INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 13, No. 4, p. 267-277, 2018

RESEARCH PAPER OPEN ACCESS

Isolation and evaluation of bacteriophage lysate specific for *Listeria monocytogenes*

Ayesha Maqbool*, Sajjad ur Rahman, Ahsan Naveed, Waqar Zaib

Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan

Key words: *Listeria monocytogenes,* Bacteriophage, Lysate, Listeriaphages.

http://dx.doi.org/10.12692/ijb/13.4.267-277 Article published on October 30, 2018

Abstract

Listeria monocytogenes is a Gram positive facultative intracellular organism and food borne infective agent that can effect severely to humans and animals. *Listeria monocytogenes* primarily causes infectious disease such as meningitis, septicemia, and meningoencephalitis that leads to 20 to 25% of death rate in humans. Ingestion of *L. monocytogenes* contaminated food such as meat, eggs, dairy products, fruits and vegetables leads to serious infectious disease Listeriosis. Most common sources of *L. monocytogenes* transmission are raw milk, dairy products and dairy plant contaminated by sick farm animals and rarely from the contaminated environment. Bacteriophages specific for *Listeria monocytogenes* may have a potential role in elimination of sources of contamination. This study was designed to isolate *L. monocytogenes* specific bacteriophage for lysing the isolate of *L. monocytogenes* from raw milk using fraser broth. Gram staining, hemolysis test on sheep blood, catalase test and sugar fermentation test were used to confirm colonies grown on plate. The colonies were further streaked on trypticase soy yeast extract and showed white shiny glass like appearance. Isolation and confirmation of bacteriophages was performed by plaque assay method. Abundant of phages are analyzed in sewage water which are vulnerable and extremely flexible host for the presence of *Listeria* phages. The overall results showed that out of 500 samples of raw milk from five different dairy farms 100 each, 80%, 60%, 32%, 54%, 85% were positive from dairy farm 1, 2, 3, 4, 5 respectively. Five sewage samples were subjected to plaque assay and lysate was prepared. The plaques formed by phages appeared in the form of spherical zone which varies in size and showed lysis of *L. monocytogenes*. The number of PFU from phage lysate was ranging from 1×10^3 to 1×10^6 and estimate plaque size was 0.2±0.5. These results demonstrate application of phages in food industry. Phages can be used individually or in combination as well for the treatment of bacterial infections by targeting cell lysis due to its lytic potential.

* **Corresponding Author:** Ayesha Maqbool ✉ ayeshamaqbool92@gmail.com

Introduction

Listeria monocytogenes transmission is usually via contaminated food in humans and causes life threatening disease Listeriosis (Farber and Peterkin, 1991). The genus *L. monocytogenes* belongs to the Kingdom Bacteria, is a Gram-positive, non-spore forming, opportunistic intracellular and facultatively anaerobes (Zhang *et al.,* 2007). Other species within the genus *Listeria* include *L. ivanovii, L. innocua, L. welshimeri, L. seeligeri* (Rocourt and Buchrieser, 2007), *L. marthii* (Graves *et al.,* 2010), *L. rocourtii* (Leclercq *et al.,* 2010), *L. weihenstephanensis, L. fleischmannii* (Bertsch *et al.,* 2013), *L. floridensis, L. aquatic, L. cornellensis, L. riparia, L. grandensis* (den Bakker *et al.,* 2014), *L. booriae,* and *L. newyorkensis* (Weller *et al.,* 2015). These pathogens are ubiquitous in the environment as they have ability to survive for long in diverse circumstances. They can grow and remain in dry environments, different active water and osmotic conditions broad range of temperatures, high salt concentration and at a wide range of pH values (Weis and Seeliger, 1975; Fenlon *et al.,* 1996; Gandhi and Chikindas, 2007). Therefore, the pathogen is considered as a potential source of contamination of raw milk of sheep, goat and cow and dairy products, and outbreaks subsequently (Hunt *et al.,* 2012; Ryser and Marth, 1999).

L. monocytogenes is the important human and animal pathogen. It causes a relatively mild gastroenteritis in healthy adults and in those individual with a severe underlying disease or having HIV/AIDS, also patients with chronic conditions such as cirrhosis, pregnant women; unborn or newborn babies are at high risk (McLauchlin *et al.,* 2004). Symptoms range from gastrointestinal symptoms like nausea, diarrhea; flu-like illness to severe complications including headache, loss of balance, stiff neck, confusion, convulsions, meningitis, septicemia, spontaneous abortion or listeriosis the infection spreads to the nervous system of the individual (Farber and Peterkin, 2000; WHO, 2008; Mead *et al.,* 2006)

Food items that are naturally prone to *L. monocytogenes* contamination include raw or processed dairy products such as eggs seafood, meat, fresh fruits and vegetables (Adzitey Huda, 2010). Milk is made up of water, protein, fat, lactose, vitamins and minerals, with the proportions varying in accordance with animal breed (Amigo and Fontecha, 2011; Ramos and Juarez, 2011; Sindhu and Arora, 2011). Milk mostly contaminated from udders and equipments while milking, from animal with listerial mastitis, by bacterial infected animal shedding faecal matter or poor silage and supports the growth of *L. monocytogenes* (Flint *et al.,* 2011; Weiler *et al.,* 2013). Cases of Listeriosis outbreak by consuming contamination food like milk or meat without enough heating and pasteurization have been reported. Studies also reveal that these food products are at risk of recontamination with *L. monocytogenes* even after heating (Karatzas *et al.,* 2001; Rocourt and Cossart, 2007).

