WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 4, 242-255. Conference Article ISSN 2277– 7105

BACTERIOPHAGES AS A BIOCONTROL AGENT AGAINST FOOD BORNE PATHOGENS

Shilpa P. Tambe* , Mansura S. Mulani, Sagar Kulkarni, Anarkali Sayyad

Department of Microbiology, Abeda Inamdar Senior College, K. B. Hidaytullah Road, Azam Campus, Camp, Pune-411001, Maharashtra, India.

Article Received on
02 Jan. 2018,

Revised on 22 Jan. 2018,
Accepted on 12 Feb. 2018,

DOI: 10.20959/wjpr20184-10778

***Corresponding Author**
Shilpa P. Tambe
Department of Microbiology, Abeda Inamdar Senior College, K. B. Hidaytullah Road, Azam Campus, Camp, Pune-411001, Maharashtra, India.

ABSTRACT

Bacteriophages are obligate intracellular parasites and rely on the host bacteria for their replication and reproduction. Recently, food industries are continuously challenged with microbial contamination of food even if modern technologies are used. Chemical compounds and physical processes used to reduce microbial contamination of food may affect natural properties of food. To overcome these problems, new methods need to be developed. Bacteriophages can be applied as biocontrol agents in food safety. In this study we have isolated three bacterial strains from poultry farm. The bacterial strains are identified till genus level based on biochemical characters and they may belong to *Escherichia*, *Enterococcus* and *Serratia* species. Isolate 2 and 3 were identified till species level by 16srRNA technique. Bacteriophages against each of these specific bacteria were isolated by membrane filtration (0.2µ) and chloroform treatment. Titre for phage against Isolate1, Isolate 2 and Isolate 3 were determined to be 1.75×10^{6}, 7.32×10^{6}, 1.48×10^{6} pfu/ml respectively. One step growth curve of all phages was done. FE-SEM of phage against Isolate 2 and Isolate 3 was done. Application of phages was done on sample whose TVC was found to be 5.2×10^{8} cfu/ml, which after treatment with cocktail of phages for 3,6 and 9 hours significant reduction was observed and found to be 4.4×10^{6}, 8.2×10^{4} and 2.3×10^{3} cfu/ml respectively.

KEYWORDS: Bacteriophages, biocontrol, food contaminants, FE-SEM.

INTRODUCTION

Bacteriophages were discovered in early 1900 by Twort (1915) and d'Herelle (1917) during early 20thcentury. Before the discovery of broad spectrum antibiotics, bacteriophages were

used as therapeutics in many countries until 2nd world war. Bacteriophages are viruses that kill the bacteria and are ubiquitous in nature. They have no known activity in animal and plant cells and are harmless to them. Bacteriophages attach to target bacterium, inject their genetic material, replicate within bacterial cell using their replication machinery and kill the bacteria by lysis. Bacteriophages are most abundant on the earth (10^{30}-10^{32} particles) and widely spread including foods of various origin (Brussow and Kutter, 2005). Most phages are 24-400nm in size and has genome encapsulated by a protein or lipoprotein capsid. Capsid is composed of many copies of protein subunits called capsomere. The genetic material can be RNA or DNA between 5 and 500 kbp long. (Eiserling F.A. 1979, Hershy and Chase, 1952). Some phages have tails and base plate and one or more tail fibers attached to it. These structures are involved in attachment of phage to bacterial cells. (Ginnoza W.1967). To enter host cells, bacteriophages attach at a specific receptors on the surface of bacteria. Nucleic acid is penetrated through tail by enzymatic digestion of bacterial cell wall. Once inside the cell, the enzyme encoded by phage genome are synthesized by using host cell's machinery resulting in diverting the host cell DNA and protein synthesis towards the generation of new phage particles. The structural components of phage are assembled to form new phage and nucleic acid is packed into the phage particles are released by lysis of cells (Blasi 1995, Kropinski 2006). Depending upon the life cycle, phages are classified as lytic or lysogenic. Lytic phages start replicating immediately after infection, releasing new phage particles by lysis of cells. Whereas lysogenic phages are able to establish stable relationship by integrating a nucleic acid in the host genome and are capable to enter into lytic cycle following induction by environmental stimulus (Stella et al., 2009).

Food borne illnesses are a major cause of morbidity and mortality worldwide. The World Health Organization estimates that globally, diarrheal diseases alone (a majority of which are caused by foodborne pathogens) kill 1.9 million children per year.[5] Foodborne diseases do not only occur in developing countries, in the United States of America for example, it is estimated that foodborne diseases result in 76 million illnesses, 325,000 hospitalizations and 5,000 deaths each year.[6] Considerable effort has been directed towards the control of the major bacterial food-borne pathogens. However, this has had little impact on addressing the problem in many countries. This is because the effectiveness of the intervention initiatives has been obscured by other changing factors. These changing factors may be associated with the pathogens, their hosts (humans) or political, economic and environmental factors. Environmental challenges have caused food-borne bacterial pathogens to evolve and the

susceptibility of human population to infections are also changing due to declining acquired immunity and increased numbers of immunocompromised individuals.

