterial infections in agriculturally important plants. The aim of present study is to treat the bacterial infections using specific bacteriophages. Since bacteria developed resistant to majority of antibiotics, phages are extensively used. Phages are more specific to pathogens that are even resistant to antibiotics. These phages are present predominantly in sewage. They are isolated, revived and used for further studies.

II. Materials And Methods

Isolation of Bacterial sample from Infected Leaves

1. Using a sterile razor blade, cut out younger portions of lesions from recently collected material (cut one half in a drop of sterile water and after several minute examine under oil immersion, if streaming bacteria are seen from the lesions, proceed for isolation)
2. Surface sterilizes it by dipping cut portions in surface sterilant for a few seconds.
3. Immediately rinse in 3 changes of sterile water
4. Take 1ml of sterile water in a Petri dish and transfer surface sterilized lesions in it.
5. Mince finely with a flamed razor blade
6. Allow it to stand for 10 minutes
7. Transfer several loop full of the suspension to another plate containing 0.5ml to 1ml of sterile water, mix thoroughly and repeat the step 7 for 2 or 3 plates.
8. Add molten cooled nutrient medium into 3 plates and mix thoroughly by rotation
9. Allow the plats to solidify
10. Incubate the plates in an inverted position at 35^{o}C for 36 to 72 hours.

Inoculation of bacterial pathogens in plant:

Pseudomonas aeruginosa

Brinjal plants (three plants) were inoculated with 0.5ml of *Pseudomonas aeruginosa* culture, one plant was acted as control plant (without bacterial inoculation). Allow the plants for incubation period and observe the symptoms.

Bacillus cereus

Brinjal plants (three plants) were inoculated with 0.5ml of *Bacillus cereus* culture one plant was acted as control plant (without bacterial inoculation). Allow the plants for incubation period and observe the symptoms.

Inoculation of bacterial pathogens into Brinjal plant

Sample collected from *Pseudomonas aeruginosa* injected plant was streaked on cetrimide agar plate. Incubate the plates at 37^{0}C for 24 hours, after incubation observe the results

Sample collected from *Bacillus cereus* injected plant was streaked on PLET agar plate. Incubate the plates at 37^{0}C for 24 hours, after incubation observe the results.

Administration of bacetiophage therapy on infected brinjal plant

Select the infected brinjal plants for phage therapy, one plant in each group were acted as bacterial control (without phage). Various concentration of bacteriophage (0.5ml, 1ml, 1.5ml) administered to *Pseudomonas aeruginosa* infected plant. Allow the plants for incubation period. After incubation the results will be observed.

Select the infected brinjal plants for phage therapy, one plant in each group were acted as bacterial control (without phage).Various concentration of bacteriophage (0.5ml, 1ml, 1.5ml) administered to *Bacillus cereus* infected plant. Allow the plants for incubation period. After incubation the results will be observed.

III. Results

The bacterial pathogens were isolated from infected plant (brinjal). Colonies formed were pure cultured by culturing them on selective medium and biochemical tests were performed, the organisms were identified as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus cereus* etc. Among them *Pseudomonas aeruginosa* and *Bacillus cereus* were selected as host for virus (phage) as they play predominant role in causing infections.

Bacteriophages specific to these host were isolated from sewage sample obtained from Sri Akilandeswari Women's College, Vandavasi. Formation of plaques (clear zone) was observed on the plates with specific bacterial host indicated the presence of phages; the phages were then enumerated and revived.

Invitro characterization of bacterial pathogens in plant

Pseudomonas aeruginosa

Brinjal plant inoculated with 0.5ml of *Pseudomonas aeruginosa* expressed symptoms like bacterial blight, necrosis, yellowing etc.

Bacillus cereus

Brinjal plant inoculated with 0.5ml of *Bacillus cereus* expressed symptoms like leaf spot, chlorosis etc.

Detection of the bacterial pathogens in the Inoculated Brinjal plant

Sample collected from *Pseudomonas aeruginosa* injected plant was streaked on cetrimide agar plate. The incubated plate shows fluorescent green colour colonies which indicate the presence of the organism and their disease causing ability.

Sample collected from *Bacillus cereus* injected plant was streaked on PLET agar plate. The incubated plate shows mucoid white colonies which indicate the presence of the organism and their disease causing ability.

Effect of Bacteriophage administration on the coarse *Pseudomonas sp & Bacillus cereus* infection

Symptoms were observed in *Pseudomonas* and *Bacillus* injected plants. Specific bacteriophages were given in 3 plants, one plant in each group was acted as bacterial control (without phage) the plant inoculated with bacterial pathogens alone were the first to express symptoms. The unprotected *Pseudomonas* and *Bacillus* injected plants developed severe symptoms. Plants treated with 0.5 ml, 1ml, 1.5ml had recovered completely within 4, 6 and 7 days respectively.

Table-1 Phage Titration *(Pseudomonas aeruginosa)*

Dilution	Number of PFU/ml
10^{-1}	TNTC
10^{-2}	TNTC
10^{-3}	TNTC
10^{-4}	194
10^{-5}	155
10^{-6}	86
10^{-7}	32
10^{-8}	20

PFU - Plaque forming unit
TNTC - Too Numerous To Count

Table-2 Phage Titration (*Bacillus cereus)*

Dilution	Number of PFU/ml
10^{-1}	TNTC
10^{-2}	TNTC
10^{-3}	275
10^{-4}	180
10^{-5}	100
10^{-6}	50
10^{-7}	20
10^{-8}	Nil

PFU - Plaque forming unit
TNTC - Too Numerous To Count

IV. Discussion

The use of bacteriophage to treat bacterial infection is a novel approach and is being seriously considered. Plant protected with phage injection exhibited no symptoms when compared to unprotected one. This protection could be attributed to the marked reduction of the pathogen load in stem of the brinjal plant in response to the presence of persistence of phage.

Although phages were discovered nearly a century ago, Western Medicine's interest in them as therapeutic agents was relatively short lived in part because of the eventual discovery and immediate success of antibiotics and in part because of the highly empirical and counter protective approach that had been used by phage practitioners in the early era. In the modern era (1980's & 1990's) some rigorously controlled plant experiments have been conducted. The experiments in the present study represent solutions to many of the problems that hindered the prior applications of the phage.

From the experimental data, injection dose of the phage seems to have a significant impact on the morbidity in the brinjal plant protected with lower dose of phage had initially exhibited symptoms such as blight, leaf spot, necrosis, yellowing etc. hence this study emphasis the need for proper optimization of phage dose for effective control of bacterial infection without the risk of onset of the disease.

V. Conclusion

Present study represents solution to many of the problems that hindered the prior applications of phage therapy. For example, the relatively narrow host range of most phages, which caused many of the early attempts of phage therapy to fail, can be overcome by isolating phages that have a broad host range within the species being targeted.

The results obtained so far showed that bacteriophages are valuable and often the only effective factor in the bacterial infections. Bacteriophages are safe, side effects are rather rare and present no danger for a patient, and hence they are transient and easy for restraint. Bacteriophages effectively control the infectious process, irrespectively of its localization, rebuilding the immunity forces of an organism. They are effective in the treatment of infections complicating immunological disorders.

Bacteriophaes can be prepared at appropriate laboratories individually for each case which ensures reliability of their effect. Worth nothing is also a low cost of their preparation. Phage therapy undoubtedly deserves special attention as an important and effective factor in the treatment of bacterial infections than to antibiotics and chemotherapeutics.

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