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Research Article

Listeria Monocytogenes as a Foodborne Pathogen: Biocontrol in Foods using Lytic Bacteriophages

Abstract

Foods are playing a significant role in human infections because they are frequent vehicles of some human pathogens, which can spread in a short time to all the animals and are associated with cross contamination during production and processing. During stable to table, in order not to take hygienic precautions, contaminations with pathogenic microorganisms such as Listeria spp. may be occurred and consumption of such food and food products can cause foodborne illnesses. L. monoctogenes is a zoonotic foodborne bacteria that leads to a variety of serious infections in humans such as encephalitis, meningitis, abortion and septicemia, and those suffering with listeriosis occurs in approximately 30% mortality. Epidemiologic studies have revealed that a significant proportion of cases of listeriosis caused by contaminated foods. The pathogen is widely distributed in the environment and well adapted to very different environmental conditions like tolerating wide temperature (0-45°C) and pH ranges (pH 4.3–9.6) make it difficult to control food-borne infections. Although there are 13 known serotypes of L. monocytogenes, according to epidemiological studies, approximately 95% of the isolates from the food and 98% of the clinical isolates that isolated from cases of listeriosis in humans belong to 1/2a, 1/2b, 1/2c and 4b serotypes. Bacteriophages can be applied to living tissues without causing any harm due to their highly selective toxicity. This is the most important advantage when they compared with antibiotics and antiseptics. Rapidly growing bacterial resistance to antibiotics and need for development of alternative methods, increasing interest in using bacteriophages in treatment or as biocontrol agents in foods nowadays. In addition to the systems like HACCP and GMP for food safety from farm to table, the use of specific virulent bacteriophages for L. monocytogenes in order to reduce the bacterial load in foods of animal origin emerges as another method. It is reported that the usage of specific virulent bacteriophages to L. monocytogenes as a biocontrol and decontamination agent of L. monocytogenes in foods, don't cause any side effects in humans.

Introduction

Significance of *Listeria monocytogenes* as a foodborne pathogen

Listeria monoytogenes is an intracellular, zoonotic foodborne bacteria which cause listeriosis in humans. Being widespread in nature, growing in refrigerator temperature and tolerating broad pH values make the bacteria difficult to control [1]. In poultry while pathogenic *Listeria* species mostly show themselves with septicemia, in humans *L. monocytogenes*, the only pathogen species for humans, causes mild flu-like symptoms to meningitis, meningoencephalitis, septicemia, conjunctivitis and pneumonia.

Listeria monocytogenes has been reported to cause approximately 1600 illnesses and 260 deaths annually in the USA. According to the statistics published by the CDC the average annual incidence of listeriosis in the United States was 0.26 cases per 100,000 individuals [2]. In the EU, a total of 1642 verified listeriosis cases were reported and 198 people were reported to have died in 2012. The incidence of the disease was determined as 0.41 cases per 100,000 individuals [3]. Although salmonellosis and campylobacteriosis are more common foodborne diseases worldwide, listeriosis is distinguished with high mortality rate up to 20-30% [4].

Although the incidence of listeriosis is low in healthy individuals (0.7/100 thousand), children (10/100 thousand) and elders (1.4/100 thousand) are more prone to this disease. Furthermore, it has been reported that pregnant women are 17 times more sensitive to listeriosis than healthy individuals [5]. Among thirteen serotypes, 1/2a, 1/2b and 4b serotypes are the major serotypes causing listeriosis in humans [6]. It was stated that the serotype 4b is responsible for 30-50% of sporadic listeriosis cases in humans worldwide. However, in most countries serotype 1/2a is more frequently isolated from

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foods [7]. Some outbreaks caused by *L. monoctogenes* were given in Table 1.

The growth potential of *L. monocytogenes* in meat and meat products depends on the type of product, the pH, the number and type of microorganisms found in flora. It has been stated that poultry meat is more favorable for *L. monocytogenes* to reproduce than other meats [7]. There are several studies indicated that chicken and turkey meat are significant sources of *L. monocytogenes* and the most common serotypes are 1/2a, 1/2b, 1/2c and 4b [16-22].

In the formation of foodborne *Listeria* infections, ready-to-eat foods, unpasteurized milk and milk products, raw meat and meat products and salads take place as the main risk groups of food [23-25]. The slaughter animals may carry *L. monocytogenes* symptomatically or asymptomatically, or the meat can be contaminated during or after the slaughter process. On the other hand, it has been reported that sea products, shellfish (especially fresh and frozen mussels) and raw, pickled or cold smoked fish meat are also risky foods for foodborne listeriosis [26,27].

Biocontrol of *Listeria monocytogenes* using bacteriophages

Since *L. monocytogenes* is common in the environment, sources of contamination are not always clearly identified. The high prevalence of *L. monocytogenes* in animals plays an important role in the occurrence of foodborne *Listeria* infections. Shoes of workers, transport vehicles, infected animals, raw meat and meat products, and subclinical infected people can be a convenient path for *L. monocytogenes* to enter the food processing plants. It has been stated that *L. monocytogenes* is generally isolated in food processing facilities from damp surfaces, dirty and stagnant water, food residues, tools and equipment [7]. On the other hand, *L. monocytogenes* is a potential source of contamination in food processing plants by

Table 1: Some outbreaks caused by *L. monoctogenes* worldwide [8-15].