There has been a continuous increase in several foodborne diseases caused by bacterial pathogens such as *Salmonella, Enterococcus, Serratia, Campylobacter, Escherichia coli* and *Listeria* despite the employment of modern technologies to inactivate these pathogens in food. These pathogens come into contact with foods during harvest or slaughtering, processing, storage and packaging. Physical treatments such as UV light, high pressure, dry heat and steam are viable strategies of reducing pathogenic bacteria in raw products. These methods have been considered because the use of antibiotics has been restricted over the years due to the risk of antibiotic-resistant bacteria entering the human food chain and causing negative impact on human antimicrobial treatment. However, physical methods of reduction of microbial load in raw foods have been known to negatively impact the organoleptic properties of the products hence reducing their acceptability. There has thus been an increasing need to develop novel strategies to reduce bacterial pathogens in foods and still satisfy consumer demand for minimally processed foods with low concentrations of chemical preservatives. Bacteriophages (phages) have found use as natural antimicrobials that can be used in controlling bacterial pathogens in foods and food processing environments. Hagens and Loesser[8] listed the nine desirable properties of phages for use as biocontrol agents in foods. We hereby discuss in detail these properties and how they influence the use of phages as biocontrol agents in different foods.

Bacteriophages have been widely used as natural antibacterial agent to control food pathogens. Phage therapy has been shown to be effective as both postharvest and pre-harvest interventions to control wide range of food-borne pathogens. Pre-harvest method is to prevent the infection of pathogenic bacteria in animals and postharvest methods involve use of bacteriophage to eliminate unwanted contaminants on the food products (Sulakvelidze).

In postharvest treatment of food, Physical processes and chemical compounds (preservatives) may not look promising and may affect natural properties of foods. The use of naturally occurring lytic phages to reduce contamination of fresh food with food-borne pathogens is advantageous over the use of chemical compounds and washes (Leverentz B., 2001). Bacteriophages can be applied to control the food contamination caused by *E.coli* (O' Flynn et al., 2003), *Campylobacter* (Atterbury et al., 2003), *Listeria* (Leverent z et al., 2003) and *Salmonella* (modi et al., 2001) on chicken and meat products.

The aim of this work is isolation, identification of food borne pathogenic bacteria such as *Enterococcus sp*. and *Serratia sp*. and isolation of lytic phages against each target bacteria in order to analyze their effectiveness in controlling contamination of food product by these bacteria.

MATERIALS AND METHODS

Collection of Sample

Poultry effluent was collected from 2 poultry farms during the month of December 2016, in pre-sterilized capped containers and transported to laboratory for further processing.

Bacteriological Analysis of effluent Sample

100µl of the water sample was diluted using sterile saline and 10^{-1} to 10^{-10}dilutions were made and was subjected to viable count studies by spreading 100µl of sample from each dilution on sterile nutrient agar plates. Plates were incubated overnight at 37^{o}C for 24 hours.

Isolation and Characterization of Target Bacteria

100µl sample was spread on selective media to isolate the target bacteria. Culture media used were 1.Eosin Methylene Blue agar, 2. Hektoen enteric agar 3.MacConkey agar. Bacterial isolates were characterized and identified as per the standard procedures.

Drug resistant profile

For testing the antibiotic resistance profile of isolated bacteria, commercially available antibiotic polydiscs, i.e. Dodeca universal I DE001 (Hi Media) were used.After spreading 0.5 ml of log phase bacterial culture onMuller - Hinton agar plate the discs were put on the media. Plates were incubated at 37^{0} C for 24 hours. Disc consist of Amikacin (AK) 30µg, Co-Tri maxazole (COT) 25µg, Colistin (CL) 10µg,Augmentin (AMC) 30µg, Netillin (NET) 30µg, Norfloxacin (NX) 10µg, Ceftriaxone (CTR) 10µg, Citrofloxacin (CIP) 5µg, Cephotaxine (CTX) 30µg, Gentamicin (GEN)10µg, Furazolidone (FR) 50µg, Amoxycillin (AMX)10µg.

Isolation of Specific Bacteriophages against Target Bacteria

Sample was filtered through coarse filter paper to remove any particulate matter. It was then transferred to sterile centrifuge tube and centrifuged at 8000 rpm for 20 min at 4°C.After centrifugation supernatant was removed and vacuum filtered through membrane filter.The filtrate was stored as lysate at 4°C.

Enrichment of Phages

In 50ml LB broth isolated bacterial strains were inoculated at 37°C to get host at log phase. 10 ml of sterile double strength PB and 10 ml lysate were added to the same flask and incubated at 37°C for 48 hours. After incubation it was centrifuged at 8000 rpm for 20 min at 4°C to remove the cell debris. Pellet was discarded and supernatant was subjected to vacuum filtration. Filtrate stored at 4ºC as enriched lysate.

