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A review on phage as biocontrol agent in food industry

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Abstract

Foodborne illnesses due to bacterial pathogens pose a significant threat to public health, resulting in tens of thousands of deaths worldwide annually. In the United States, cost of healthcare related to foodborne disease are estimated to be $75 billion per year with combining multiple financial losses from food scraps, culled farm animals, and food recalls. Conventional biological control of pathogenic bacteria had also depended on broad-spectrum approaches such as antimicrobials or pasteurization, which differ in efficacy, have an impact on food natural microflora, and can negatively impact quality of food. Hence researchers looking for alternative approach while using phage. The most ubiquitous and diverse biological species in the biosphere are bacteriophages (phages). In the last ten years, multi-drug resistant bacterial strains have become more prevalent, which has sparked interest in phages. The potential of bacteriophages and their derivative in the fields of healthcare and biotechnology is a topic of current investigation. Currently, phage treatments that target dangerous food-borne bacteria are used to treat and decontaminate crops and livestock as well as a biocontrol tool after harvest. So we take a look to see how deep researchers go in the topic and their findings in their literature.

Introduction

Biofilms are sessile populations of bacteria; biofilms develop on surfaces and are enmeshed in an extracellular matrix that they are self-replicate [1]. They are made up of multiple bacteria adhered to the surface, which are enveloped in an extracellular matrix made up of a variety of proteins, polysaccharides, and extracellular DNA. Bacterial biofilms must be controlled in the food industries since its existence on the instrument surface and in the facilities can seriously impair people's health. Antibiotic and disinfectants are ineffective against bacteria fixed in biofilms even more than against planktonic bacteria [2]. It is worthy to note that biological control with bacteriophages in the milk products have already had varying degrees of achievement. Despite the fact that the mechanism remains unclear, studies have discovered that certain milk constituents play a significant role in bacteriophage activities in dairy foods and products [3]. On the contrary, studies indicate that phage effectiveness is heavily influenced by the composition of a food material. The diary food (product) is typically complicated matrix whose microstructures alter due to the storage and processing of dairy, that potentially influencing phage-bacterial cell interaction [4]. Foodborne outbreak is frequently caused by processed foods. For instance, hemolytic uremic syndrome and hemorrhagic colitis caused by *E. Coli O157:H7*, *Listeriosis,* and *Salmonellosis* outbreaks have indeed been connected to foods such as sausages, processed meat, yoghurt, and milk powder [5]. Keeping these foods free from foodborne pathogens paved the way for the application of phages as biocontrol, emphasizing the variety of the foods that can be applied with bacteriophages as well as versatility of the phage as antimicrobials [6].

Results

By producing surface-ripened reddish mozzarella and infecting it with small amounts of *Listeria Monocytogenes* at the beginning of the ripening process, virulent *P100 Listeriophage* demonstrated its effectiveness as an antibacterial agent. It infects and kills the large bulk of Listeria Monocytogenes. During the peel washings, *P100 Listeriaphages* were applied to the surfaces. The researchers saw a significant decrease of the *Listeria Monocytogenes* bacteria tend to range from 3.5 logs to fully elimination based on the intervals, repetitions, and doses of the bacteriophage applications. Researchers noted *no Listeria Monocytogenes* resistant cells in the samples they examined [7, 4]. This study suggested that phages can be used to manage *Listeria Monocytogenes* surface contamination on cheeses, which has been the root of numerous *listeriosis* outbreaks in cheese [8]. Modi et al., investigated the impact of phages on the continued existence of *S. Enteritidis* during the production and stockpiling of cheddar cheese [9]. The researchers discovered that adding phages to raw and pasteurized milk greatly decreased the number of *S. Enteritidis* in cheddar cheese products from the milk [6]. Milk, in addition is a biological fluid that serves as a food source in which so many immunoglobulins and biologically active molecules that are naturally produced. Those biomolecules are essential in the immune mechanism against a variety of microorganisms, including viruses and bacteria. Antibodies are quite well understood for their capacity to suppress phage-bacteria interactions by anchoring to bacteriophage tail. These interactions between phages and antibodies, however, don't always indicate a reduction in viability of phage [3]. A review article of phage endolysins have shown potential novel agent for the biological control of food - borne pathogens, especially in preservation of food and processing applications [10]. Due to its strong host specificity, they are only able to manage the targeted pathogens and not the good bacteria, such probiotics in food. Endolysins should, however, be used with caution because their enzymatic characteristics can alter depending on a variety of physicochemical factors, including NaCl concentrations, temperature and pH. Additionally, endolysins can stop the global problem of bacteria that resistant to antibiotic from developing [11, 15]. Endolysins could be used on the surfaces of facilities that produce food because they can also remove biofilm. Although there have historically been issues with endolysin use, particularly in Gram negative bacteria, numerous studies now developed unique techniques that employ endolysins as biological controls against Gram negative infections. As a result, endolysins have the promise to be potent enzymes that reduce the risk of foodborne illness and increase food science safety [12]. Gram negative bacteria are resistant to exterior endolysins in contrast to Gram positive bacteria because they have an outside barrier on their cell wall which blocks the contact between peptidoglycan layer and endolysins [10]. Even though Gram positive endolysins that have been employed as biocontrol agents, more recently research has revealed ways to lyse and kill Gram negative bacteria by breaking through the outer membrane barrier [13]. The most popular method for enhancing the efficiency of Gram negative endolysins as biological control agents is the use of outer membrane-permeabilizing chemicals like chelators. For instance, citric and malic acids and chelators like ethylenediaminetetraacetic acid (EDTA) have typically been employed as permeabilizers of the outer membrane. Particular evidence derives from the endolysin OBPgp279, which when combined with EDTA was said to exhibit bactericidal effect of about a 1-log reduction in activity within 30 min against *Salmonella Typhimurium* cells [1].

