
TOXICOLOGICAL STUDIES OF VEGSAVPHAGE7 AGAINST *PECTOBACTERIUM CAROTOVORUM* SUBSP. *CAROTOVORUM***¹V. Ponvizhi Ramya, ²S. Gunasekaran and ³M. Senthil Kumar**

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ABSTRACT

Vegsavphage7 that was isolated against *Pectobacterium carotovorum* subsp. *carotovorum* was tested for toxicity. It is important because *Pectobacterium* is an opportunistic human pathogen, hence toxicity study may be helpful to analyze whether pathogenic gene was inserted into that isolated phages. Repeated oral toxicological study was conducted on wister rats for *Vegsavphage7* and that was not toxic to rats and hence, they will be safe to humans. Hispathopathological and haematological profile was normal when compared with control. Non *Vegsavphage7* could be better exploited as a novel biopreservative for vegetables.

INTRODUCTION**Spoilage bacteria in vegetables**

The bacterium *Pectobacterium carotovorum* subsp. *carotovorum* is a highly effective spoilage microbe that causes soft rot across a broad host range of vegetables and some fruits (Lund *et al.*, 1983). *P. carotovorum* subsp. *carotovorum* is one of that infect and destroy plant tissues both pre and postharvest and is the species that causes the greatest damage to harvested vegetables. *P. carotovorum* subsp. *carotovorum* is a plant pathogen which causes soft rot in a number of economically important crops, such as potatoes, carrots and beets (Pérombelon, 1992).

Safety of current bacteriophage-based food applications

Commercial production of the first phage for use in foods has begun in the Netherlands (www.ebifoodsafety.com), with their Listex™ P100 product (Carlton *et al.*, 2005) launched to control *Listeria* in cheese and meat. Phages may also be seen as a natural alternative to chemical preservatives. Early phage therapy pioneers demonstrated safety by ingesting preparations themselves, and a recent publication reported the safe intake of phage T₄ by volunteers (Bruttin and Brussow, 2005). Another approach is the application of purified phage-derived antimicrobials. These are enzymes (endolysins) produced by lytic phages which degrade the cell wall from the inside out, allowing phages to burst out of the cell. These enzymes are generally very host specific (Fischetti, 2005) and can also work from the outside in. The use of endolysins is therefore best suited for Gram positive pathogens, because Gram negative hosts have an outer membrane which hinders access by the enzyme to the cell wall. However, this barrier is not insurmountable, with peptidoglycan degradation by a *Pseudomonas* endolysin recently demonstrated (Paradis-Bleau *et al.*, 2007). Whatever the perceptions concerning safety it is a fact that phages are a normal component of foods (Tsuei *et al.*, 2007) and so are being ingested by everyone every day. While the use of phages to kill *Salmonella* in host guts has historically met with mixed results, the inactivation of both *Salmonella* and *Campylobacter* in chickens has been demonstrated (Atterbury, 2007). Humans have been exposed for decades to phages, when they eat raw produce (Kennedy *et al.*, 1986), water (Bergh *et al.*, 1989), raw milk (Whitman and Marshall, 1971), other foods such as fermented products fresh and oysters (Denis, 1975).

Only in the last few years has there been an extension of lab-based trials into the food production environment, where perhaps the best example is the recognition of the efficacy and the granting of 'generally recognized as safe' (GRAS) status to bacteriophages targeting *Listeria monocytogenes* by the United States Food and Drug Administration. There are two products of note now available commercially-List- Shield™ (Intralytix Inc., USA) a phage cocktail comprising virulent phages with broad activity against *L. monocytogenes*, and Listex P100™ (EBI Food Safety, Netherlands). Following GRAS classification both List- Shield™ and Listex P100™ are now viewed as safe to be applied as food biopreservatives on ready-to-eat food in the USA. Phage P100 (the active component of Listex P100) was initially characterized at the genetic level and in oral toxicity studies by Carlton *et al.* (2005). These studies showed that P100 had no undesirable genes within its genome, and caused no ill effects when administered to rats. Many studies reporting the efficacy of Listex P100 against *L. monocytogenes* in various food production settings are now available in the literature (Guenther *et al.*, 2009, Soni *et al.*, 2010, Soni and Nannapaneni, 2010).