Conformational Test for Presence of Bacteriophages: (By Spot Test)

100µl log phase host was spread on sterile NA plate and 20µl lysate was spotted on the plate. Incubated at 37°C for 24 hours.

Purification of Bacteriophages

The phage purification was done by repeated passaging of the plaques.The plaques formed in the plaque assay were picked using a cut micropipette tip.Inoculated in a fresh culture of the respective host for 24 hours.Lysate was prepared as mentioned earlier by vacuum filtration method.All Ø1, Ø2 and Ø3 were passaged till P2.Purified phage lysate stored at -20ºC.

Determination of pfu/ml

100 µl of phage lysate was serial diluted from 10^{-1} to 10^{-10}.100µl of each dilution was incubated with 500µl of log phase host culture for 30 minutes at 37ºC.1ml of host and the mixture was added to SA butt mixed well. Content of tube was poured on NA base agar plate. Plates were incubated at 37^{0}C for 24 hours. Pfu/ml were determined.

Host Range Determination

The host specificity was determined by infecting three different bacterial strains i.e. *Enterococcus spp. Serratia spp. Escherichia spp.* with Ø1, Ø2&Ø3.

The assay was done using phage spot test by spreading 100µl log phase host on sterile nutrient agar plate.20µl lysate was spotted on the plate and incubated at 37°C for 24 hours.

Characterization of phage by FE-SEM

Ø lysate was re-filtered and spot was placed on Silicon wafer after it dried and submitted for FE-SEM analysis.

Application of bacteriophage on Chicken

Chicken obtained from the market was cut into 1×1 cm squares placed in sterile glass tubes.

Chicken squares were disinfected by washing with sterile distilled water, Hypochloride and exposing to U.V radiation for 20 mins.100µl of 10^6log phase cultures of isolated hosts were carefully poured by using pipette on the surface of chicken. Incubated for 10mins at RT.100µl of diluted phage lysate (10^9 PFU/ml) was poured. For un-inoculated and host-only (Positive) controls same volume of saline (0.8%Nacl) was added instead of phage suspension. Experiment was performed in triplicates. At each sampling time of 3, 6 and 9 hour, the chicken squares were transferred to a sterile tube; 5ml of sterile saline was added and mixed thoroughly for 2mins at R.T. The liquid portion was transferred to a sterile tube and centrifuged at 3000rpm for 10mins. 100µl of supernatant was spread on EMB agar (for *E.coli)*, Brain- Heart infusion agar (for *Enterococcus)* and Nutrient agar (for *Serratia sp.*).Plates were incubated at 37^{o}C for 24 hours. CFU was determined.

RESULTS

Bacteriological Analysis of Sample

TVC of the sample was found to be 5.2×10^8cfu/ml.

Isolation and Characterization of Target Bacteria

Three bacterial strains were isolated and identified till genus level by referring Bergey's Manual of Determinative Bacteriology. Isolate I, II and III were found to be *Enterococcus spp.*, *Serratia spp.* and *E.coli* respectively.

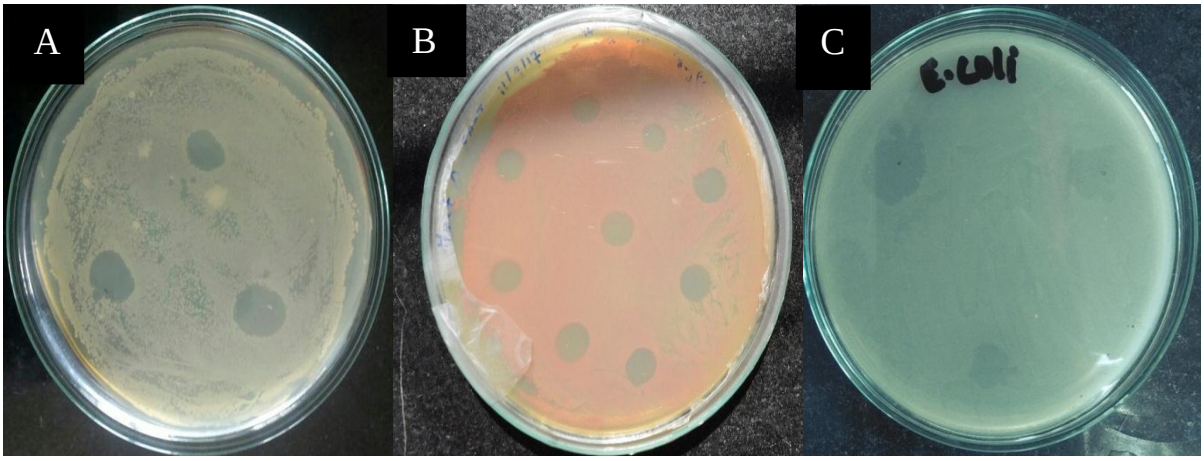
Drug resistant profile of host

Antibiotics / Organisms	CIP	AMX	NX	AMC	CTX	GEN	Azt	NET	TE	FR
Enterococcus	+	-	+	-	-	-	-	-	-	+
Serratia	+	-	-	+	+	-	+	-	-	-
E.coli	+	+	-	-	-	-	-	-	-	-

