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Application of Phages

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Abstract: A bacteriophage is type of virus, which can infects bacteria, either kill a bacterial cell or integrate its DNA into the host bacterial chromosome. For the first time d'Herelle called the virus bacteriophage or bacteria-eater. Phages can replicate only inside host cells. They are associated with most bacterial families and use the ribosomes, protein-synthesizing factors, amino acids and energy-generating systems of the host cell to replicate. Indeed, phages can multiply only in metabolizing host bacteria. Nowadays, using bacteriophage in different purpose viz., phage typing, Phages as indicators, as food preservatives and decontaminants, transducer and therapeutic agents considered as tremendous investigate to make facility for human being. Hence, this study conducted to application of phage in order to prepare compact information concerning to the same.

Key words: Bacteriophage, application, exploitation

Phage Typing

The limited host range of many phages makes them useful for distinguishing different strains within the same bacterial species. The great advantage of typing bacteria by phage in this way is that it will detect differences between strains that are identical by serological and other tests, so that precise surveys can be made of the distribution and spread of a given phage type of pathogen within a community. Phage typing for epidemiological purposes has been particularly successful in *Salmonella* infections, notably typhoid fever and in staphylococcal infections in hospital.

The technique consists in inoculating the surface of a nutrient agar plate with the test strain and adding each member of a set of different typing phages. After incubation, the confluent bacterial growth is interrupted by patches of lysis by the phages able to attack the strain. The phage type is empirically established by the phage, or combination of phages, which attack the strain.

In phage-typing it is essential to use the phage in a concentration that reveals its specificity. In addition, the phage preparations used for typing are impure because of spontaneous variation of the phage. Each typing phage preparation is, therefore, used in the highest dilution, which produces a patch of confluent lysis on its corresponding phage type, a dilution known as the routine test dilution (Topley and Wilson, 1990).

Sakamoto *et al*. (1975) had studied phage typing method for *P. aeruginosa* by using phage groups instead of the individual phages. The results were more clear and reproducible. They concluded that this system was specific for *P. aeruginosa* or its closely related species. On the other hand, phage typing was also used to track outbreak, epidemic and community strains of *S. aureus* effectively (Blair and Williams, 1961; O'Brien *et al*., 1999; O'Neill *et al*., 2001).

Besides, Zadoks *et al*. (2002) studied *S. aureus* isolates from bovine milk by phage typing, Pulsed Field Gel Electrophoresis (PFGE) and binary typing and concluded that PFGE was more discriminatory than phage typing. In addition, Gustafson *et al*. (2003) had used phage typing and *Sma*I

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chromosomal RLFPs to study relatedness between a collection of 12 well-characterized *in vitro* selected vancomycin intermediate *Staphylococcus aureus* (VISA) strains and their seven vancomycin susceptible parent strains. Thus, findings indicated inappropriate relationship between VISA and vancomycin susceptible parents if phage typing is utilized to determine epidemiological relationship.

For decades, bacteriophage typing was the standard method for typing of *S. aureus* and is widely used today despite a number of drawbacks (Struelens *et al*., 1996; Walker *et al*., 1999 ; Van Belkum, 2000). In the past decades PFGE has replaced bacteriphage typing as the gold standard for typing *S. aureus* isolates. It is because PFGE is known to be more discriminatory than phage typing. (Bannerman *et al*., 1995; Shimizu *et al*., 1997; Givney *et al*., 1998; Walker *et al*., 1999; Van Belkum, 2000; Buzzola *et al*., 2001; Zakoks *et al*., 2002; Gustafson *et al*., 2003).

Phages as Indicators

Guelin (1948) was the first to recognize the potential of bacteriophage as an indicator and, numerous reports have indicated the potential of bacteriophage/coliphage as indicators of microbiological quality of water (Hilton and Stotzky, 1973; Grabow *et al*., 1987; Kennedy *et al*., 1985; Borrego *et al*., 1987). They have suggested that coliphages can be used as indicators of water pollution and as possible models for enteroviruses during treatment of drinking water and wastewater (Hilton and Stotzky, 1973; Kott *et al*., 1978; Scarpino, 1978).

In addition, Coliphages have been suggested to be better indicators of enteroviruses as they have been found to be removed at comparable rates with enteroviruses during treatment processes, to exhibit a seasonal variation similar to that of enteroviruses and certain coliphages are at least as resistant to environmental stresses and to chlorination as enteroviruses (Kott *et al*., 1974; Settler, 1984). Reports suggest that coliphages and enteroviruses are removed at comparable rates during treatment process and that coliphage exhibit a seasonal variation similar to that of enteroviruses (Berg, 1974; Kott *et al*., 1974; Simokova and Cervenka, 1981).