If more phage-based applications are to reach standards where they are deemed fit for human/animal consumption then certain matters pertaining to safety must be taken into consideration. For instance, phages that show potential during preliminary studies must adhere to strict criteria if they are to be developed further as

antimicrobial agents (Merabishvili *et al.*, 2009). Most importantly, the selected phages must not possess genes associated with virulence, or those that may enhance the pathogenic profile of its target (Gill and Hyman, 2010). The use of virulent bacteriophages (phages) as biological control (biocontrol) agents against bacterial pathogens is an expanding field of research aimed at producing sustainable solutions for the control of these pathogens, and to circumvent problems such as those associated with the development of multidrug-resistant bacteria (Mahony *et al.*, 2011). Bacteriophages in bioprocessing are used to reduce the bacterial load in foods usually in the minimally processed foods to avoid cooking associated flavor or texture (García *et al.*, 2010).

MATERIALS AND METHODS

Toxicity analysis of bacteriophages

Toxicity analysis of bacteriophages was conducted in Kovai Medical College and Hospital (KMCH) College of Pharmacy, Coimbatore.

Culture conditions

The concentrated phage solutions 5×10^{15} were then stored at -4°C until they were utilized for assays.

Experimental animals and housing conditions

This study was conducted according to the current principles of good laboratory practice. A total of 10 healthy male and 10 healthy female Wistar albino rats (Origin Laboratory) of about 8 weeks of age were used, with a pre-test body weight range of g 130 -160 g per male, and 130–170 g per female. Animals were randomly selected and assigned to three groups of five males and five females per group, and individually identified by ear tags. The rats were housed 1 per cage in stainless steel wire bottom cages, in a temperature controlled animal room, with a 12 h light/dark cycle.

Diet

Fresh rodent chow diet was provided, except for the fasting period of one day prior to sacrifice. Fresh water was provided.

Repeated oral toxicity study

As test material for the oral studies, purified and concentrated (5×10^{11} pfu/ml) Vegsavphage 7 in SM buffer saline pH 7 was used. The slightly cloudy liquid was aliquot in five tubes containing 12ml each, and stored at 4°C for the duration of the experiment. The phage suspension and control liquid (SM) were orally administered once daily, over a five day period, using a syringe and 16 gauge ball-tipped feeding needle. Control group I was not treated. Animals in group 2 were given a dose of 1.0ml of Vegsavphage 7. Animals in group 3 received 1.0ml of SM buffer only. Body weights were recorded pre-test and prior to termination. The animals were observed once daily for toxicity and pharmacological effects, and twice daily for morbidity and mortality.

Food consumption was calculated daily and also at the end of the study. On day 8, all animals were anesthetized with ether, sacrificed, and exsanguinated. All animals were examined for gross pathology. The Kidney, Brain. Lungs, liver, esophagus, stomach, duodenum, jejunum, ileum, cecum, and colon were preserved in 10% neutral buffered formalin. Histopathologic preparation (cross-sections and longitudinal sections) and microscopical analysis were performed according to standardized procedures. All results were evaluated based on the relationship between the dose levels and incidents or severity of responses.

Thirty rats were randomly divided into six groups with five animals in each group. The male and female rats were randomly assigned to control and treatment groups. The rats were fed with one ml of bacteriophages (6×10^{13} PFU ml^{-1}) orally to assess the cumulative effect of high doses. The following general behaviors were observed for first 1h and after 24 h of test drug administration *viz.*, (hypoactivity, passivity, muscle relaxation, narcosis, and ataxia), CNS activity (hyperactivity, irritability, stereotypy, tremors, convulsions, straub reaction, and analgesia) and ANS activity (ptosis, exophthalmia, urination, salivation and lacrimation). Rats were observed thoroughly for onset of any immediate toxic signs. Body weight gain was monitored and all the animals were observed thoroughly for the onset of any toxicity. At the end of the experimental period all the animals were sacrificed under light ether anesthesia.