(+) = Resistant (-) = Susceptible

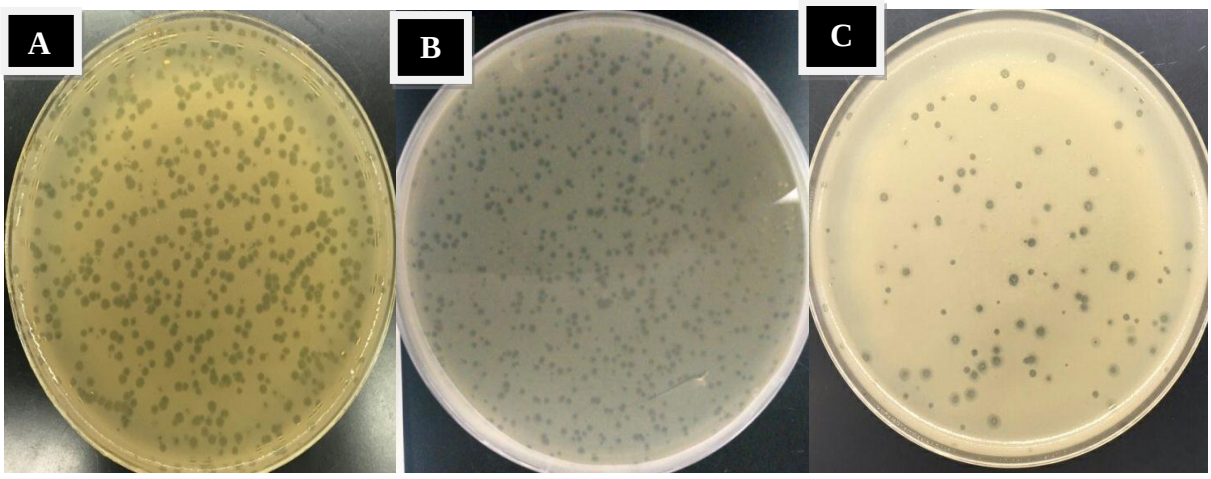
Conformational Test for Presence of Bacteriophages: (By Spot Test)

20µl of lysate was spotted on plates spread with three isolated bacteria and all showed zone of lysis on plate.



Spot of Ø1 on lawn of *Enterococcus sp.;* B- spot of Ø2on lawn of *Serratia sp.;* C- spot of Ø3 on lawn of *E.coli*

Purification of the phages



Purification of the phages: A - Passage 2 of Ø1

B - Passage 2 of Ø2

C - Passage 2 of Ø3

Determination of pfu/ml: (By Soft Agar Overlay method)

In the soft agar overlay method, isolates showed plaques on the plate and PFU/ml found to be

Table Ø Titre value

Ø against	Pfu/ml
A-Isolate 1	1.75×10^{6} pfu/ml
B-Isolate 2	7.32×10^{6} pfu/ml
C-Isolate 3	1.48×10^{6}pfu/ml

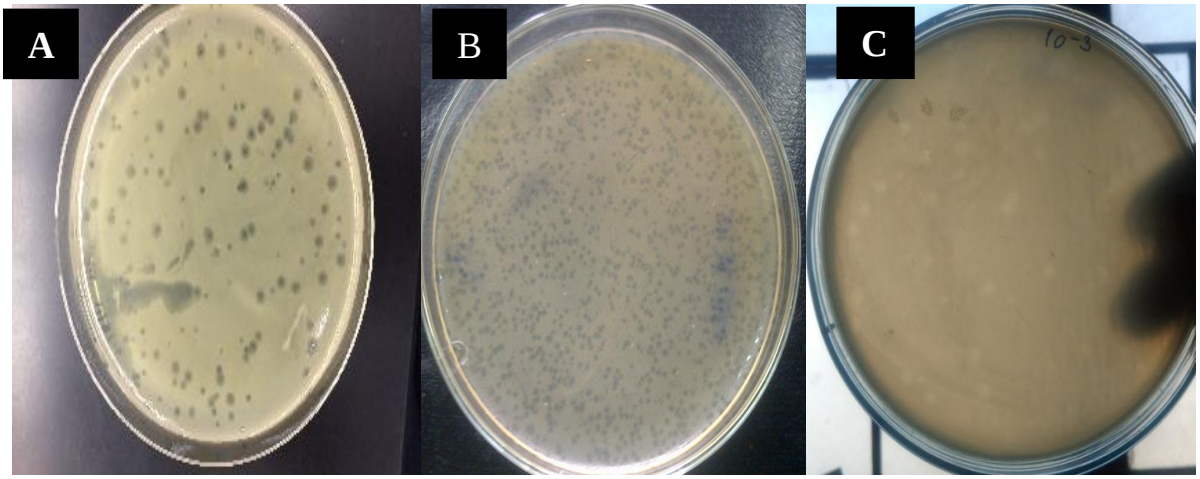


Fig Plaques on bacterial lawns A- Ø1 against isolate I *Enterococcus* spp., B- Ø2 against isolate II *Serratia Spp.*and C- Ø3 against Isolate III *E.coli.*

