

A Study On Application Of Listeria Monocytogenes Bacteriophage As Biocontrol Agents For Dairy Products.

Mahananda B Math¹, Bharati S Meti², Virupakshaiah DBM^{3*}.

¹Department of Microbiology, Davangere University, Davangere, Karnataka – 577007, India.

²Department of Microbiology, Davangere University, Davangere, Karnataka – 577007, India.

³Department of Microbiology, Davangere University, Davangere, Karnataka – 577007, India.

*Corresponding Author
Virupakshaiah DBM.

Email:
virudbm@gmail.com

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Abstract: *Background:* Listeriosis is epidemiologically linked to the consumption of foods contaminated with *Listeria monocytogenes* (LM), especially ready-to-eat dairy food products that are stored for extended periods at refrigeration temperatures and do not require heating for consumption. The use of lytic bacteriophages has been successfully tested for the biocontrol of *L. monocytogenes* in different food matrices such as artificially contaminated dairy products. Hence, this study was conducted with the main objective to evaluate the application of *L. monocytogenes* bacteriophage as biocontrol agents for dairy products such as milk, butter milk, and curd. The samples of dairy products viz. milk, butter milk, and curd were collected, and observed for morphological changes like texture, odour and colour before and after two days of incubation. Dairy products underwent treatment with bacteriophage (107PFU/g), while an uninoculated sample served as control. Each treatment was sealed with sterile aluminium foil under various conditions viz. room temperature, 15-18°C, 4°C, and deep freezer (-20°C). Subsequently, the samples were subjected to microbiological examination. Results revealed that there was statistically significant ($p < 0.001$) decrease in *L. monocytogenes* colony count was observed in all the dairy product's samples viz. milk, butter milk, and curd following treatment with *L. monocytogenes* bacteriophage for two consecutive days. In conclusion, *L. monocytogenes* bacteriophage treatment of dairy products like milk, butter milk, and curd significantly controls the *L. monocytogenes* infections. Hence, bacteriophages could be explored as biocontrol agents for the prevention of dairy products from getting infected with pathogenic *L. monocytogenes* infections.

Keywords: *Listeria monocytogenes*, Bacteriophage, Biocontrol agents, Milk, Butter milk, Curd.

INTRODUCTION

Dairy farming in India predominantly involves numerous smallholder dairy farmers and entrepreneurs, making it a subsidiary activity. The economic significance of the dairy sector is evident, with the value of milk alone exceeding Rs 35 thousand crores. Dairying plays a key role in Indian agriculture by efficiently utilizing low-valued agricultural by-products and engaging an underemployed labor force for milk production.¹

Milk consumption in India is linked to health benefits. The intake of milk can serve as an indicator of overall good nutrition, and dairy products offer essential nutrients such as calcium in milk, that support the growth and development of young children.² The majority of the milk produced is used domestically in various forms, including liquid milk and dairy-products like butter, clarified butter (ghee), yogurt, curd, cheese, paneer, flavored milk, and ice cream. Consumption patterns exhibit diversity across the country, influenced by regional or community-specific factors, including levels of deprivation/food security and adherence to traditional practices.³

The composition of milk encompasses water (86-88%), fat (3-5%), protein (3-4%), lactose (4.5-5%), and minerals (0.7%). Additionally, milk contains minor components such as phospholipids, sterols, vitamins, enzymes, and pigments. In dairy products, these components are present in significant amounts, playing

crucial roles in maintaining and developing the human body while offering therapeutic and other beneficial effects. Andersen and colleagues emphasize about the interdependence of food and health, asserting that the consumption of nutritious food leads to good health. In line with this understanding, Indian dairy products inherently contain various health-promoting components with therapeutic values, such as antiallergenic qualities, prevention of osteoporosis, reduced risk of heart disease, antimicrobial properties, immune enhancement, anti-diarrheal effects, and alleviation of lactose intolerance.⁴ In the 1920s, listeriosis was identified as an infection brought on by a Gram-positive bacterium that was subsequently identified as *Listeria monocytogenes* (LM).⁵ *L.*

monocytogenes is extensively distributed in a wide range of agricultural environment such as soil, manure, and water.⁶ Furthermore, *L. monocytogenes* is a global occurrence with widespread distribution in the environment, posing a potential threat as a contaminant in various dairy products, including raw milk. While pasteurization is known to eliminate *L. monocytogenes*, instances of post-pasteurization contamination may arise due to inadequate temperature control and technical errors.⁷ Microbes, especially bacteria, exhibit varying responses to antibiotics and anti-microbial compounds, due to inherent differences or the progress of resistance through application or genetic exchange. Resistant

bacteria will cause severe health effects directly or by transmitting antibiotic resistance.⁸