Another study on salmonella phages have done for the purpose of effectively reducing the growth of *Salmonella spp*. on a range of fresh and fresh fruit and vegetables, many phages that are unique to *Salmonella spp* have been studied [14]. Using only *phage-lA* on mustard seeds led to a 1.37 Log decrease in *Salmonella* growth, but combining *phage-**A*** and *phage-**B*** led to a 1.50 Log decrease in CFU of *Salmonella* growth in the soaking water of broccoli seeds. Kocharunchitt et al. employed two *Salmonella* phages, *SSP5* and *SSP6*, to reduce *Salmonella Oranienburg* on alfalfa seeds because chemical disinfectants did not work well. Spricigo et al., also conducted tests on freshly cut romaine lettuces for the presence of *Salmonella enterica serovars Enteritidis* and *Typhimurium* [15]. The *Salmonella* concentration was greatly decreased by the phage cocktail. *Salmonella enterica Enteritidis* populations in freshly cut melons and apples were examined by Leverentz et al. using lytic phages [16]. A common zoonotic bacterial infection, such as *Campylobacter Jejuni*, is found in raw poultry. *C. Jejuni* is harmless to birds and is a normal component of their gut microbiota [17]. The bacterium contaminates the meat when birds are slaughtered because it is expelled from the intestines. The foods that have been cross-contaminated while being processed with meat can give humans diarrhea and in rare instances, post-infectious consequences like rheumatism and peripheral nerve damage [18].

Table 1. Provides a summary of how endolysins have been used to combat different foodborne pathogens in foods [1]

Endolysins	Organism	Food application	Characteristics
Ctp1L	*Clostridium Perfringens*	Caw milk	About 1-log CFU/mL reduction in 2 h.
LysZ5		Soy milk	Reduction of more than 4-log CFU/mL in 3 hours at 4 °C.
Ply500	*Listeria Monocytogenes*	Iceberg lettuce	Effect of nisin with bacteria in a synergistic manner.
Plyp100		Queso fresco	24-hour reduction of about 4-log CFU at 25 °C (free or immobilized endolysins).
PlyP825		Milk	3.5-log CFU/g reduction at 4 °C during a period of 4 weeks.
		Mozzarella	Combined with high hydrostatic pressure, antibacterial action.
LysH5. Ply187AN -KSH3b λSA2-E-LysO-SH3b.		Milk	At 37 C, there is an immediate 8-log CFU/mL reduction in6 hours.
λSA2-E-LysK- HydH5Lyso.		Milk	Nisin has a synergistic antibacterial action.
HydH5SH3b, CHAPSH3b. LysSA97		Caw milk	At 37 C, there is an immediate 3-log CFU/mL reduction.
LysSA11	*Staphylococcus aureus*	Milk	At 37 degrees Celsius, the drop in CFU/mL is around 3-log.
Phi11-481		Milk, Beef	
			After 15 minutes of CHAPSH3b treatment at 37°C, there was a 4-log CFU/mL reduction.
		Milk, Ham	Carvacrol has a synergistic antibacterial action.
		Milk	At 25°C, the decrease is around 4-log CFU/cm3 in 15 minutes.
			At 2-3 mM CaCl2, there was a lot of activity.

Table 2. lists of commercially available phages products for application in food and animal products [16]

Product	Application	Characteristic	Company
AgriPhage Food	Food (tomato)	Targets Xanthomonas campestris pv. Vesicatoria or Pseudomonas syringae pv. Vesicatoria bacterial specks or spots on crops.	Omnilytics, Inc. USA
EcoShield	Food	Aims to reduce Escherichia coli O157:H7 contamination in food and food processing plants.	Intralytix, Inc. USA
ListShield	Food	*Targets Listeria Monocytogenes Contamination within food and food production sites.*	Intralytix, Inc. USA
SalmoShield	Food	*Targets the contamination of specific highly pathogenic Salmonella-serotypes in food and food production sites.*	Intralytix, Inc. USA
Shigashield	Food	*Targets the contamination of foods and food production sites by Shigella spp...*	Intralytix, Inc. USA
Listex P100	Food	Target contamination with L. monocytogenes on food goods.	Micreos Netherlands
Ecolicide	Animal feeds	Targets infection with Escherichia coli O157:H7 on live animal before slaughter.	Intralytix, Inc. USA
SalmoLyse	Animal feeds	Targets *Salmonella* contamination in pet food	Intralytix, Inc. USA
BioTector	Animal feeds	Salmonella control in the poultry	CheilJedang Co. Korea
SalmoFresh	Food	Targets *Salmonella Enterica* in various foods	Intralytix, Inc. USA

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