Country	Year	Food	Serotype	Cases	Deaths
Germany	1953	Raw milk	-	2	1
Sweden	1959	Poultry meat	-	4	2
Germany	1966	Milk product	-	279	109
Canada	1979-1981	Cabbage salad	4b	41	0
New Zealand	1980	Shellfish	-	22	6
Switzerland	1983	Soft cheese	4b	57	32
USA	1983	Pasteurized milk	4b	49	29
USA	1985	Mexican cheese	4b	142	34
USA	1986-1987	-	x	44	36
England	1987-1989	Pate	4b	366	63
USA	1988	Turkey sausage	-	1	-
Finland	1989	Salted mushroom	4b	1	0
USA	1989	Shrimp	4b	2	1
France	1993	Cooked meat	4b	38	32
Italy	1993	Rice salad	1/2b	39	0
USA	1994	Chocolate milk	1/2b	45	0
France	1995	Soft cheese	-	20	20
Italy	1997	Corn	4b	1566	0
Finland	1998	Rainbow trout	1/2a	5	0
Finland	1998-1999	Butter	3a	25	24
USA	1998-1999	Sausage/deli meat	4b	50	16
France	1999-2000	Cooked meat	4b	10	10
France	1999-2000	Pork tongue	4b	32	31
USA	2000	Turkey deli meat	1/2a	30	7
USA	2001	Sliced turkey meat	1/2a	16	0
USA	2002	Turkey deli meat	-	54	11
USA	2003	Mexican cheese	4b	12	2
Canada	2008	Meat product	-	57	22
USA	2011	Cantaloupe	1/2a, 1/2b	147	33
USA	2013	-	-	124	24
USA	2014	Ice cream	-	8	5

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forming biofilms with attaching onto the equipment surfaces [28]. In addition to the systems like HACCP and GMP for food safety, the use of bacteriophages for *L. monocytogenes* in order to reduce the bacterial load in foods and food processing plants emerges as an alternative method.

Bacteriophages were first described as bacteria-eating viruses in the early 1900s. Compared to antibacterial agents such as antibiotics and antiseptics, bacteriophages have a different and functional reproductive status. They can be applied without damaging live tissues due to high selective toxicity [29]. Two types of bacteriophages with very high host specificity have been demonstrated; temperate phages and lytic phages. In food safety approaches lytic phages which attach to bacteria, transfer its genetic material, multiply in it and lyse the bacteria in about 20-60 minutes are preferred as a biocontrol agent [30].

Today, there are many studies on the use of bacteriophages in foods. *Campylobacter* [31], *Escherichia coli* O157:H7 [32], *Staphylococcus aureus* [33], *Salmonella*, and *Listeria* [34] are among the bacteria for testing the bacteriophages on experimental contaminated foods.

Virulent bacteriophages are derived from different environments and the importance of controlling *L. monocytogenes* in food is increasing consequently [35]. Listex™, containing the P100 phage isolated from a milk processing plant in Germany [36] and ListShield™, consisting of six different phages, isolated from the Baltimore inner harbor water in the USA [37] are listeriophage preparations which are currently approved for use commercially in food [38].

Bacteriophages have several advantages in food safety applications. They show a high specificity to their host bacteria which is determined by target bacteria cell wall receptors. This causes not damaging the remaining microbiota and not causing any side effects or toxicity to humans. Besides, phages can adapt to alternating host systems easily while bacteria develop phage defense mechanisms to survive. As long as the target bacteria exist in the environment, phages continue to multiply [39]. Being cheap and easy to isolate make bacteriophages appropriate to be used in various forms such as mixing in food, spraying, attaching to food packaging material or dipping food in phage water [40]. Also it is stated that phages prolonged shelf life [41].

Difficulties in using bacteriophages in food safety

Although there are many advantages of using bacteriophages in food safety, some difficulties are encountered in practice. The ability to transfer resistance genes or virulence genes between the bacteriophages and the bacteria, limits the use of lysogenic phages. However, this disadvantage is overcome by the use of lytic phages instead of lysogenic phages [42]. On the other hand, in cases when lytic bacteriophages cannot be used at very high concentrations, the target pathogen does not completely disappear, but the number of pathogens in the food decreases to levels that do not cause problems in the consumption of food [43].

The use of phages in food safety is a new application, therefore producer and consumer approaches are different in commercial applications. While consumers concern about the safety of consumption of phage applied foods, producers are worried about increased production costs [43]. This situation can be overcome by popularized the phage preparations, understanding that it is a cheap and easily producible agent, and announcing by the authorities that there is no inconvenience in consumption phages. Making legal arrangements for the application of phages in food safety will facilitate the usage widespread [44].

Another difficulty encountered in using phages is the variation of phage activity in different food matrixes and storage temperatures [45]. Bacterial specificity of phages is also a limiting factor in their use. Although this seems to be a disadvantage compared to antimicrobial chemistries, the factors that phages only affect the current target pathogen and because they do not harm microorganisms such as starter cultures that are desired to be found in food make bacteriophages an advantageous agent in food safety [42].

Conclusion

L. monocytogenes is a ubiquitous foodborne pathogen that is frequently isolated from foods and causes serious illnesses and deaths in the risk groups. Its growing ability during cold storage makes the pathogens difficult to control, especially in meat and meat products. For this purpose development of control methods which do not make any changes in the structure of the product plays an important role. At this point, previous studies revealed that lytic bacteriophages which are natural enemies of bacteria can reduce the level of *L. monocytogenes* in foods. Although there are many advantages of bacteriophages such as specificity, effectiveness and showing no toxicity to humans, they have some disadvantages that limit their usage as a decontamination agent. So, further studies should focus on improving the efficiency of lytic activity of bacteriophages to increase the reduction level of the bacterium in different food models. In addition to use of bacteriophages in food safety, they can also be used for the decontamination of the surfaces of food processing plants, for treatment of wastewater, and also for phage therapy in human and animal listeriosis since the world is entering a post antibiotic era because of rapid increase in antibiotic resistance.

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