On the other hand, Dana *et al*. (2003) reported that, Male-specific (F^+) coliphages have been investigated as viral indicators of fecal contamination that may provide source-specific information for impacted environmental waters. In their study, they examined the presence and proportions of the different subgroups of F^+ coliphages in a variety of fecal wastes and surface waters with well-defined potential waste impacts. The results indicated that, municipal wastewater samples had high proportions of F^+ DNA and group II and III F^+ RNA coliphages. Bovine wastewaters also contained a high proportion of F^+ DNA coliphages, but group I and IV F^+ RNA coliphages predominated. Swine wastewaters contained approximately equal proportions of F^+ DNA and RNA coliphages and group I and III F^+ RNA coliphages were most common. Waterfowl (gull and goose) feces contained almost exclusively F^+ RNA coliphages of groups I and IV. No F^+ coliphages were isolated from the feces of the other species examined. F^+ coliphage recovery from surface waters was influenced by precipitation events and animal or human land use. They have reported, there were no significant differences in coliphage density among land use categories, but significant seasonal variation was observed in the proportions of F^+ DNA and RNA coliphages. Hence, they believed that, monitoring of F^+ coliphage groups can indicate the presence and major sources of microbial inputs to surface waters, but it must be mentioned that, environmental effects on the relative occurrence of different groups need to be considered.

In addition, Sharon *et al*. (2006) reported that, Male-specific (F^+) coliphages have been proposed as a candidate indicator of fecal contamination and of virus reduction in waste treatment. They have isolated coliphages from municipal wastewater sludge and from biosolid samples after thermophilic anaerobic digestion to evaluate the susceptibility of specific groups to thermal inactivation. The results indicated that, similar numbers of F^+ DNA and F^+ RNA coliphages were found in untreated sludge, but the majority of isolates in digested biosolids were group I F^+ RNA phages. Separate experiments on individual isolates at 53°C confirmed the apparent heat resistance of group I F^+ RNA coliphages as

well as the susceptibility of group III F^+ RNA coliphages. Although few F^+ DNA coliphages were recovered from the treated biosolid samples, thermal inactivation experiments indicated heat resistance similar to that of group I F^+ RNA phages. Therefore, in this study with a laboratory thermophilic anaerobic digester, a heat-resistant fraction of F^+ coliphage populations indigenous to municipal wastewater and sludge was evident.

Although some studies have shown that faecal coliforms and faecal streptococci do not provide adequate information about viruses, particularly in terms of their fate in the environment and their resistance to treatment (Geldenhuys and Pretorius, 1989; Merrett *et al*., 1989; Havelaar *et al*., 1993), three types of bacteriophages have been proposed as specific indicator of viral contamination: the somatic coliphage (Morinigo *et al*., 1992), the F-specific RNA bacteriophage (Havelaar, 1986) and the *Bacteroides fragilis* phages (Jofre *et al*., 1986). On the other hand, Gantzer *et al*. (1998) in their study evaluated that whether somatic coiphages, *B. fragilis* phages and the enterovirus genome could be used as indicators of enterovirus contamination. They concluded that in the three different types of wastewater tested, *B. fragilis* phages were good indicators of enterovirus contamination.

Moreover, Durán *et al*. (2003) reported that the three groups of bacteriophages (somatic coliphages, F-specific RNA and bacteriophage infecting *Bact. fragilis*.) studied were resistant to chlorination than bacteria and some of them were more resistant than enteroviruses. Thus, the bacteriophages offer a wide range of resistance to chlorination that may represent most of the viruses that can be found in water. Hence, data reported in this study support the inclusion of bacteriophages as additional indicators of the efficiency of water chlorination processes and water quality.

Besides, Lucena *et al*. (2003) believed that the number of bacterial indicators and bacteriophages were relatively similar in 22 sampling sites in 10 rivers in Argentina, Colombia, France and Spain and they observed that bacteriophage persist in rivers much longer than bacterial indicators. They illustrated that in surface fresh water; somatic coliphages will provide additional information than bacterial indicator. Consequently, the detection and counting of one bacterial indicator and somatic coliphages will be more informative about the presence of persistent pathogen than the enumeration of two bacterial indicators.

Phages as Food Preservatives and Decontaminants

Greer (1986) illustrated that at highest concentration of phages (10^8 pfu mL^{-1}) the steak case life was significantly increased from 1.6 to 2.9 day and concluded that phages could multiply on the steak surface and have potential for the biological control of beef spoilage. Then, he with his colleague in 1990 evaluated biological control of beef spoilage with bacteriophage pool (7 phages) under simulated retail conditions and they concluded that the phage pool produced a significant reduction in Pseudomonas growth (Greer and Dilts, 1990). Besides, they studied control of *Brochothrix thermospactos* spoilage of pork adipose tissue using bacteriophage at low temperature (2°C). They illustrated that phages may provide a novel approach to extend the storage quality of chilled meat (Greer and Dilts, 2002).