Organ weight and histopathological studies

The following vital organs of each rat such as brain, liver, lungs and kidney were excised and weighed. Histopathological investigation of Lungs, Liver, Brain, and Kidney of all experimental and control rats were performed after sacrificing them at the end of 8 days of administration. These tissues were separately sliced in pieces, fixed in 10 per cent formaline for 3 days; processed; stained using hematoxylin, eosin reagent and diphenyl xylene mounting fluid; mounted on glass slides and observed under power microscope.

Hematological study

Blood samples were collected from at least 3 rats of each group and were examined for hematological profiles like haemoglobin concentration (Hb), erythrocytes / Red Blood Cell (RBC), leucocytes / White Blood Cell (WBC), Packed Cell Volume (PCV), differential count and total platelet count were calculated according to the method of Bharucha *et al.* (1976).

Biochemical analysis

Plasma samples were used to estimate the various biochemical parameters such as aspartate transaminase (SGOT), alanine transaminase (SGPT), alkaline phosphatase, cholesterol and urea by using standard protocols.

Determination of Aspartate Transaminase (AST) or SGOT activity

AST catalyzes the transfer of amino group between L-aspartate and α -ketoglutarate to form oxaloacetate and glutamate. The oxaloacetate formed reacts with NADH in the presence of malate dehydrogenase to form NAD. The rate of oxidation of NADH to NAD, is measured as a decrease in absorbance which is proportional to AST activity in the sample (Moss and Henderson, 1999).

Determination of Alanine Transaminase (ALT) or SGPT activity

ALT catalyzes the transamination from L-alanine to 2-oxoglutarate, forming L-glutamate and pyruvate. The pyruvate formed is reduced to lactate by LD with simultaneous oxidation of reduced NADH to NAD. The change in absorbance with time due to the conversion of NADH to NAD is directly proportional to AST activity (Moss and Henderson, 1999).

Estimation of alkaline phosphatase

The method is similar to that of King and Armstrong in which disodium phenyl phosphate is hydrolysed with liberation of phenol and inorganic phosphate. The liberated phenol is measured at 700 nm with Folin-Ciocalteu reagent (King and Armstrong, 1964).

Estimation of cholesterol

The extraction and oxidation of cholesterol was done by acid solution of ferric chloride. Addition of H_2SO_4 to the cholesterol and ferric chloride mixture forms coloured complex which is read at 560 nm (Zak and Ressler, 1955).

Estimation of urea

Urease breaks down urea into ammonia and carbon dioxide in alkaline medium. Ammonia liberated from the breakdown of urea reacts with hypochlorite and salicylate to form dicarboxyindophenol. This reaction is catalysed by the presence of nitroprusside. The intensity of the colour produced by the reaction is directly proportional to the concentration of urea present in the sample and it is measured photometrically at 600 nm (Fawcett and Scott, 1960).

RESULTS**Repeated oral toxicity study****Clinical signs and mortality**

All the mice were observed daily with the purpose of recording any symptoms of ill-health or behavioral changes. These observations were also performed on week-ends. The observations included changes in skin and fur, in the eyes and mucous membranes, in the respiratory, circulatory, central nervous and autonomous systems, soma to motor activity and behavior

All the mice survived until scheduled necropsy and showed normal growth and appeared healthy throughout the study period. Daily general observations, ophthalmoscopy and clinical examinations revealed no treatment – related changes (Data not shown) occurred.

Body weight, Food intake and water intake

Oral feeding of bacteriophages and SM buffer did not significantly affect food intake and water intake in male and female rats of treatment groups and were comparable to that of control group. No difference in food and water intake was observed in treatment groups of male rats during the 8 days of experimental period.

The bodyweight of each rat was recorded before the start of the treatment and the changes of body weight recorded every at the end of the study. The changes of body weight in bacteriophages treated male rats were slightly increased tend to be higher than the control values.

Hematological profile

Haemoglobin, red blood cells, white blood cells, packed cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, differential count, mean corpuscular volume, red blood cell distribution width, platelet count and mean platelet volume did not show any biologically or statistically significant differences between control and treatment groups.