One-step Growth Curve

Lytic life cycle of bacteriophage was characterized using their specific host that is *Enterococcus spp,Serratia spp. E.coli.*

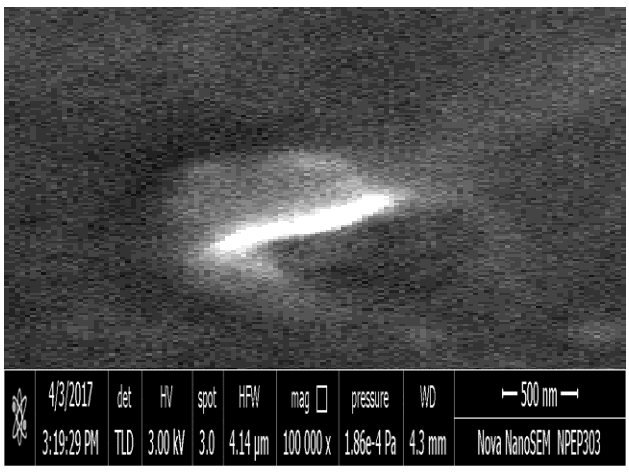
Host range determination

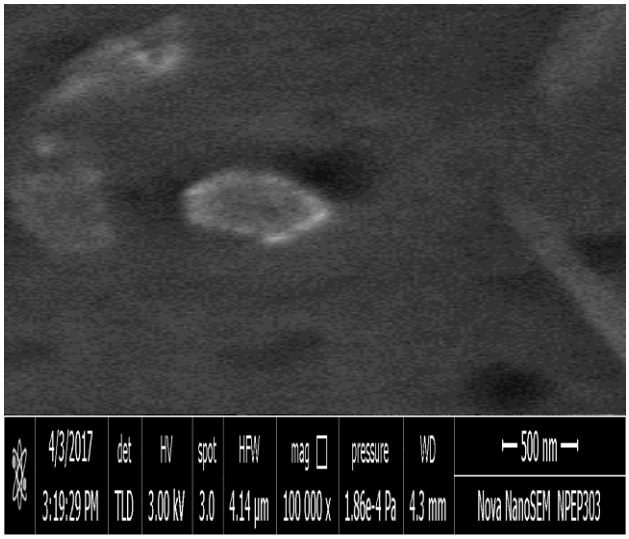
Host range of the phages

	Phage Infectivity			
No.	**Culture**	**Ø1**	**Ø2**	**Ø3**
1.	***E.coli***	-	-	+
2.	***Serratia spp.***	-	+	-
3.	***Enterococcus spp.***	+	-	-

Characterization of bacteriophage by FE-SEM

Icosahedral symmetry was observed of both the phages at 500nm.The size of head of **Ø1**was 250nm and tail was 300nm.The size of head of Ø2 was 200nm and tail was 250nm.