On the other hand, effect of bacteriophages of *Pseudomonas solanacearum* and their potential for biological control of potato bacterial wilt was studied by Mosa *et al*. (1996). In their study they showed that in the presence of the bacteriophage population of *Pseudomonas solanacearum* greatly reduced and phage treatment could reduce and limit survival of *Pseudomonas solanacearum* in soil. In addition, several reports published regarding effect of bacteriophages on control of bacterial spot on tomato (Somodi *et al*., 1997; Flaherty *et al*., 2000; Balogh, 2002). They concluded that application of phages to field grown tomatoes significantly reduced disease severity in field trials on tomato compared to the standard copper-mancozeb treatment.

Besides, Modi *et al*. (2001) studied effect of phage on survival of *Salmonella enteritidis* during manufacture and storage of cheddar cheese. They demonstrated that the addition of phage may be a useful adjunct to reduce the ability of *Salmonella* to survive in cheddar cheese made from raw and pasteurized milk.

Biocontrol of *Salmonella* and *Listeria monocytogenes* on fresh-cut fruit was studied by Leverentz *et al*. (2001 and 2003). They found that the phage mixture reduced *Salmonella* population on honeydew melon slices. However, the phages did not significantly reduce *Salmonella* populations on the apple slices because of acidic pH of apple slice. In addition, they showed that spray application of the phage and phage plus nisin reduced population of *L. monocytogenes*. In 2004 they applied a phage cocktail to honeydew melon pieces before and after contamination with *L. monocytogenes*.

The result indicated that the effectiveness of the phage application on honeydew melon pieces can be optimized by using a phage concentration of at least 10^8 pfu mL^{-1} applied up to 1 h after processing of the honeydew melons (Leverentz *et al*., 2004).

In another study lytic bacteriophages, applied to chicken skin that had been experimentally contaminated with *Salmonella enteritica* serovar Enteritidis or *Campylobacter jejuni* at a multiplicity of infection (MOI) of 1, increased in titer and reduced the pathogen numbers by less than 1 $\log_{10}$ unit. Phages applied at a MOI of 100 to 1000 rapidly reduced the recoverable bacterial numbers by up to 2 $\log_{10}$ units over 48h. When the level of *Salmonella* contamination was low (<$\log_{10}$ 2 per unit area of skin) and when the MOI was 10^5, no organisms were recovered. By increasing the number of phage particles applied (i.e., MOI of 10^7), it was also possible to eliminate other *Salmonella* strains that showed high levels of resistance because of restriction but to which the phages were able to attach (Goode *et al*., 2003).

On the other hand, lytic phages against human isolates of *E. coli* O157: H7 were isolated and evaluated for their ability to lyse the bacterium *in vivo* and *in vitro*. For this experiment 18 pieces of meat were inoculated with rifampin -resistant derivative of *E. coli* O 157: H7 strain P1432. A phage cocktail composed of phages e11/2, e4/1c and pp01 was pipetted onto nine pieces of meat and no phages were pipetted onto another nine pieces, which acted as controls. The enrichment step was included to permit the detection of any surviving *E. coli* cells. All nine-control pieces of meat were positive, exhibiting counts of *E. coli* O157:H7 of around 10^5 cfu mL^{-1} while, in the samples where phage cocktails had been added; out of nine samples seven were completely free of *E. coli* O157:H7. Hence, O'Flynn *et al*. (2004) suggested that O157 antigen specific phages could be applied for biocontrol of *E. coli* O157:H7 in animal and fresh foods.

In addition, Bahador (2006) has studied the effect of coliphage and staphylophage on survival of *E. coli* and *S. aureus* in river water and raw milk respectively. The results obtained form the study illustrated that, number of *E. coli* in river water reduced indicating activity of coliphage however, reduction of *S. aureus* in presence of staphylophage in raw milk was not significant.

Phage as Transducer

Bacteriophages that can package host DNA are called transducing phages. There are two types of these phages viz., 1. Generalized transducing phage such as the *E. coli* phage P1 and the *S. typhimurium* phage P22 occasionally package host DNA fragments randomly during lytic growth. 2. Specialized transducing phages such as λ are formed as a result of inexact excision of the prophage following induction (Campbell, 1976).

Generalized transduction discovered in *Salmonella typhimurium* more than 50 years ago by Zinder and Lederberg (1952). They showed that genetic material of strain LT22 could be transferred to recipient cells of strain LT2 by means of a temperate bacteriophage, PLT-22 (now called P22). Then Lennox (1955) realized that *E. coli* phage P1 was able to transfer the genetic material of host cells and since that time, generalized transducing phages have become invaluable tools for fine mapping of genes.