Researchers and industries have found more effective methods to overcome the tolerance for low temperatures and to combat this pathogen as it has the ability to grow and proliferate in refrigerator temperature (Jawetz *et al.,* 2001).

Bacteriophages are phages that infect bacteria, acts against food pathogens in the food industry as natural antimicrobial agent (Goodridge *et al.,* 2003). Treatments of bacteriophages that are environmental friendly as they are free of chemical preservatives, do not affect flavor, color or other physical properties of food and are less destructive treatments. Bacteriophages rely on host bacteria as they do not have their own metabolism for multiplication which exhibits less range of hosts for targeting specific strains (Guenther *et al.,* 2009).

Bacteriophages specific to *Listeria* was published in 1945 (Schultz, 1945). Approximately, 500 phages have been isolated from the various sources of food, silage, sewage, and lysogenic strains (Briers *et al.,* 2011; Loessner and Rees, 2005). Recent studies suggests that phages have potential to act as biocontrol agent for *Listeria* in foods and in the food processing plants (Hudson *et al.,* 2005; Leverentz *et al.,* 2003; Leverentz *et al.,* 2004). In 2006, the FDA (U.S. Food and Drug Administration) approved the

application of a first two commercial phage products Listex™ and ListShield™ that targets *L. monocytogenes* and granted GRAS (generally regarded as safe) status for use commercially in food products (Sulakvelidze, 2013). In Germany, P100 phage was isolated from sewage effluent of dairy industry and used in Listex™. In USA, combination of six phages were isolated from the Baltimore inner harbor water in the USA to form ListShield™ suspension. (Carlton *et al.,* 2005; Pasternack and Sulakvelidze, 2009; Micreos Food Safety, 2010). *Listeria monocytogenes* are one of the most studied foodborne pathogens and has a major detrimental influence that causes disease outbreaks such as listeriosis. Reviews regarding the use of bacteriophages are mostly discussed but numerous reviews regarding bacteria as a biocontrol agent in the food industry have been published. Therefore it is needed to study the antimicrobial potential of bacteriophages (Cossart, 2007). This study aims to evaluate the presence of *L. monocytogenes* in the raw milk collected from various dairy farms and the feasibility of using bacteriophages as a biocontrol agent.

Materials and methods

Sample Collection and Transportation

Total 10 raw milk samples were randomly collected from dairy farms and dairy shops at various locations in the Faisalabad city, Pakistan. Samples were packed into sterile containers and transported in icebox to the laboratory of institute of microbiology, University of Agriculture, Fsd.

Isolation of Listeria monocytogenes

In this research, method used for isolation and identification of *L. monocytogenes* was ISO 11290 described by Becker (Becker *et al.,* 2006). In brief, 25ml of raw milk samples added to 225ml of half Fraser broth (Oxoid, Basingstoke, UK) for the first enrichment culture and incubated. Then a loopful of enriched broth culture was streaked on *Listeria* Selective Agar plate and incubated again. On the other hand, half Fraser broth added to Fraser broth as a second enrichment culture and incubated. Then, inoculum from second enriched Fraser broth culture was streaked onto *Listeria* selective agar (Oxford Formulation) (Oxoid, Basingstoke, UK) and incubated

Identification

Presumptive colonies from *Listeria* selective agar were again streaked on Tryptic soy yeast extract agar for identification and confirmation of *L. monocytogenes* (Oxoid, Basingstoke, UK). TSYEA was used as a nonselective medium and incubated. The colonies were identified by Gram staining, motility test, catalase reaction, hemolysis on blood agar medium, CAMP test, MRVP, TSI, esculin hydrolysis test, sugar fermentation test for glucose, maltose, mannitol, rhamnose (Murray *et al.,* 2009; Aygun and Pehlivanlar, 2006).

Preparation of Bacteriophage lysate specific to L. monocytogenes

For listeriaphage isolations, sewage water samples from hospital waste water plant in Pakistan were used. The sampling containers were placed in ice box and transported to the laboratory. Usually, samples were processed for phage isolation within 48 h of collection. Sewage water samples were transferred into centrifuge tube and centrifuged at 2,500 RPM for 5 minutes. Then pipette the supernatant into a syringe barrel fitted with a 0.45 micron filter. Remaining bacteria from the phage sample were removed by gently moving the plunger, allowed the flow to drip into the storage tube and then stored at 4°C which is stable for 3 to 4 months. Then nutrient broth as equal volume of filtrate was added and overnight culture of indicator strain *Listeria monocytogenes* was added. The mixture was then incubated in shaking water bath for 5 to 6 hours. After incubation, the mixture was again centrifuged and filtrate was used in a plaque assay (Kim *et al.,* 2008).