Biochemical parameters

No marked alteration in any of the specific activities of enzymes in bacteriophages pigments treated rats was noticed. The record of biochemical parameters in treatment groups of rats were not significantly different in comparison with the control group indicating that bacteriophages have no adverse effect on liver and kidney functioning.

A decrease and increase in urea and serum cholesterol level was observed in male and female rats fed with bacteriophages discretely. The decrease in urea of male and female rats was 36.56 per cent and 29.60 respectively. The increase in serum cholesterol levels of male and female rats 148.68 per cent and 159.52 respectively. The results indicate that bacteriophages invariably decreased and increased the urea and serum cholesterol in experimental rats.

Organ weights and histopathology

Relative organ weight of brain, heart, liver, lungs and kidney (left and right), of treated groups were comparable to that of controls. No histopathological alterations were observed on repeated oral application.

Organ weight

There were no significant difference in the group mean relative weights of various vital organs (brain, liver, lungs, kidney (left and right) in the bacteriophages treated male and female rats compared to that of control rats.

Histopathological examinations

No abnormality was observed between the control and pigment (red and reddish brown) fed rats indicating no treatment related histological changes in various tissues or organs examined. Both of these two pigments have no adverse effect on cellular structure of these organs. Gross examinations of vital organs during autopsy did not reveal any abnormalities that could be attributed to repeated oral dose of bacteriophages in male and female rats. Further on microscopic examinations, no treatment related histopathological alterations were observed in any of the vital organs. Detailed histopathological examinations of each vital organ are shown in Table 1 and 2. Liver was characterized by normal hepatic tissue with distinct nuclei and normal eosinophilic cytoplasm with normal sinusoids. There was no inflammation, fatty change or fibrosis. Kidney displayed normal renal parenchyma with normal glomerules, proximal tubules and collecting ducts. Normal cerebral cortex was observed in brain cells. The cerebellum showed normal purkinjie cells and molecular layer. The hippocampus displayed normal morphology. Lungs showed normal bronchioles. The alveolar spaces were unremarkable. The interstitial were normal. There were no inflammations or fibrosis.

DISCUSSION**Safety of bacteriophages**

Phages are highly specific and can infect only a very limited range of host bacteria. All available evidence indicates that their oral consumption (even at high levels) is entirely harmless to humans. The phages were mostly administered orally or superficially but they were also injected intramuscularly, intravenously, and even into the pericardium and carotid artery (Ackermann and DuBow, 1987). Most noteworthy, none of the reports mentioned significant phage related undesirable side effects. Safety studies have been performed for example with the *Listeria*-phage P100, in which rats were fed high doses of phages with no measurable effects compared to the control group (Carlton *et al.*, 2005).

A study with *E. coli* phages both in mice and in human volunteers also showed no significant effects on the test subjects (Chibani-Chennouf *et al.*, 2004), (Bruttin and Brüssow, 2004). Perhaps the most remarkable aspect of this latter study was the fact that although these phages were able to infect commensal *E. coli* strains *in vitro*, they seemed to have little effect on the *E. coli* occurring in the gut ecological systems of the animals or human volunteers. In the mouse model, only *E. coli* cells implicated in an artificial infection model were affected by the phages. It was speculated that the commensal *E. coli* population lives in niches not easily accessible to phages.

Since the human intestinal tract generally hosts a plethora of phages (which nonetheless does not result in dysbiosis of the gut), this speculation makes sense and is likely true for also for the other bacteria and their bacteriophages living in the gut.

Overwhelming additional (albeit more circumstantial) evidence exists corroborating the results observed in specific safety studies. For example, even though phage therapy was not used extensively in the Western world over the past more than 50 years (except for France), thousands of people have received phage therapy in other countries, especially the former Soviet Union and Poland (Kutter *et al.*, 2010).