One promising strategy to improve food safety is the use of bacteriophages to specifically eliminate unwanted bacteria like *Listeria*. Phages contribute to the natural microflora present in foods, are extensively dispersed throughout the environment, and provide their hosts with a high degree of specificity.⁹ Because of this, they are perfect for controlling infections without affecting the resident microbiota or starter culture organisms needed to produce fermented foods.¹⁰ Hence, current study was conducted with the main purpose of evaluation of application of *Listeria monocytogenes* bacteriophage as biocontrol agents for dairy products like milk, butter milk, and curd.

MATERIALS AND METHODS

The samples of dairy products viz. milk, butter milk, and curd were collected KMF outlets located around the Bagalkot district, Karnataka, India and transferred to the laboratory.

Morphological characterization

Dairy products were observed for morphological changes like texture, odour and colour before and after two days of incubation and recorded.

Preparation of bacteriophages

Bacteriophages specific to *L. monocytogenes* were introduced at a dilution of 1:100 in 200 ml of *L. monocytogenes* and then subjected to incubation at 37°C in a shaking incubator set at 240 rpm. Once the optical density at 600nm reached 0.1, stock phages were introduced at a concentration of 10⁷ plaque-forming units (PFU/g).

Application of LM bacteriophage as biocontrol agents for dairy products

Dairy products underwent treatment with LM Bacteriophage (10⁷PFU/g), while an uninoculated sample served as control. Each treatment was sealed with sterile aluminium foil under various conditions i.e., room temperature, 15-18°C, 4°C, and deep freezer (-20°C). Subsequently, the samples were subjected to microbiological examination i.e., *L. monocytogenes* colony count.

Statistical analysis

The data were expressed as the mean. Data were subjected to statistical analysis using unpaired t-test to draw a comparison of the *L. monocytogenes* colony count between control and treatment groups on both the incubations periods i.e., 0th day and 2nd day. $p < 0.05$ was considered as statistically significant. Statistical analyses were conducted using SPSS (Statistical Package for Social Sciences) version 21 software (IBM SPASS statistics [IBM corp. released 2011]).

RESULTS AND OBSERVATIONS:

Dairy products viz. milk, butter milk, and curd were observed for morphological changes like texture, odour and colour changes before and after two days of incubation. Results revealed that after two days of incubation the normal consistency of dairy products was disturbed and observed odour of microbial infection in the dairy products (Table 1 and Figure 1 and 2).

Table 1. Morphological characterization of dairy products

Days	Appearance/ Colour	Texture	Odour	Taste
Milk				
0 th Day	White	Watery Consistency	No smell	Normal
2 nd Day	Yellowish	Semisolid Consistency	Smell	Bitter
Butter milk				
0 th Day	White	Watery Consistency	No smell	Normal
2 nd Day	Yellowish	Semisolid Consistency	Smell	Bitter
Curd				
0 th Day	White	Normal consistency	No smell	Normal
2 nd Day	Yellowish	Hard consistency	Smell	Bitter