Plaque Assay

Bacteriophage suspension was successively diluted in sterile phosphate buffer saline, containing phage suspension. A single colony of overnight culture of *L. monocytogenes* was inoculated into LB broth and incubated. 3ml of soft agarose was added into tubes placed in a water bath with phage suspensions and *L. monocytogenes*.

The soft agar mixture was gently vortexed prior to pouring onto a TSYEA plate, and then distributed evenly by gentle rotation of the agar plate. These plates were then incubated in an inverted position after solidifying of soft agar for about 30 min at room temperature. After the incubation, plaques were visible and the resulting number of plaques were multiplied by a dilution factor to obtain the counts called as PFU per milliliter (Akhtar *et al.,* 2014; Soni and Nannapaneni, 2010).

Preservation of Phage lysate

Phosphate buffer saline was pipetted into a microfuge tube. Then a drop of chloroform was added. Select typical plaque and cut the agar surrounding using broad end of a Pasteur pipette, and plucked out the agar containing the plaque. Place the agar into PBS. Vortex for 3 min, centrifuge and stored the supernatant in the refrigerator (Tan *et al.,* 2008).

Results

In the current study, *Listeria monocytogenes* were isolated and identified by morphological, cultural and biochemical methods from raw milk in the region of Faisalabad, Pakistan. Patterns of *L. monocytogenes* lysis by phages isolated from sewage water were also determined. To confirm and observe the effectivity of bacteriophage lysate against lysis of *L. monocytogenes*, plaque assay was performed.

Total 500 raw milk samples were collected from 5 different dairy farms and only 350 *Listeria monocytogenes* species were identified when plated on *Listeria* selective agar. Raw milk samples were enumerated in half fraser broth and fraser broth respectively, appeared as black color indicating esculin hydrolysis by *Listeria monocytogenes*. The suspension was then streaked over the surface of *Listeria* Selective agar as a selective media. Colonies of *L. monocytogenes* displayed grayish and black colonies with black halos as shown in Fig 1. The prevalence of *L. monocytogenes* was 91/350, 65/350, 37/350, 60/350, 97/350 from dairy farm 1, 2, 3, 4, 5 respectively.

Morphological evaluation by Gram staining was used as presumptive confirmation of the colonies, showed Gram positive rods. The size, shape and color of colonies were macroscopically analyzed. Then these colonies were further cultured on trypticase soy yeast extract agar. Colonies appeared as white shiny like crushed glass as shown in fig. 2.

Fig. 1. Black colonies of *L. monocytogenes* isolated from raw milk on *Listeria* selective agar.

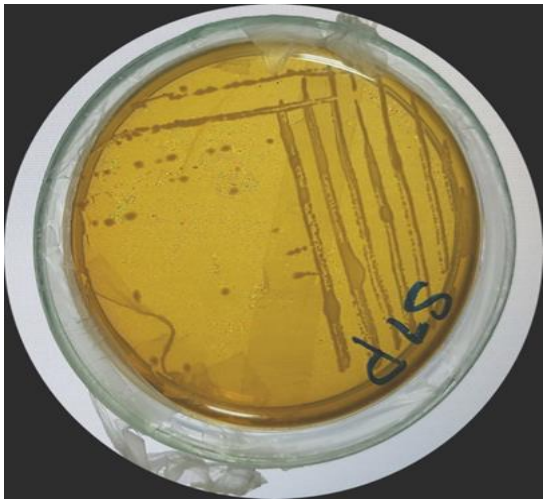

Fig. 2. Purified *L. monocytogenes* growth on Trypticase soy yeast extract agar (TSYEA). Purified *Listeria monocytogenes* colonies are dense white and shiny white appearing as crushed glass on TSYEA at 37°C for 24 hrs.

Biochemical identification confirmed positive catalase reaction, observation of trembling motility in a wet mount, clear and complete zone of beta hemolysis around the streak line of the *L. monocytogenes* on sheep blood agar medium, methyl red vogues proskauer showed A/A. All isolates showed positive reactions for triple sugar iron and esculin hydrolysis.

The occurrence of *L. monocytogenes* species by morphological and biochemical analysis were 80/91, 60/65, 32/37, 54/60, 85/97 respectively. The highest prevalence of *L. monocytogenes* was observed in farm 5 as shown in Table 1.

Table 1. Raw milk samples from various dairy farms indicating positive samples for *L. monocytogenes* confirmed by biochemical tests.

Number of dairy farms	Sample size	Positive samples in *Listeria* selective agar	Positive samples confirmed by biochemical tests	Percentage of *L. monocytogenes* species
1	100	91	80	80
2	100	65	60	60
3	100	37	32	32
4	100	60	54	54
5	100	97	85	85

Five sewage waste water samples collected from various hospitals for isolation of phage lysate specific for *Listeria monocytogenes*. Sewage samples were subjected to plaque assay and lysate was prepared. The plaque which were formed by the lysis of *L. monocytogenes* appeared as spherical zone and varies in size as shown in fig. 3. The number of PFU from phage lysate was ranging from 1×10^3 to 1×10^6 and estimate plaque size was 0.2±0.5 (Table 2).

