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Phages as Biocontrol Agents to Control and Detect Pathogens in the Dairy Industry

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Food safety and sustainable food production is an important part of the Sustainable Development goals aiming to safeguard the health and wellbeing of humans, animals and the environment. The demand for food produce of superior quality is on the rise in order to cater to the requirements of a continuously expanding human population. Sustainability difficulties arise when the expansion of food production has adverse effects on biodiversity and animal well-being, both of which are significant focal points within the One Health agenda. Foodborne illness is a significant contributor to both illness and death, especially in light of the escalating global issue of antibiotic resistance. The WHO states that 600 million cases of foodborne illness occur yearly with 420,000 deaths globally with a prevalence of 30% to 40% among children < 5 years (Moye *et al.,* 2018). To effectively progress towards sustainable food production, it is crucial to use green biocontrol measures to avoid and alleviate the occurrence of infectious diseases in the realm of food production. Substituting existing chemical pesticides, antimicrobials, and disinfectants with environmentally friendly alternatives like biopesticides is a progressive stride towards achieving a sustainable future. The impact of food production systems on developing infectious diseases in humans and animals, as well as their contribution to zoonotic transmission, has been well-documented (Rohr *et al.,* 2019). Moreover, the presence of food microbial contamination leads to the occurrence of disease outbreaks, morbidity, and mortality on a global scale, hence giving rise to food safety concerns that pose challenges in terms of monitoring, prediction, and surveying. In conjunction with the emergence of antimicrobial resistance (AMR), it is evident that foodborne infectious illness will inevitably impose a more substantial economic burden and pose a heightened risk to public health. According to Fenibo *et al.* (2021), the present biocontrol strategies rely on antimicrobial agents such as disinfectants, antibiotics, antifungals, and pesticides (fungicides). However, these treatments are not regarded as environmentally safe or sustainable green approaches. Several bacteria classified as "ESKAPE" by the World Health Organization (WHO) are commonly linked to the transmission of foodborne illnesses. These pathogens include *Enterococcus spp., Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter spp*.

Bacteriophages as Bio-Control

Bacterophages, a type of virus that infects and eliminates bacteria, hold significant promise as biocontrol agents in the dairy industry. It is also known as phages, have been extensively explored as biocontrol agents in the food industry as a means of controlling pathogenic bacteria. Bacteriophages are being increasingly acknowledged as an environmentally friendly biocontrol technology that specifically targets bacterial pathogens found in various environments, including food production. The use of phages as biocontrol agents to prevent infectious diseases in food-producing animals and crops, as well as subsequent foodborne zoonosis, is a highly intriguing field. Phage genomes vary in size, ranging from 3.4 kb to around 500 kb. It is important to note that not all phage genomes include a single gene. This indicates that phages encompass a wide array of genes and proteins that have not yet been thoroughly studied. Phages exhibit a life cycle characterized by either lytic or lysogenic processes. The lytic phase leads to the lysis and subsequent death of host cells, whereas the lysogenic phase involves the insertion of the viral genome into the host genetic material following infection. According to Batinovic *et al.* (2019), phages have a limited host range and heightened specificity as they adhere to the host cell through diverse host receptors, including proteins, carbohydrates, and lipopolysaccharides.

Phages have shown effectiveness as agents for disinfecting and preserving food against several foodborne pathogens, and they also exhibit activity against biofilm communities. Moreover, the therapeutic potential of phages has been well acknowledged, both in terms of prophylaxis and metaphylaxis (O'Sullivan *et al.*, 2020).