Fig 1. Showing dairy products before being infected with *L. monocytogenes*

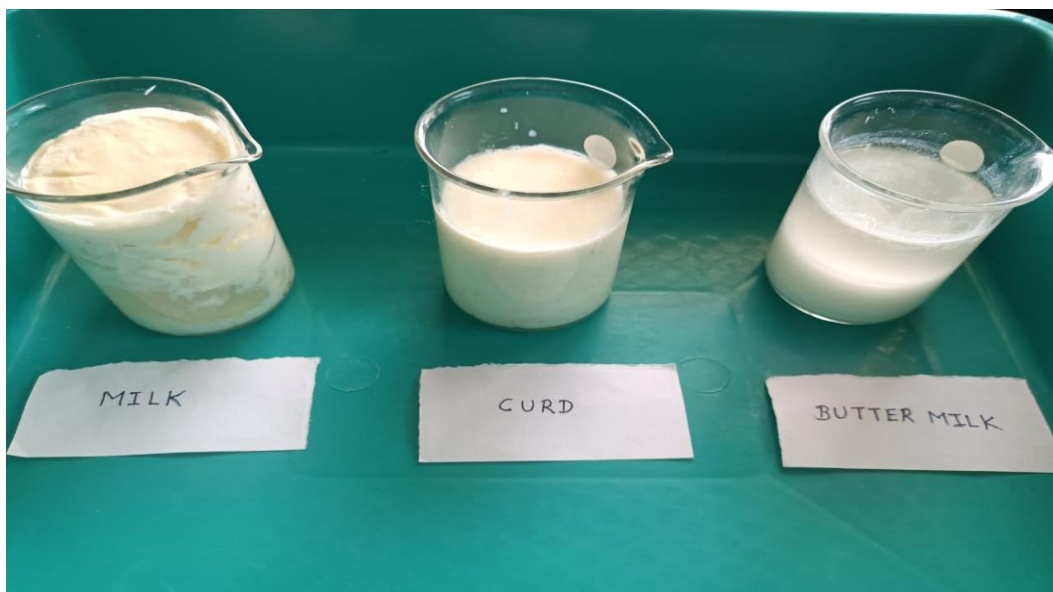


Fig 2. Showing dairy products after being infected with *L. monocytogenes*

Dairy products were treated with bacteriophage (10^7 PFU/g) and uninoculated sample served as control. Each treatment was closed with sterile aluminium foil, and incubated at different conditions viz. room temperature, at 15-18°C, at 4°C and deep freezer (-20°C) and then were examined by microbiologically for *L. monocytogenes* colony count. Results depicted that on day 2, there was a statistically significant ($p < 0.001$) decrease in *L. monocytogenes* colony count was observed in milk samples treated with LM bacteriophage (Table 2).

Table 2. Effect of LM bacteriophage treatment on *L. monocytogenes* colony count in milk sample

Incubation Period	Milk Sample		
	<i>L. monocytogenes</i> Colony Count		
	Control	Milk + LM Phage	p-value
0 th Day	360	355	0.062
2 nd Day	365	310	<0.0001

Values are expressed as mean; n=3

In butter milk sample also, there was a statistically significant ($p < 0.001$) decrease in *L. monocytogenes* colony count was observed on day 2 (Table 3).

Table 3. Effect of LM bacteriophage treatment on *L. monocytogenes* colony count in butter milk sample

Incubation Period	Butter Milk Sample		
	<i>L. monocytogenes</i> Colony Count		
	Control	Milk + LM Phage	p-value
0 th Day	380	365	0.051
2 nd Day	390	325	<0.0001

Values are expressed as mean; n=3

Furthermore, there was a statistically significant ($p < 0.001$) decrease in *L. monocytogenes* colony count was observed on day 2 in curd sample also (Table 4).

Table 4. Effect of LM bacteriophage treatment on *L. monocytogenes* colony count in curd sample

Incubation Period	Curd Sample		
	<i>L. monocytogenes</i> Colony Count		
	Control	Milk + LM Phage	p-value
0 th Day	428	425	0.1401
2 nd Day	410	335	<0.001

Values are expressed as mean; n=3

DISCUSSION

The dairy industry is among the largest food industries in the world, producing a wide range of perishable and semi-perishable products that are widely demanded. It is estimated that, by 2026, the global dairy trade will exceed one billion USD.¹¹ Ensuring the quality and safety of these products is a major concern for dairy companies. However, due to their highly nutritious nature, dairy products are excellent environments for the growth of a wide variety of microorganisms, and undesirable (spoilage-inducing and pathogenic) microbes can be introduced into the dairy chain during any step, from milking to serving.¹²⁻¹⁴ As a result, many milk products have a relatively short shelf life or are associated with cases of foodborne disease, such as listeriosis.¹¹ *L. monocytogenes* is the causative agent of listeriosis, a disease that mainly affects specific groups of individuals. It is especially concerning due to the severity of its sequelae and high mortality rate (up to 30%).^{15,16}