Table 2. Results of phage titer calculation obtained from sewage waste water.

No. of sewage waste water samples	Dilution factor	Average no. of plaques	Total no. of phages PFU/ml	Plaque size (cm)
1	10^3	5	5×10^3	(0.2 ±0.5)
2	10^2	6	6×10^4	(0.1±0.3)
3	10^1	4	4×10^5	(0.2±0.3)
4	10^4	2	2×10^6	(0.2±0.3)
5	10^3	5	5×10^3	(0.2±0.4)

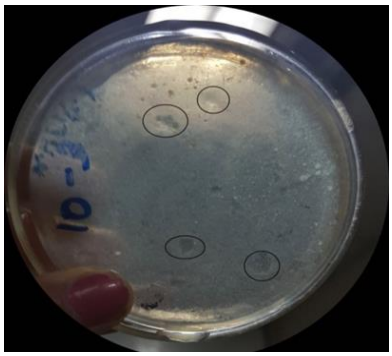

Fig. 3. Clear zones showing Lysis of *L. monocytogenes* by bacteriophages. Lysis of *Listeria monocytogenes* by bacteriophages on nutrient agar at 37°C for 24 hrs.

Listeria monocytogenes, contamination in food is more likely to occur during processing, consequently this is the most suitable time for phage to act as a biocontrol agent against pathogen. As phages are most abundant in nature so they can be utilized to prepare phage lysate and eradicate *Listeria monocytogenes* from raw milk and subsequently reduces the chances of Listeriosis and other related diseases. In this perspective, the FDA has approved the use of List Shield and Listex 100 as an anti Listerial phage food additives, and regarded as generally recognized safe status. Alternatively, phages can also be applied or mixed directly onto or into the food product. On the other hand, the use of bacteriophages as an antimicrobial agents is the possible replacement option for antibiotics Bacteriophages are also very specific to their hosts, that minimizes the chance of secondary infections, however antibiotics do target both pathogens and friendly bacterial flora of patients, which can lead to the secondary infections. Also, bacteriophages replicate at the site of infection where they are generally expected to lyse the pathogens, but antibiotics travel throughout the body and do not concentrate at the site of infection. Most importantly, no side effects have been reported during or after phage application, but commonly antibiotic treatment shows reaction in the form of resistant bacteria, allergies, and secondary infections.

Discussion

From the previous studies it is assumed that milk is a main reservoir for *L. monocytogenes* appearance due to greater chances for contamination by poor handling and presence of higher nutrients (James *et al.,* 1983; Schlech *et al.,* 1983; Beckers *et al.,* 1987; Farber *et al.,* 1987; Pintado *et al.,* 2007; Makino *et al.,* 2005). In our study, the incidence of *L. monocytogenes* was evaluated in raw milk which was collected from various dairy farms. The above results indicate the need of control strategy to prevent the contamination of *L. monocytogenes*.

Oftenly, isolation of *L. monocytogenes* is challenging and time consuming process. In this research, it was attempted to isolate *L. monocytogenes* using fraser broth as an enrichment.

In addition, for preventing growth and proliferation of unwanted bacteria and fungi, antibiotics and substances such as cycloheximide, acriflavine hydrochloride and nalidixic acid were added to enrichment culture medium. The enriched medium was then streaked over the surface of *Listeria* selective agar. The colonies were further confirmed by microscopic and macroscopic examination, and by biochemical tests.

Due to importance of *L. monocytogenes* in public health and food products, several studies have been done in various countries on this microbe. In 2001, Listeriosis 3 cases were observed during 2 weeks due to contaminated homemade Mexican style cheese (Anonymous, 2000; MacDonald *et al.,* 2005). In Brazil, out of 103 cheese samples, 11 samples were infected by *L. monocytogenes* (Da Silva *et al.,* 1998). Jamishidi (Jamshidi and Khanzadi, 2011) studies indicated the rate of contamination was 4% in northeast of Iran Mashhad, 2% in South of Iran Noor Abad city (Mahmoodi, 2010) and 1.6% in West of Iran Shahr-e-Kord city (Moshtaghi and Mohamadpour, 2007).

The main source of many diseases caused by *Listeria monocytogenes* is the consumption of raw milk . Listeriosis is one of the dangerous disease that occur in humans by using contaminated milk and milk products by *L. monocytogenes*. In 2014, Usman and Mukhtar stated the results that 7.5% of raw milk samples were positive for *Listeria* spp. and 2.25% for *Listeria monocytogenes*. Mahmood *et al.,* 2003 study was performed 320 samples of poultry meat and poultry meat products, collected from various poultry supermarkets and shops at Faisalabad. *Listeria* species isolated from all the examined samples were ranging from 10 to 37.5% and *L. monocytogenes* percentages were ranging from 2.5 to 17.5% from poultry meat and poultry meat samples. Our study also reports the raw milk contamination of *L. monocytogenes* in Faisalabad, Pakistan. The rate of contamination in this study is 80%, 60%, 32%, 54%, 85% from five various dairy farms in Faisalabad. *Listeria monocytogenes* was differentially identified by beta haemolysis, characteristic tumbling motility, sugar fermentation tests, aesculin test, and catalase test. Recently, Gohar *et al.,* 2017 stated the prevalence of *Listeria* species were 16.8%, *Listeria monocytogenes* was 13.6% in raw milk and dairy products.