FE-SEM images of Ø2 and Ø3

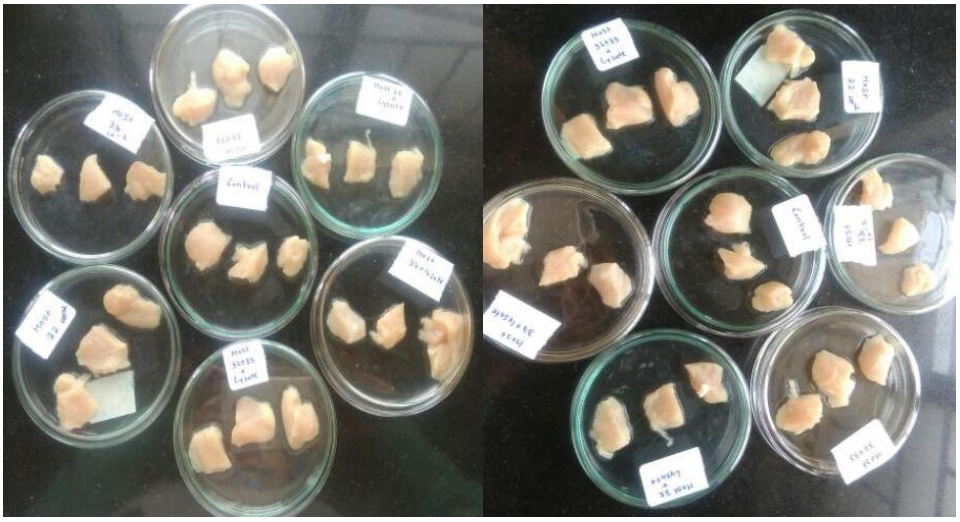
Application of bacteriophage on Chicken

TVC at 3rd, 6th and 9th hour before Ø1, Ø2, Ø3 and Ø cocktail inoculation

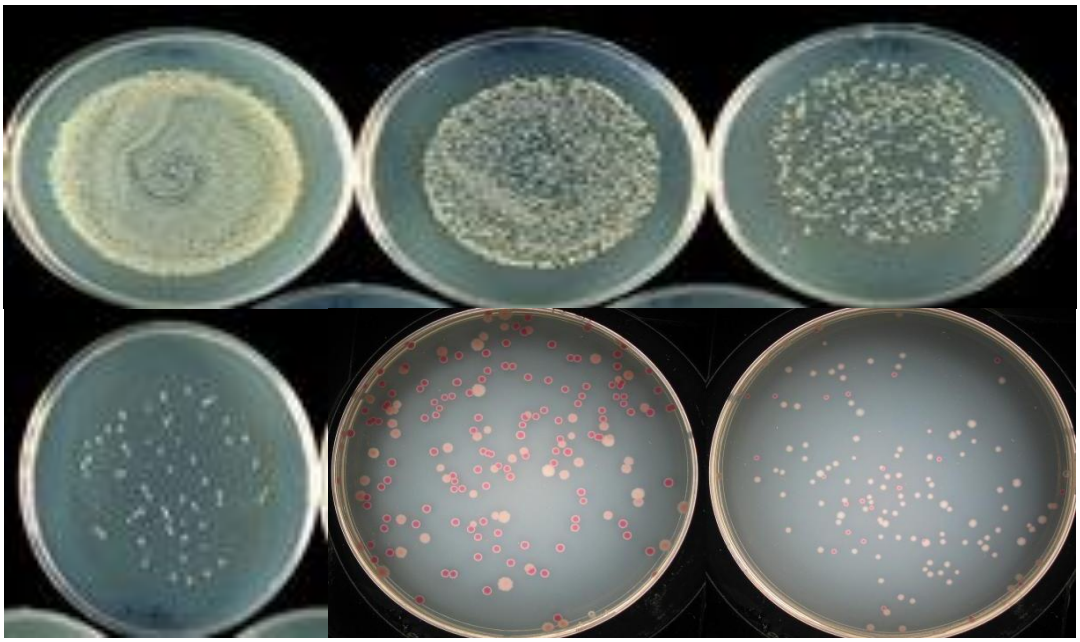
Time ➡ / Culture ⬇	Host TVC cfu/ml			
	0 hour	**3 hour**	**6 hour**	**9 hour**
E.coli	5.3×10^{6}	5.8×10^{6}	7.8×10^{6}	6.4×10^{7}
Serratia sp.	4.5×10^{6}	4.7×10^{6}	5.1×10^{6}	8.4×10^{6}
Enterococcus sp.	4.8×10^{6}	5.2×10^{6}	5.7×10^{6}	7.8×10^{6}
Host cocktail	5.6×10^{6}	8.2×10^{6}	6.2×10^{7}	2.3×10^{8}

TVC at 3rd, 6th and 9th hour after Ø1, Ø2, Ø3 and Ø cocktail inoculation

Time ➡ / Culture ⬇	Host+ Ø lysate TVC cfu/ml			
	0hour	**3 hour**	**6 hour**	**9 hour**
E.coli	5.3×10^{6}	4.8×10^{6}	5.8×10^{5}	4.9×10^{4}
Serratia sp.	4.5×10^{6}	3.2×10^{6}	6.1×10^{5}	2.4×10^{4}
Enterococcus sp.	4.8×10^{6}	3.3×10^{6}	7.7×10^{5}	3.8×10^{4}
Host + Ø cocktail	5.6×10^{6}	4.4×10^{6}	8.2×10^{4}	2.3×10^{3}



Artificially contaminated Chicken sample before and after inoculation of Ø1, Ø2, Ø3 and Ø cocktail inoculation



Plates showing reduction in growth

DISCUSSION

Biocontrol by bacteriophages in meat products was developed in last few years and are generally recognized as safe due to their host specificity. Phages are naturally present in significant numbers in water and food (Sulakvelidze, 2011). In fresh cut meat and meat products more than 10^3 phages per gram may be present (Kennedy and Bilton, 1987). Previous studies have shown that phages are very effective in treating meat products to control contamination caused by food borne pathogens, several examples of such application have been published. Goode et al. (2003) inactivated the *Campylobacter jejuni* by phages on

chicken skin of the order of 95 to 99%, and Higgins et al. (2005) used a specific phage to reduce *Salmonella* in poultry products. Phages may also be exploited in preventing colonization of pathogens in animals. Use of phages as biocontrol agent in food seems to be promising alternative for the management of food contamination as the use of chemical preservative are not able to retain foods natural properties.