Listeriosis is epidemiologically linked to the consumption of foods contaminated with *L. monocytogenes*, especially ready-to-eat foods that are stored for extended periods at refrigeration temperatures and do not require heating for consumption.^{16,17} The use of lytic bacteriophages has been successfully tested for the biocontrol of *L. monocytogenes* in different food matrices such as artificially contaminated dairy products.^{18,19} With this scenario, present study was conducted with the main purpose of evaluation of application of LM bacteriophage as biocontrol agents for dairy products such as milk, butter milk, and curd.

Our study findings showed deteriorated morphology of dairy products under investigations viz. milk, butter milk, and curd following 2 days incubation due to pathogenic *L. monocytogenes* infections. However, upon treatment with bacteriophage at 107PFU/g the colony count of *L. monocytogenes* count significantly decreased. These findings implied that bacteriophages could be used as biocontrol agents for the prevention of dairy products from getting infected with pathogenic *L.*

monocytogenes infections. In concurrence with our study results, literature reports evidenced that lytic bacteriophages hijack the metabolic mechanisms of their hosts for their growth and multiplication, leading the death of host cells due to lysis, without disturbing the normal microbiota of the food, as there is a high specificity of phage-host interactions.¹⁹ Furthermore, Lee et al., showed that different lytic bacteriophages inhibited the growth of *L. monocytogenes* in a culture medium and milk at various temperatures.²⁰ Moreover, Silva et al., observed that the application of a lytic phage in soft cheeses reduced the initial pathogen counts only at the beginning of the cold storage of the products, indicating that the effectiveness of the phage treatment was dependent on the initial contamination of the product with *L. monocytogenes*.²¹

Furthermore, Guenther and colleagues used a lytic bacteriophage to control *L. monocytogenes* during the production and ripening of soft-ripened cheeses, and observed that a single larger dose of the phage was more effective than repeated smaller applications at reducing the pathogen counts.¹⁰ In addition, bacteriophages were able to reduce *L. monocytogenes* counts below the detection limit when low initial counts of the pathogen

were added to cheeses, which was in accordance with Silva et al., observations.²¹

CONCLUSION

In conclusion, *L. monocytogenes* specific bacteriophage treatment of dairy products like milk, butter milk, and curd significantly controls the *L. monocytogenes* infections. Hence, bacteriophages could be explored as biocontrol agents for the prevention of dairy products from getting infected with pathogenic *L. monocytogenes* infections. However, although the use of bacteriophages for the biocontrol of *L. monocytogenes* may offer a safe and ecological approach to reduce the contamination of foods with the pathogen, further studies are warranted to optimize their use in different food products and environments.