Bacteriophage lysate was prepared and antimicrobial effects are observed in terms of lysis of *L. monocytogenes*. Our research reports valuable information in terms of antimicrobial properties of bacteriophage against *L. monocytogenes* and the use of phage lysate as a biocontrol agent in dairy food. By following bacteriophage isolation protocol, phages were isolated and phage titer was confirmed by plaque assay. For isolation of phage lysate specific for *L. monocytogenes,* six sewage water samples were anaylzed. The plaques caused by the phages appeared as spherical zone which varies in size. The number of PFU from phage lysate was ranging from 1×10^3 to 1×10^6 pfu/ml.

Leverentz *et al.,* 2003 stated that treatment by phages depends upon the intial concentration of *L. monocytogenes*. Likewise, Bigot *et al.,* 2011 reported the prevention of *L. monocytogenes* growth on the surface of ready to eat and vacuum sealed pack of chicken breast rolls. At the beginning of the ripening period, Carton *et al.,* 2005 contaminated cheese with small concentration of *L. monocytogenes*. Later on, during the rind washings, P100 was applied to surface. In 2010, Nannapaneni and Soni also studied the effect of bacteriophage P100 against *L. monocytogenes* serotypes 4b and 1/2a. They conducted the experiment on the surface of raw salmon fish tissue in a broth medium.

Guenther *et al.,* 2009, implies that the successful infection of phages and subsequent eradication of the target cells primarily depends on the environmental conditions. Authors emphasize that the ratio of bacterial cells that could be infected is determined by several factors. First, the binding of phages to their receptors on the bacterial surfaces is influenced by intrinsic parameters, such as ionic strength, pH and other substances, which may have impact on this process. The food itself define these parameters and it may vary in production, ripening, and storage stages. Secondly, the effectiveness of phage significantly relies on its concentration at the time of application.

Generally, the higher concentrations of phage resulted in increased inactivation of host cell.

According to researchers, the infection of bacterial cells depend on several factors. Firstly, The intrinsic factors that may disturb the process such as pH, ionic strength influence the binding of phages to their receptors on bacterial cells. The food itself defined these parameters and may vary while production, ripening and storage. Secondly, the concentration of phage application play an crucial role in the process efficacy. More concentration of phages results in greater inactivation of bacterial cell.

Between 2004–2008, *L. monocytogenes* was found in 55 out of 2180 analyzed food samples. All types of foods was analyzed for *L. monocytogenes,* however it was most frequently detected in fresh fruits. In 2007, European Union region reported highest presence of *L. monocytogenes* in fish products (18.3%), particularly in smoked fish (EFSA, 2009). In some states, *L. monocytogenes* was detected in 13% of ready-to-eat products from the supermarkets (Chao *et al.,* 2006).

Several studies have discovered that bacteriophages are natural enemies of bacteria that could reduce the extent of *L. monocytogenes* counts in food. However, there are many pros of phage application but some cons as well that limits their usage as decontaminating agent. So, future studies should emphasize on improving the efficiency of lytic activity of bacteriophages to boost up the reduced levels of the bacterium in various food products. One of the main advantage of using phages is that they have specificity, effectiveness and zero toxicity to humans. Besides the use of bacteriophages in food safety, they can also be used for other applications as well i.e. wastewater treatment, phage therapy of animal and human listeriosis and sterilization of food processing plants. In addition, experiments with various dairy products are needed to test the efficacy of phages against the wide range of *L. monocytogenes* isolates that frequently originate in dairy processing facilities.

Conclusion

Above findings concluded the effectiveness of bacteriophage for reduction of *L. monocytogenes* in raw milk samples. These results demonstrate application of phages in food industry. Lysate of phages could help to cure a wide range of bacterial infections that are otherwise resistant to antibiotics. Due to lytic potential of phages, they can be used individually to treat a bacterial infection by lysing the bacterial cell. Phages have also been proven effective to deal with the food spoilage problem, and to treat the bacterial infection of human and animals.

Acknowledgements

I gratefully acknowledge the support of my family and technical help by my lab mates and friends.

References

Adzitey F, Huda N. 2010. *Listeria monocytogenes* in foods: incidences and possible control measures. Afr. J. Microbiol. Res **4,** 2848-2855.

Akhtar M, Viazis S, Diez-Gonzalez F. 2014. Isolation, identification and characterization of lytic, wide host range bacteriophages from waste effluents against *Salmonella enteric* serovars. Food Control **38,** 67-74.

Amigo L, Fontecha J. 2011. Milk: Goat Milk. In: JW Fuquay editors. Encyclopedia of Dairy Sciences (Second Edition). San Diego: Academic Press.