On the other hand, Ikeda and Tomizawa (1965) and Schmieger (1968) illustrated that it is possible to transduce effectively with clear plaque mutants of the known generalized transducing phages, such as P1and P2. Therefore, they concluded that reasonable transduction results with clear plaque mutants can only be obtained by using lysogenic recipients.

In addition, Schicklmaier and Schmieger (1995) examined frequency of generalized transducing phages in natural isolates of the *Salmonella typhimurium* complex. A total of 46 different phages be assayed and 41 of them (89%) were able to transduce the his+ marker. On the other hand, all phages, which were able to transduce the his+ marker also transferred the trp+ marker and all his+ and trp+ transducing phages were also able to encapsulate and transfer this plasmid. Therefore, they concluded that all phages tested were generalized transducing phages.

Phages as Therapeutic Agents

Bacteria resistant to most or all available antibiotics are causing increasingly serious problems, raising widespread fear of returning to a pre-antibiotic era of untreatable infections and epidemics. Despite intensive work by drug companies, very few classes of antibiotics have been found in recent years.

There are hopes that the new found ability to sequence entire microbial genomes and to determine the molecular bases of pathogenecity will open new avenues for treating infectious disease; but other approaches are also being sought with increasing fervor. One result is a renewed interest in the possibilities of bacteriophage therapy the harnessing of specific kind of viruses that attack only bacteria, to kill pathogenic microorganisms (Levin and Bull, 1996; Lederberg, 1996; Radestky, 1996; Barrow and Soothill, 1997).

During last two decades data have been accumulated to show that phage therapy became important alternative to antibiotics in the treatment of bacterial infections. In many cases successful results have been obtained in combating infections in humans and animals (Weber-Dabrowska *et al*., 2000).

The first known report of successful phage therapy came from Bruynoghe and Maisin,(1921) who used phage to treat staphylococcal skin infections. Phage therapy and sanitation measures were the primary tools in d'Herelle (1926) arsenal to deal with major outbreaks of infectious diseases throughout the Middle East and India. The most detailed publications documenting phage therapy have come from Stefan Slopek's Group at the Institute of Immunology and Experimental Medicine of the Polish Academy of Science in Wroclaw. This group published a series of detailed papers in the Archivum Immunologiae et therapie Experimentalists (Slopek *et al*., 1983; 1985; 1987), describing the results of phage treatments carried out from 1981 to 1986 with 550 patients.

The bacteriophages were used in phage therapy all were virulent. Soothill (1994) carried out a series of very nice studies preparatory to using phages for infections of burn patients. Using guinea pigs, he showed that skin-graft rejection could be prevented by prior treatment with phage against *Pseudomonas aeruginosa*. He also saw excellent protection of mice against systemic infections with both *Pseudomonas* and *Acinetobacter* when appropriate phages were used (Soothill, 1992). Then Ahmad (2002) studied treatment of post-burns bacterial infections by bacteriophages. Results have shown that phage therapy can effectively be used for the treatment of post-burn infection particularly the ubiquitous opportunistic pathogen *Pseudomonas* sp.

In addition, Perepanova *et al*. (1995) inferred that phage therapy is effective and safe therapeutic modality in the treatment of urinary infection in monotherapy and in combination with antibiotics. Merrill and Adhya (1996) have carried out a series of experiments designed to better understanding of the interactions of phages with the human immune system. Recently, *E. coli* O157 has been the subject of much concern, with contamination of such products as hamburgers and unpasteurized fruit juices leading to serious epidemics (Grimm *et al*., 1995). Deaths had occurred, particularly in young children and the elderly, usually from hemorrhagic colitis (bloody diarrhea) or hemolytic-uremic syndrome, where the kidneys are affected. Antibiotic therapy has shown no benefit (Greenwald and Brand, 1997). Scientific finding that the version of O157 from the Seattle fast-food-chain epidemic, at least, is susceptible to several of T4-related phages. It is interesting to consider their potential use in feedlots and meat-packing plants and in prophylaxis and therapy during outbreaks.

Phage therapy was tried extensively and many successful cures were reported for a variety of diseases, including dysentery, typhoid and paratyphoid fevers, cholera and pyogenic (pus-producing) and urinary tract infections. Phages were poured into lesions, given orally or applied as aerosols or enemas. They were also given as injections-intradermal, intravascular, intramuscular, intraduodenal and intraperitoneal, even into the lung, carotid artery and pericardium.

It should be highlighted that in many cases following bacteriophage therapy, an increased protection against subsequent bacterial and viral infections was observed. Thus, it may be that the bacteriophage therapeutic effect (disappearance of clinical symptoms and negative bacteriologic tests) is not the result of the destruction of bacterial cells at the infection sites but also a consequence of bacteriophage on regulation of the immune response. While monitoring the immune status of patients receiving bacteriophage it was noted that effective bacteriophage therapy is associated with normalization of cytokine production by blood cell cultures (Weber-Dabrowska *et al*., 2000).

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