Phages are Ubiquitous in Foods

Our environment features a massive abundance (both in numbers and variety) of phage particles, with aquatic environments currently holding the record - up to 10^9 phages per milliliter have been reported for certain freshwater environments, and up to 10^7 phage-like-particles per millilitre were found in marine surface systems. Similar numbers have been reported for terrestrial ecosystems such as topsoil. In fact, with estimates of 10^{31} (or more) in total, phages represent the by far most abundant form of self-replicating units in the biosphere (Rohwer and Edwards, 2002). Apart from environmental sources, humans are constantly exposed to contact with phages by way of their food. Bacteriophages are associated with bacteria and any foodstuff that has not undergone extensive processing will contain phages, with fermented food of course having especially high numbers of those phages infecting the fermentation flora. Fresh vegetables are also a rich source of bacteriophages. Several studies have been undertaken to enumerate phages in food but it should be kept in mind that only specific phages were investigated, always employing only a specific and limited sets of host bacteria.

Because of this bias, total phage populations are not properly reflected; even some of the species-specific phages under investigation may have been missed due to a lack of susceptible host bacteria used in the study. A few examples: In Argentina, phages infecting thermophilic lactic acid bacteria have been isolated from dairy plant samples at numbers of up to 10^9 PFU/ml, though these were from batches that failed to achieve the desired fermentation levels (Suarez *et al.*, 2002). Fermenting cabbage (Sauerkraut) is a good source of phages (Barrangou *et al.*, 2002, Yoon *et al.*, 2002), with one study describing 26 different phages isolated from commercial Sauerkraut fermentation plants (Lu *et al.*, 2003).

Also, *Campylobacter* phages have been isolated at levels of 4×10^6 PFU from chicken (Atterbury *et al.*, 2003), and *Brochothrix thermosphacta* phages. Phage biocontrol of foodborne pathogens is a rather more recent idea, which seems to be gaining momentum. Perhaps more importantly, phages can also be isolated from non-fermented foods. *E. coli* phages have been recovered from fresh chicken, pork, ground beef, mushrooms, lettuce, raw vegetables, chicken pie, and delicatessen food, with counts as high as 10^4 phages per gram (Allwood *et al.*, 2004).

Toxicity evaluation of bacteriophages

Oral toxicity evaluation for 8 days toxicity test is an accustomed practice in a repeated oral toxicity study. Safety of bacteriophages was analyzed for oral acute toxicity which is a prerequisite for application as a biopreservative. In the studies no treatment related adverse effects were observed for any parameters in male and female rats. Although the body weights were increased, there were no significant changes of body weights in female rats and control groups. The RBC and other hematological parameters did not show any biologically or statistically significant differences between rats fed with both pigments individually or controls.

There were no significant changes in histological findings in lungs, liver, brain and kidneys and were comparable in incidence and severity in control and treatment groups. Minor changes observed were common in rats and were not considered to be treatment related. No other significant or treatment related increases in incidence of abnormalities were observed in any of the other tissues examined. Gross examination and microscopy examination also revealed that administration of bacteriophages could not be lethal. Incorporation of bacteriophages did not significantly affect food and water intake in treatment groups and were comparable to that of control group. Clinical chemistry evaluations in 8 days oral dosing groups did not show any statistical significant difference between treatment and control groups.

Other studies on the application of phage to animals also reported no adverse or unexpected effects of bacterial viruses on animals (Berchieri *et al.*, 1991; Biswas *et al.*, 2002; Cerveny *et al.*, 2002; Chibani-Chennou *et al.*, 2004; Merrill *et al.*, 1996). In line with this, a recent a study with human volunteers receiving phage T₄ indicated that it is safe for oral administration; and no phage or phage-specific antibodies could be detected in the serum of the human subjects (Bruttin and Brussow, 2005). In conclusion, there is no reason to assume that the intake of phage with food may possibly have any negative effects on humans. With respect to phage P100, the available data suggest that its use as an additive for biopreservation of foods can be expected to be safe for consumers as well as for the environment (Carlton *et al.*, 2005). Hence vegsavphage 7 could be better safer to humans and could be coated over vegetables.