Anonymous A. 2000. Outbreak of Listeriosis associated with home made Mixican-style cheese in North Carolina. MMWR. Morb. Mortal. Wkly. Rep **26,** 560-2.

Aygun O, Pehlivanlar S. 2006. *Listeria* spp. in the raw milk and dairy products in Antakya, Turkey. Food Control **8,** 676–679.

Becker B, Schuler S, Lohneis M, Sabrowski A, Curtis GD, Holzapfel WH. 2006. Comparison of two chromogenic media for the detection of *Listeria monocytogenes* with the plating media recommended by EN/DIN 11290-1. Int. J. Food Microbiol **1,** 127-131.

Beckers HJ, Soentoro PSS, Delgou-van Asch. 1987. The occurrence of *Listeria monocytogenes* in soft cheeses and raw milk and its resistance to heat. Int. J. Food Microbiol **3,** 249-256.

Bertsch D, Rau J, Eugster MR, Haug MC, Lawson PA, Lacroix C, Meile L. 2013. *Listeria* fleischmannii sp. nov., isolated from cheese. Int. J. Syst. Evol. Micr **2,** 526-532.

Bigot B, Lee WJ, Mcintyre L, Wilson T, Hudson JA, Billington C, Heinemann JA. 2011. Control of *Listeria monocytogenes* growth in a ready-to-eat poultry product using a bacteriophage. Food Microbiol **8,** 1448–52.

Briers Y, Klumpp J, Schuppler M, Loessner MJ. 2011. Genome sequence of *Listeria monocytogenes* Scott A, a clinical isolate from a food-borne listeriosis outbreak. J. Bacteriol **16,** 4284-4285.

Carlton RM, Noordman WH, Biswas B, De Meester ED, Loessner MJ. 2005. Bacteriophage P100 for control of *Listeria monocytogenes* in foods: genome sequence, bioinformatic analyses, oral toxicity study, and application. Regul. Toxicol. Pharm **3,** 301-312.

Carlton RM, Noordman WH, Biswas B, De Meester ED, Loessner MJ. 2005. Bacteriophage P100 for control of *Listeria monocytogenes* in foods: genome sequence, bioinformatic analyses, oral toxicity study, and application. Regul. Toxicol. Pharm **3,** 301-312.

Chao G, Deng Y, Zhou X, Xu Q, Qian X, Zhou L, Zhu B. 2006. Prevalence of *Listeria monocytogenes* in delicatessen food products in China. Food Control **12,** 971–974.

Cossart P. 2007. Listeriology (1926–2007): the rise of a model pathogen. Microb. Infect **10,** 1143-1146.

Da Silva MCD, Hofer E, Tibana A. 1998. Incidence of *Listeria monocytogenes* in cheese produced in Rio de Janeiro, Brazil. J. Food Protect **3,** 354-356.

den Bakker HC, Warchocki S, Wright EM, Allred AF, Ahlstrom C, Manuel CS, Stasiewicz MJ, Burrell A, Roof S, Strawn LK, Fortes E. 2014. *Listeria* floridensis sp. nov., *Listeria* aquatica sp. nov., *Listeria* cornellensis sp. nov., *Listeria* riparia sp. nov. and *Listeria* grandensis sp. nov., from agricultural and natural environments. Int. J Syst Evol Micr **6,** 1882-1889.

EFSA. 2009. The community summary report on trends and sources of zoonoses and zoonotic agents in the European Union in 2007. The EFSA Journal 223.

Faber JM, Terkin PI. 2000. *Listeria monocytogenes*, In: Lund B.M., Baird-Parker, The microbiological safety and quantity of foods. Gaithersubug (MD), Aspen.

FAO/WHO Microbiological Risk Assessment Series. Risk Assessment of *Listeria monocytogenes* in ready–to–eat foods: Interpretative Summary. 2008. http:// www.who.int/foodsafety/micro/jemra/ assessment/ listeria/en. Accessed on 24 April 2008.

Farber JM, Peterkin PI. 1991. *Listeria monocytogenes*, a food-borne pathogen. Microbiol. Rev **3,** 476-511.

Farber JM, Johnson MA, Purvis U, Loit A. 1987. Surveillance of soft and semi-soft cheese for the presence of *Listeria* spp. Int. J. Food Microbiol **2,** 157-163.

Fenlon DR, Wilson J, Donachie W. 1996. The incidence and level of *Listeria monocytogenes* contamination of food sources at primary production and initial processing. J. Appl. Microbiol **6,** 641-650.

Flint S, Palmer J, Bremer P, Seale B, Brooks J, Lindsay D, Burgess S. 2011. Biofilm formation. In: Fuquay J.W editors. Encyclopedia of Dairy Sciences (Second Edition). San Diego: Academic Press.

Gandhi M, Chikindas ML. 2007. *Listeria*: a foodborne pathogen that knows how to survive. Int. J. Food Microbiol **1,** 1-15.