This study we have demonstrated the application of virulent phage in preventing food contamination by pathogens such as *E.coli, Serratia* and *Enterococcus* and the results of this study are very promising. In this study we have isolated 3 strains of pathogenic bacteria, characterized till genus level viz. *E.coli, Serratia sp.* and *Enterococcus sp.* bacteriophages against these isolates were isolated from the same sample viz. Ø1, Ø2and Ø3. Titer of Ø1, Ø2 and Ø3 was found to be 1.75×10^6, 7.32×10^5 and 1.48×10^6 respectively. Significant reduction of these pathogens was observed by plate count method. The phages and their cocktail were applied to artificially contaminated meat by these pathogens and significant reduction in CFU's was observed. The result indicates that the bacteriophage have the potential of preservation and/or treatment of bacterial contamination on food.

In conclusion, we believe that the application of virulent bacteriophages for control of food contamination by pathogens represent a specific, effective, and environmentally friendly path toward the supply of safer food.

Phages may also be helpful in decontaminating food-processing equipment where *E.coli., Serratia and Enterococcus* may be present as a "house flora."

REFERENCES

1. Mansura S. Mulani*, Syed Azhar, Shaikh Azharuddin and ShilpaTambe (2015). Harnessing the Power of Bacteriophage for Pathogen Reduction in Wastewater Int. J. Curr. Microbiol. App. Sci Special, 2015; 2: 152-161.
2. 1Neha Bhardwaj, 1Sanjeev K. Bhardwaj, 1Akash Deep, 2Swati Dahiya and 2Sanjay Kapoor (2015). Lytic Bacteriophages as Biocontrol Agents of Foodborne Pathogens. Asian Journal of Animal and Veterinary Advances, 10(11): 708-723.
3. Tan Loh Teng-Hern1, 2, Chan Kok-Gan3 and Lee Learn Han1 Application of Bacteriophage in Biocontrol of Major Foodborne Bacterial Pathogens. J Mol Biol & Mol Imaging, 2014; 1(1).

4. Craig Billington J Andrew Hudson Elaine D'Sa Prevention of bacterial food borne disease using nanobiotechnology. Nanotechnology, Science and Applications 2014: 7: 73–83.
5. Mathias Schmelcher* and Martin J Loessner Application of bacteriophages for detection of foodborne pathogens. Bacteriophage, 2014; 4: e28137.
6. KamalpreetKaur Sangha, 1 B. V. Sunil Kumar,1 Ravi Kant Agrawal,2 Dipak Deka,1 and Ramneek Verma1Proteomic Characterization of Lytic Bacteriophages of Staphylococcus aureus Isolated from Sewage Affluent of India. Hindawi Publishing Corporation International Scholarly Research Notices, 2014; Article ID 265298.
7. Sabah A. A. Jassim • Richard G. Limoges Natural solution to antibiotic resistance: bacteriophages 'The Living Drugs' World J Microbiol Biotechnol, 2014; 30: 2153–2170.
8. Lorraine Endersen, 1 Jim O' Mahony, 1 Colin Hill,2,∗R. Paul Ross,2,3 Olivia McAuliffe, 3 and Aidan Coffey 1 Phage Therapy in the Food Industry. The Annual Review of Food Science and Technology, 2014; 5: 327–49.
9. Spardha Jhamb Biopreservation of Food Using Bacteriocins, Bacteriophages And Endolysins. Bombay Tech., 2014; 64(1).
10. Humberto Moreira Hungaro a Regina Célia Santos Mendonça a, Delaine MeirelesGouvêa Maria Cristina DantasVanetti b, CláudiaLúcia de Oliveira Pinto c. Use of bacteriophages to reduce Salmonella in chicken skin in comparison with chemical agents. Food Research International, 2013; 52: 75–81.
11. Agata Kęsik-Szeloch1, Zuzanna Drulis-Kawa1*, Beata Weber-Dąbrowska 2, Jerzy Kassner1, Grażyna Majkowska-Skrobek1, Daria Augustyniak1, Marzanna Łusiak-Szelachowska2, Maciej Żaczek2, Andrzej Górski2,3 and Andrew M Kropinski4,5 Characterising the biology of novel lytic bacteriophages infecting multidrug resistant Klebsiella pneumonia. Kęsik-Szeloch et al. Virology Journal, 2013; 10: 100.
12. DavidTomat1*, Leonel Migliore1, VirginiaAquili 1, AndreaQuiberoni2 and Claudia Balagué1 Phage biocontrol of enteropathogenic and shigatoxin-producing Escherichia coli in meat products. Frontiers in Cellular and Infection Microbiology, 2013; 3: 20.
13. HuiZhang ,1 Ran Wang , and Hongduo Bao Phage inactivation of foodborne Shigella on ready-to-eat spiced chicken. Poultry Science, 2013; 92: 211–217.
14. Alexander Sulakvelidze Using lytic bacteriophages to eliminate or significantly reduce contamination of food by foodborne bacterial pathogens. Wiley Online Library, 2013.
15. Sanna M. Sillankorva, Hugo Oliveira, and Joana Azeredo Bacteriophages and Their Role in Food Safety. International Journal of Microbiology, 2012; Article ID 863945.