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REFERENCES

1. Ackermann, H.W., and DuBow, M. S., Eds.; CRC Press: Boca Raton, FL, 1987, Vol. I, pp. 143-172.
2. Allwood, P. B., Malik, Y. S., Maherchandani, S., Vought, K., Johnson, L. A., Braymen, C., Hedberg, C. W., Goyal, S. M. 2004. Occurrence of *Escherichia coli*, noroviruses, and F-specific coliphages in fresh market-ready produce. *J. Food Prot.*, **67 (11)**: 2387-2390.
3. Allwood, P. B., Malik, Y. S., Maherchandani, S., Vought, K., Johnson, L. A., Braymen, C., Hedberg, C. W., Goyal, S. M. 2004. Occurrence of *Escherichia coli*, noroviruses, and F-specific coliphages in fresh market-ready produce. *J. Food Prot.*, **67 (11)**: 2387-2390.
4. Atterbury R.J, Van Bergen M.A, Ortiz F, Lovell M.A, Harris J.A, De Boer A, Wagenaar J.A, Allen V.M and Barrow P.A. 2007. Bacteriophage therapy to reduce salmonella colonization of broiler chickens. *Appl. Environ. Microbiol.*, **73**: 4543-4549.
5. Atterbury, R. J., Connerton, P. L., Dodd, C. E. R., Rees, C. E. D., Connerton, I. F. 2003. Isolation and characterization of *Campylobacter* bacteriophages from retail poultry. *Appl. Environ. Microbiol.*, **69 (8)**: 4511-4518.
6. Barrangou, R., Yoon, S. S., Breidt, F., Jr., Fleming, H. P and Klaenhammer, T. R. 2002. Characterization of six *Leuconostoc fallax* bacteriophages isolated from an industrial sauerkraut fermentation. *Appl. Environ. Microbiol.*, **68 (11)**: 5452-5458.
7. Berchieri A, Jr, Lovell M A, Barrow P A. 1991. The activity in the chicken alimentary tract of bacteriophages lytic for *Salmonella typhimurium*. *Res. Microbiol.*, **142**: 541-549.
8. Bergh, O., Borsheim, K. Y., Bratbak, G. and Heldal, M. 1989. High abundance of viruses found in aquatic environments. *Nature*, **340**: 467-468.
9. Biswas, B., Adhya, S., Washart, P., Paul, B. Trostel, A.N., Powell, B., Carlton, R., and Merrill, C. 2002. Bacteriophage Therapy Rescues Mice Bacteremic from a Clinical Isolate of Vancomycin-Resistant *Enterococcus faecium*. *Infection and Immunity.*, **70**: (1) 204-210.
10. Bruttin, A and Brüßow, H. 2005. Human volunteers receiving *Escherichia coli* phage T₄ orally: a safety test of phage therapy. *Antimicrob. Agents Chemother.*, **49 (7)**: 2874-2878.
11. Bruttin, A and Brüßow, H. 2005. Human volunteers receiving *Escherichia coli* phage T₄ orally: a safety test of phage therapy. *Antimicrob. Agents Chemother.*, **49 (7)**: 2874-2878.
12. Carlton, R. M., Noordman, W. H., Biswas, B., de Meester, E. D., Loessner, M. J. 2005. Bacteriophage P100 for control of *Listeria monocytogenes* in foods: genome sequence, bioinformatic analyses, oral toxicity study, and application. *Regul. Toxicol. Pharm.*, **43(3)**: 301-312.
13. Cervený, K. E., de Paola, A., Duckworth, D. H. and Gulig, P. A. 2002. Phage therapy of local and systemic disease caused by *Vibrio vulnificus* in iron-dextran-treated mice. *Infect. Immun.*, **70**: (62) 51-62.
14. Chibani-Chennou, S., Sidoti, J., Bruttin, A., Kutter, E., Sarker, S., Brüßow, H. 2004. *In vitro* and *in vivo* bacteriolytic activities of *Escherichia coli* phages: implications for phage therapy. *Antimicrob. Agents Chemother.*, **48 (7)**: 2558-2569.
15. Denis, F. A. 1975. Contamination of shellfish with strains of *Pseudomonas aeruginosa* and specific bacteriophages. *Can. J. Microbiol.*, **21**: (10) 55-57.
16. Fawcett J.K, and Scott J.E. 1960. A rapid and precise method for the determination of urea. *J. Clin. Path.* **3**:156-159.
17. Fischetti V. A. 2005. Bacteriophage lytic enzymes: novel antiinfectives. *Trends Microbiol.*, **13**: 491-496.
18. García P, Rodríguez L, Rodríguez A, and Martínez B. 2010. Food biopreservation promising strategies using bacteriocins, bacteriophages and endolysins. *Trends Food Sci. Technol.*, **21**: 373-382.
19. Gill J.J and Hyman P. 2010. Phage choice, isolation, and preparation for phage therapy. *Curr. Pharm. Biotechnol.*, **11**: 2-14.