REFERENCES

1. Mahin S, Dixit PK, Krishnadas M, Sivaraman M. Understanding Water Productivity in Milk Production Some Economic and Ecological Reflections. Compendium of Model Training Course on Management Strategies for Sustainable Livestock Production, Organized by NDRI, during. 2013:18-25.
2. Herber C, Bogler L, Subramanian SV, Vollmer S. Association between milk consumption and child growth for children aged 6–59 months. *Sci Rep.* 2020;10(1):2–10.
3. Bowen L, Ebrahim S, de Stavola B, Ness A, Kinra S, Bharathi AV, Prabhakaran D, Reddy KS. Dietary intake and rural-urban migration in India: a cross-sectional study. *PLoS ONE.* 2011;6(6):1–8.
4. Andersen PP. Leveraging Agriculture for Improving Nutrition and Health, 2020 Conference Brief – February 13, 2011.
5. Todd ECD, Notermans S. Surveillance of listeriosis and its causative pathogen, *Listeria monocytogenes*. *Food Control.* 2011;22: 1484-1490.
6. Jeyaletchumi P, Tunung R, Selina PM, Chai LC, Radu S, Farinazleen MG, Cheah YK, Mitsuaki N, Yoshitsugu N, Kumar MP. Assessment of *Listeria monocytogenes* in salad vegetables through kitchen simulation study. *J Trop Agric Food Sci.* 2012; 40:55-6.
7. Rousham EK, Unicomb L, Islam MA. Human, animal and environmental contributors to antibiotic resistance in low-resource settings: integrating behavioural, epidemiological and One Health approaches. *Proc Biol Sci.* 2018;285(1876):20180332.
8. Economou V, Gousia P. Agriculture and food animals as a source of antimicrobial-resistant bacteria. *Infect Drug Resist.* 2015; 8:49-61.
9. Chibani-Chennoufi S, Bruttin A, Dillmann ML, Brüssow H. Phage-host interaction: an ecological perspective. *J Bacteriol* 2004; 186:3677-86.
10. Guenther S, Loessner MJ. Bacteriophage biocontrol of *Listeria monocytogenes* on soft ripened white mold and red-smear cheeses. *Bacteriophage.* 2011;1(2):94-100.
11. Bhosale S, Desale RJ, Fulpagare YG. Biofilm: An Overview with Respect to Dairy Industry. *Int J Curr Microbiol App Sci.* 2020;9(10):150-60.
12. Gopal N, Hill C, Ross PR, Beresford TP, Fenelon MA, Cotter PD. The Prevalence and Control of *Bacillus* and Related Spore-Forming Bacteria in the Dairy Industry. *Front Microbiol.* 2015; 6:1418.
13. Machado SG, Baglinière F, Marchand S, Van Coillie E, Vanetti MC, De Block J, Heyndrickx M. The Biodiversity of the Microbiota Producing Heat-Resistant Enzymes Responsible for Spoilage in Processed Bovine Milk and Dairy Products. *Front Microbiol.* 2017; 8:302.
14. Owusu-Kwarteng J, Akabanda F, Agyei D, Jespersen L. Microbial Safety of Milk Production and Fermented Dairy Products in Africa. *Microorganisms.* 2020;8(5):752.
15. Inoue T, Itani T, Inomata N, Hara K, Takimoto I, Iseki S, Hamada K, Adachi K, Okuyama S, Shimada Y, Hayashi M, Mimura J. *Listeria Monocytogenes* Septicemia and Meningitis Caused by *Listeria Enteritis* Complicating Ulcerative Colitis. *Intern Med.* 2017;56(19):2655-2659.
16. Choi MH, Park YJ, Kim M, Seo YH, Kim YA, Choi JY, Yong D, Jeong SH, Lee K. Increasing Incidence of Listeriosis and Infection-associated Clinical Outcomes. *Ann Lab Med.* 2018;38(2):102-109.
17. Buchanan RL, Gorris LG, Hayman MM, Jackson TC, Whiting RC. A review of *Listeria monocytogenes*: An update on outbreaks, virulence, dose-response, ecology, and risk assessments. *Food control.* 2017; 75:1-3.
18. Kawacka I, Olejnik-Schmidt A, Schmidt M, Sip A. Effectiveness of phage-based inhibition of *Listeria monocytogenes* in food products and food processing environments. *Microorganisms.* 2020;8(11):1764.
19. Stone E, Lhomet A, Neve H, Grant IR, Campbell K, McAuliffe O. Isolation and Characterization of *Listeria monocytogenes* phage vB_LmoH_P61, a phage with biocontrol potential on different food matrices. *Front Sustain Food Syst.* 2020; 4:521645.
20. Lee S, Kim MG, Lee HS, Heo S, Kwon M, Kim G. Isolation and Characterization of *Listeria* phages for Control of Growth of *Listeria monocytogenes* in Milk. *Korean J Food Sci Anim Resour.* 2017;37(2):320-328.
21. Silva EN, Figueiredo AC, Miranda FA, de Castro Almeida RC. Control of *Listeria monocytogenes* growth in soft cheeses by bacteriophage P100. *Braz J Microbiol.* 2014;45(1):11-6.