Gohar S, Abbas G, Sarfraz M, Ali S, Ashraf M, Aslam R, Yaseen K. 2017. Prevalence and antimicrobial resistance of *Listeria monocytogenes* isolated from raw milk and dairy products. Matrix Science Medica **1,** 10-14.

Goodridge L, Abedon ST. 2003. Bacteriophage biocontrol and bioprocessing: application of phage therapy to industry. SIM news **6,** 254-262.

Graves LM, Helsel LO, Steigerwalt AG, Morey RE, Daneshvar MI, Roof SE, Orsi RH, Fortes ED, Milillo SR, Den Bakker HC, Wiedmann M. 2010. *Listeria marthii* sp. nov., isolated from the natural environment, Finger Lakes National Forest. Int. J. Syst. Evol. Micr **6,** 1280-1288.

Guenther S, Huwyler D, Richard S, Loessner MJ. 2009. Virulent bacteriophage for efficient biocontrol of *Listeria monocytogenes* in ready-to-eat foods. Appl. Environ. Microbiol **1,** 93-100.

Guenther S, Huwyler D, Richard S, Loessner MJ. 2009. Virulent bacteriophage for efficient biocontrol of *Listeria monocytogenes* in ready-to-eat foods. Appl. Environ. Microbiol **1,** 93-100.

Halter EL, Neuhaus K, Scherer S. 2013. *Listeria weihenstephanensis* sp. nov., isolated from the water plant Lemna trisulca taken from a freshwater pond. Int. J. Syst. Evol. Micro **2,** 641-647.

Hudson JA, Billington C, Carey-Smith G, Greening G. 2005. Bacteriophages as biocontrol agents in food. J. Food Protect **2,** 426-437.

Hunt K, Drummond N, Murphy M, Butler F, Buckley J, Jordan K. 2012. A case of bovine raw milk contamination with *Listeria monocytogenes*. Irish Vet. J **1,** 13.

James SM, Fannin SL, Agree BA, Hall B, Parker E, Vogt J, Run G, Williams J, Lieb L, Salminen C, Prendergast T, Werner SB, Chin J. 1983. Listeriosis outbreak associated with Mexican-style cheese: California. MMWR Morb Mort Wkly Rep **34,** 357-359.

Jamshidi A, Khanzadi S. 2004. The presence of *Listeria monocytogenes* in raw milk samples in Mashhad, Iran. Iranian J. Vet. Res **4,** 363–367.

Jawetz E, Melnick JL, Adelberg EA. 2001. Medical Microbiology, pp 179-194. 22nd ed. Appleton and Lang.

Karatzas AK, Kets EPW, Smid EJ, Bennik MHJ. 2001. The combined action of carvacrol and high hydrostatic pressure on *Listeria monocytogenes* Scott A. J. Appl. Microbiol **3,** 463-469.

Kim J, Siletzky RM, Kathariou S. 2008. Host Ranges of Listeria-Specific Bacteriophages from the Turkey Processing Plant Environment in the United States. Appl. Environ. Microbiol **21,** 6623–6630.

Leclercq A, Clermont D, Bizet C, Grimont PA, Le Flèche-Matéos A, Roche SM, Buchrieser C, Cadet-Daniel V, Le Monnier A, Lecuit M, Allerberger F. 2010. *Listeria rocourtiae* sp. nov. Int. J. Syst. Evol. Micr **9,** 2210-2214.

Leverentz B, Conway WS, Camp MJ, Janisiewicz WJ, Abuladze T, Yang M, Saftner R, Sulakvelidze A. Biocontrol of *Listeria monocytogenes* on fresh-cut produce by treatment with lytic bacteriophages and a bacteriocin. Appl. Environ. Microbiol **8,** 4519-4526.

Leverentz B, Conway WS, Camp MJ, Janisiewicz WJ, Abuladzey T, Yang M, Saftner R, Sulakvelidze A. 2003. Biocontrol of *Listeria monocytogenes* on fresh-cut produce by treatment with lytic bacteriophages and a bacteriocin. Appl. Environ. Microbiol **8,** 4519-4526.

Leverentz B, Conway WS, Janisiewicz W, Camp MJ. 2004. Optimizing concentration and timing of a phage spray application to reduce *Listeria monocytogenes* on honeydew melon tissue. J. Food Protect **8,** 1682-1686.

Loessner MJ, Rees CED. 2005. *Listeria* phages: basics and applications, pp 362–379. In: Waldor M.K., Friedman D.I. Adhya S.L editors. Phages: their role in bacterial pathogenesis and biotechnology, 1st ed. Washington, DC: ASM Press.

MacDonald PD, Whitwam RE, Boggs JD, MacCormack JN, Anderson KL, Reardon JW, Saah JR, Graves LM, Hunter SB, Sobel J. 2005. Outbreak of listeriosis among Mexican immigrants as a result of consumption of illicitly produced Mexican-style cheese. Clin. Infect. Dis **5,** 677-682.

Mahmood MS, Ahmed AN, Hussain I. 2003. Prevalence of *Listeria monocytogenes* in poultry meat, poultry meat products and other related inanimates at Faisalabad. Pak. J. Nutr **2,** 346-349.