20. Guenther S, Huwyler D, Richard S and Loessner M.J. 2009. Virulent bacteriophage for efficient biocontrol of *Listeria monocytogenes* in ready-to-eat foods. *Appl. Environ. Microbiol.*, **75**: 93-100.
21. Kennedy, J. E., Jr., Wei, C. I., and Oblinger, J. L. 1986. Distribution of coliphages in various foods. *J. Food Prot.*, **49**: 944-951.
22. King, E.J. and A.R. Armstrong. 1964. Determination of serum and alkaline phosphatase activity. *Canadian Medical J.*, **31**: 376.
23. Kutter, E. M.; De Vos, D.; Gvasalia, G.; Alavidze, Z.; Gogokhia, L.; Kuhl, S.; Abedon, S. T. 2010. Phage therapy in clinical practice: treatment of human infection. *Curr. Pharm. Biotechnol.*, **11(1)**, 69-86.
24. Lu Z., Breidt F., Plengvidhya V., Fleming H. P. 2003. Bacteriophage ecology in commercial sauerkraut fermentations. *Appl. Environ. Microbiol.*, **69**: 3192–3202.
25. Lund, B. M. Baird-Parker, and Gould. 1983. Bacterial spoilage. In C. Dennis (Ed.), Post-harvest pathology of fruits and vegetables (pp. 218–257). London: Academic Press.
26. Mahony J, McAuliffe O, Ross R.P and van Sinderen D. 2011. Bacteriophages as biocontrol agents of food pathogens. *Curr. Opin. Biotechnol.*, **22**:157-163.
27. Merabishvili M, Pirnay J.P, Verbeke G, Chanishvili N, Tediashvili M, Lashkhi N, Glonti T, Krylov V, Mast J, Van Parys L, Lavigne R, Volckaert G, Matthews W, Verween G, De Corte P, Rose T, Jennes S, Zizi M, De Vos D, and Vanechoute M. 2009. Quality-controlled small-scale production of a welldefined bacteriophage cocktail for use in human clinical trials. *PLoS One*, **4**:E4944.
28. Merrill, C.R., Biswas, B., Carlton, R., Jensen, N.C., Creed, G.J., Zullo, S., and Adhya, S., 1996. Long-circulating bacteriophage as antibacterial agents. *Proc. Natl. Acad. Sci.*, **93**: 3188–3192.
29. Paradis-Bleau C, Cloutier I, Lemieux L, Sanschagrin F, Laroche J, Auger M, Garnier A and Levesque R.C 2007. Peptidoglycan lytic activity of the *Pseudomonas aeruginosa* phage fKZ gp144 lytic transglycosylase. *FEMS Microbiol. Lett.*, **266**: 201-209.
30. Perombelon M.C.M. 1992. Potato blackleg: epidemiology, host–pathogen interaction and control. *Netherlands J. Plant Pathol.*, **98(Suppl. 2)**, 135-146.
31. Rohwer, F and Edwards, R. 2002, The phage proteomic tree: a genome based taxonomy for phage. *J. Bacteriol.*, **184(6)**: 4529-4535.
32. Soni K.A, and Nannapaneni R. 2010. Removal of *Listeria monocytogenes* biofilms with bacteriophage P100. *J. Food Prot.*, **73**:1519-1524.
33. Soni KA, Nannapaneni R, and Hagens S. 2010. Reduction of *Listeria monocytogenes* on the surface of fresh channel catfish fillets by bacteriophage Listex P100. *Foodborne Pathog. Dis.*, **7**:427-434.
34. Suarez, V. B., Quiberoni, A., Binetti, A. G and Reinheimer, J. A. 2002. Thermophilic lactic acid bacteria phages isolated from Argentinian dairy industries. *J. Food Prot.*, **65 (10)**: 1597-1604.
35. Tsuei A. C, Carey-Smith G.V, Hudson J. A, Billington C and Heinemann J. A. 2007. Prevalence and numbers of coliphages and *Campylobacter jejuni* bacteriophages in New Zealand foods. *Int. J. Food. Microbiol.*, **116**:121-125.
36. Whitman, P. A. and Marshall, R. T. 1971. Isolation of psychrophilic bacteriophage-host systems from refrigerated food products. *Appl. Microbiol.*, **22**: 220-223.
37. Yoon, S. S.; Barrangou-Pouey, R.; Breidt, F., Jr.; Klaenhammer, T. R.; Fleming, H. P. 2002. Isolation and characterization of bacteriophages from fermenting sauerkraut. *Appl. Environ. Microbiol.*, **68 (2)**: 973-976.
38. Zak, B. and N. Ressler. 1955. Methodology in determination of cholesterol: A review. *Amer. J. Clin. Path.*, **25**: 433.