16. Lawrence D. Goodridge* and BledarBisha Phage-based biocontrol strategies to reduce foodborne pathogens in foods. Bacteriophage, 2011; 1(3): 130-137.
17. Spencer D. Heringa,1 JinKyung Kim,2 Xiuping Jiang,2* M. P. Doyle,3 and M. C. Erickson3 Use of a Mixture of Bacteriophages for Biological Control of Salmonella enterica Strains in Compost. Applied And Environmental Microbiology, 2010; 76(15): 5327–5332. 0099-2240/10/$12.00 doi:10.1128/AEM.00075-10.
18. P. Garcı´a, B. Martı´nez, J.M. Obeso and A. Rodrı´guez Bacteriophages and their application in food safety. Letters in Applied Microbiology, 2008; 47: 479–485.
19. L.R. Bielke1, S.E. Higgins1, A.M. Donoghue2, D.J. Donoghue1, B.M. Hargis1 and G. Tellez1 Use of Wide-Host-Range Bacteriophages to Reduce Salmonella on Poultry Products. International Journal of Poultry Science, 2007; 6(10): 754-757.
20. R. J. Atterbury,1†* M. A. P. Van Bergen,2† F. Ortiz,1 M. A. Lovell,3 J. A. Harris,1 A. De Boer,2 J. A. Wagenaar,2 V. M. Allen,1 and P. A. Barrow3 Bacteriophage Therapy To Reduce Salmonella Colonization of Broiler Chickens. Applied and Environmental Microbiology, 2007; 4543–4549.
21. R. J. Atterbury,1†* M. A. P. Van Bergen,2† F. Ortiz,1 M. A. Lovell,3 J. A. Harris,1 A. De Boer,2 J. A. Wagenaar,2 V. M. Allen,1 and P. A. Barrow3 Bacteriophage Therapy To Reduce Salmonella Colonization of Broiler Chickens. applied and environmental Microbiology, 2007; 4543–4549.
22. G. O' Flynn, 1, 2 R. P. Ross,1,3* G. F. Fitzgerald,2,3 and A. Coffey4 Evaluation of a Cocktail of Three Bacteriophages for Biocontrol of Escherichia coli O157:H7. Applied and Environmental Microbiology, 2004; 70(6): 3417–3424.
23. G. O' Flynn, 1,2 R. P. Ross,1,3* G. F. Fitzgerald,2,3 and A. Coffey4 Evaluation of a Cocktail of Three Bacteriophages for Biocontrol of Escherichia coli O157:H7. Applied and Environmental Microbiology, 2004; 70(6): 3417–3424.
24. D. Goode,1 V. M. Allen,2 and P. A. Barrow3 Reduction of Experimental Salmonella and Campylobacter Contamination of Chicken Skin by Application of Lytic Bacteriophages. Applied and Environmental Microbiology, 2003; 69: 5032–5036.
25. Agata Kęsik-Szeloch1, Zuzanna Drulis-Kawa1*, Beata Weber-Dąbrowska2, Jerzy Kassner1,Grażyna Majkowska-Skrobek1, Daria Augustyniak1, Marzanna Łusiak-Szelachowska2, Maciej Żaczek2, Andrzej Górski2,3 and Andrew M Kropinski4,5 Characterising the biology of novel lytic bacteriophages infecting multidrug resistant Klebsiella pneumonia. Kęsik-Szeloch et al. Virology Journal, 2013, 10: 100.

26. Gwen Wendelschafer-Crabb,1 S. L. ERLANDSEN,2 AND D. H. WALKER, JR.* Conditions Critical for Optimal Visualization of Bacteriophage Adsorbed to Bacterial Surfaces by Scanning Electron Microscopy JOURNAL OF VIROLOGY, June 1975; 1498-1503.
27. Lubov Y. Brovko*,1, Hany Anany*,†, Mansel W. Griffiths*. Bacteriophages for Detection andControl of Bacterial Pathogens in Food and Food-Processing Environment. Paul Barrow,1* Margaret Lovell,1 And Angelo Berchieri, Jr.2 Use of Lytic Bacteriophage for Control of Experimental Escherichia coli Septicemia and Meningitis in Chickens and Calves. Clinical and Diagnostic Laboratory Immunology, 5(3): 294–298.