Mahmoodi MM. 2010. Occurrence of *Listeria monocytogenes* in raw milk and dairy products in Noorabad, Iran. J. Anim. Vet. Adv **1,** 16-19.

Makino SI, Kawamoto K, Takeshi K, Okada Y, Yamasaki M, Yamamoto S, Igimi S. 2005. An outbreak of food-borne listeriosis due to cheese in Japan, during 2001. Int. J. Food Microbiol **2,** 189-196.

McLauchlin J, Mitchell R, Smerdon WJ, Jewell K. 2004. *Listeria monocytogenes* and listeriosis: a review of hazard characterization for use in microbiological risk assessment of foods. Int. J. Food Microbiol **1,** 15-33.

Mead PS, Dunne EF, Graves L, Wiedmann M, Patrick M, Hunter S, Salehi E, Mostashari F, Craig A, Mshar P, Bannerman T. 2006. Nationwide outbreak of listeriosis due to contaminated meat. Epidemiol. Infect., **4,** 744-751.

Micreos Food Safety. 2010. Use of the bacteriophage preparation ListexP100" as an antimicrobial intervention method against *Listeria monocytogenes* in or on variety of meat, poultry, seafood and cheese.

Moshtaghi H, Mohamadpour AA. 2007. Incidence of *Listeria* spp. in raw milk in Shahrekord, Iran. Foodborne Pathog. Dis **1,** 107-110.

Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH. 2003. Manual of Clinical Microbiology, 8th edition. Washington DC: American Society for Microbiology.

Pasternack GR, Sulakvelidze A. 2009. *Listeria monocytogenes* bacteriophage and uses thereof. US Patent No. US **7,** 507-571 B2.

Pintado CMBS, Oliveira A, Pampulha ME, Ferreira MASS. 2005. Prevalence and characterization of *Listeria monocytogenes* isolated from soft cheese. Food Microbiol **1,** 79-85.

Ramos M, Juarez M. 2011. Milk: Sheep Milk. In: Fuquay JW editors. Encyclopedia of Dairy Sciences (Second Edition). San Diego: Academic Press.

Rocourt J, Buchrieser C. 2007. The genus *Listeria* and *Listeria monocytogenes*: phylogenetic position, taxonomy, and identification. Food Sci. Technol **1.**

Rocourt J, Cossart P. 1997. *Listeria monocytogenes*. In: L.R. Beuchat, M. Doyle, T.J. Montville, editors. Food Microbiology: Fundamentals and Frontiers pp. 337-352. Washington D.C: ASM Press.

Ryser ET, Marth EH. 1999. *Listeria*: Listeriosis, and Food Safety, 3rd edition, CRC Press, Marcel Dekker Inc, New York, USA.

Schlech III WF, Lavigne PM, Bortolussi RA, Allen AC, Haldane EV, Wort AJ, Hightower AW, Johnson SE, King SH, Nicholls ES, Broome CV. 1983. Epidemic Listeriosis evidence for transmission by food. J. Med **4,** 203-206.

Schultz EW. 1945. Listerella infections: a review, pp 135–151. Stanford Med. Bull.

Sindhu JS, Arora S. 2011. Milk: Buffalo milk. In: Fuquay JW editors. Encyclopedia of Dairy Sciences (Second Edition). San Diego: Academic Press.

Soni KA, Nannapaneni R. 2010. Bacteriophage significantly reduces *Listeria monocytogenes* on raw salmon fillet tissue. J. Food Protect **1,** 32-38.

Soni KA, Nannapaneni R. 2010. Bacteriophage significantly reduces *Listeria monocytogenes* on raw salmon fillet tissue. J. Food Protect **1,** 32-38.

Sulakvelidze A. 2013. Using lytic bacteriophages to eliminate or significantly reduce contamination of food by foodborne bacterial pathogens. J. Sci. Food Agr **13,** 3137-3146.

Tan GH, Nordin MS, Napsiah AB. 2008. Isolation and characterization of lytic bacteriophages from sewage water. J. Trop. Agr. Food Sci **2,** 1-5.

Usman M, Mukhtar N. 2014. Prevalence of *Listeria monocytogenes* in Raw Milk in Faisalabad, Pakistan. J. Agri. Vet. Sci **7,** 8–11.

Weiler C, Ifland A, Naumann A, Kleta S, Noll M. 2013. Incorporation of *Listeria monocytogenes* strains in raw milk biofilms. Int. J. Food. Microbiol **2,** 61-68.

Weis J, Seeliger HPR. 1975. Incidence of *Listeria monocytogenes* in nature. Appl. Microbiol **1,** 29-32.

Weller D, Andrus A, Wiedmann M, den Bakker HC. 2015. *Listeria* booriae sp. nov. and *Listeria* newyorkensis sp. nov., from food processing environments in the USA. Int. J. Syst. Evol. Micr **1,** 286-292.

Zhang Y, Yeh E, Hall G, Cripe J, Bhagwat AA, Meng J. 2007. Characterization of *Listeria monocytogenes* isolated from retail foods. Int. J. Food Microbiol **1,** 47-53.