Table 1. Histopathological examination of male rats fed with Bacteriophages

Organ / findings	No. of mice affected / group*		
	Control	Bacteriophages	SM buffer
Kidney			
Eosinophilic body, proximal tubule	0/2	0/2	0/2
Tubular Regeneration	0/2	0/2	0/2

Mineralization, cortico – medullary junction	0/2	0/2	0/2
Liver			
Renal parenchyma with glomereular congestion	0/2	0/2	0/2
Hepatic tissue with dilation	0/2	0/2	0/2
Brain			
Gliosis, edema / degeneration	0/2	0/2	0/2
Lungs			
Inflammation / fibrosis	0/2	0/2	0/2
Oesophagus			
Inflammation / Edema	0/2	0/2	0/2
Liver			
Lobular Inflammation, necrosis Granulomas	0/2	0/2	0/2
Stomach			
Inflammation, Cryptitis	0/2	0/2	0/2
Jejunum			
Cryptitis	0/2	0/2	0/2
Colon			
Cryptitis, crypt abscess	0/2	0/2	0/2
Duodenum			
Inflammation , Ulceration	0/2	0/2	0/2
Ileum			
Inflammation , Ulceration	0/2	0/2	0/2
Rectum			
Lymphocytic infiltration	0/2	0/2	0/2

*2 Rats per group

Table 2. Histopathological examination of female rats fed with Bacteriophages

Organ / findings	No. of mice affected / group*		
	Control	Bacteriophages	SM buffer
Kidney			
Eosinophilic body, proximal tubule, Tubular Regeneration, Mineralization, cortico – medullary junction	0/2	0/2	0/2
	0/2	0/2	0/2
	0/2	0/2	0/2
Liver			
Renal parenchyma with glomereular congestion	0/2	0/2	0/2
Hepatic tissue with dilation	0/2	0/2	0/2
Brain			
Gliosis, edema, degeneration	0/2	0/2	0/2
Lungs			
Inflammation , fibrosis	0/2	0/2	0/2
Oesophagus			
Inflammation , Edema	0/2	0/2	0/2
Liver			
Lobular Inflammation, necrosis Granulomas	0/2	0/2	0/2
Stomach			
Inflammation, Cryptitis	0/2	0/2	0/2
Jejunum			
Cryptitis	0/2	0/2	0/2
Colon			
Cryptitis, crypt abscess, inflammatory infiltrates	0/2	0/2	0/2
Duodenum Inflammation , Ulceration	0/2	0/2	0/2
Ileum Inflammation , Ulceration	0/2	0/2	0/2
Rectum			
Lymphocytic infiltration	0/2	0/2	0/2

*2 Rats per group