Food Borne Pathogen	Route of Transmission	Phage Demonstrating Efficacy
Shigella spp. *(S. flexneri, S. sonnei, S. boydii* and *S. dysenteriae)*	Soft cheese, **dairy,** vegetables, meat products, water, contact via fomites	lytic phage Sfk20 ShigaShield™ cocktail
Acinetobacter baumannii	Fruit, vegetables, meat, fish, **dairy,** water	pIsf-AB02 via endolysin activity
E. coli species (STEC, O157:H7) *Pseudomonas aeruginosa*	Fruit, vegetables, meat, fish, **dairy,** water transmission	Pyo-bacteriophage, Intesti-bacteriophage, EcoShield, Ecolicide® (Ecolicide PX™), Secure Shield E1
Salmonella spp. *(S. enterica, S. thymiurium)*	Fruit, vegetables, seafood, **dairy,** poultry	Salmonella typing phage 12, SJ2, SCPLX-1, SalmoFresh™ SalmoPro
Staphylococcus species	Unwashed handled foods, meat and meat products, poultry, egg products, milk, **dairy products,** salads	vB_SauS-phi-IPLA35, vB_SauS-phi-SauS-IPLA88, SES-bacteriophage, Intesti-bacteriophage
Listeria monocytogenes	Fish and fish products, mixed meat, **cheese,** ready to eat food, **pasteurized milk, ice cream**, raw vegetables.	ListShield™ (formerly LMP-102), PhageGuard Listex™ (formerly Listex™; P100)

Exploitation of Phages in Pathogen Bio-control

Dairy products may have some benefits among the many dietary materials that could be candidates for phage-mediated pathogen biocontrol. For solid products like cheeses, when phages have been added to the surface, it is essential to have high phage titres to guarantee adequate coverage. According to reports, the utilization of phage lysins in conjunction with HHP led to enhanced eradication of pathogens compared to using either HHP or phages alone (Misiou *et al.*, 2018). Unpasteurised milk and raw milk soft cheeses are frequently associated with *L. monocytogenes*. Additionally, lysins have demonstrated potential in enhancing the microbiological safety of dairy products against *Listeria*. In their study, Van Tassell *et al.* (2017) showed that the development of *L. monocytogenes* was hindered when the lysin PLY100 was added to contaminated Queso Fresco and stored in a refrigerator for a period of 3 weeks. Ibarra-Sánchez *et al.* (2018) subsequently ascertained that PlyP100 exhibited stability in cheese for a duration of up to four weeks. Furthermore, they observed a synergistic antilisterial activity when supplied in conjunction with the bacteriocin nisin. The addition of HHP to milk, together with the inclusion of lysin PlyP825, effectively eliminated *Listeria* in 89% of milk samples. Misiou *et al.* (2018) demonstrated a comparable synergistic antilisterial efficacy in Mozzarella. Human illness is caused by foodborne *S. aureus* by the consumption of enterotoxins produced by the bacterium. Staphylococcal enterotoxins (SEs) exhibit resistance to heat treatments, and certain superantigens have the ability to last in milk even following pasteurization (Necidova *et al.*, 2016). The enzymatic domain of lysin Ply187 was fused to the LysK SH3b CBD in pasteurized milk, as reported by Mao *et al*. in 2013. In addition to lysins, VAPGHs have been employed in in vitro trials to decrease *S. aureus* burdens, suggesting their potential application as a food bio preservative.

The ingestion of unpasteurized milk raw milk products has been linked to numerous instances of illness connected to *E. coli* (Mungai *et al.*, 2015). In a study conducted by it was demonstrated that a combination of three coliphages effectively eliminated two strains of *E. coli* in raw milk. This eradication occurred at a temperature of 25 °C, when the cells were metabolically active, as well as following prolonged refrigeration storage. Tomat *et al.* (2013) employed phages to effectively decrease the presence of *E. coli* 0157:H7 during milk fermentations for the purpose of yogurt manufacture. *Enterococcus faecalis and Yersinia* *enterocolitica* are among the pathogens that have been targeted for phage biocontrol in the dairy industry. *Enterococcus faecalis* is linked to the synthesis of tyramine, an amine that induces hypertension and migraines in dairy products such as cheeses. In the study conducted, the utilization of the enterococcal phage Q69 in a cheese model yielded a notable reduction in the buildup of tyramine. Jun *et al.* (2018) conducted a study on the zoonotic disease Y. enterocolitica, wherein phages were effectively utilized to manage this pathogen in milk that had been intentionally contaminated.

Conclusion

In the dairy industry, products contaminated with pathogenic bacteria increase the likelihood of food wastage and economic losses along with the risk to human health. The utilization of phages in biocontrol and bio detection encompasses a diverse range of applications. Phages have the potential to be employed in various biocontrol applications throughout the entire dairy processing chain, encompassing the entire journey from farm to fork.In order to uphold food safety, environmental safety, and sustainability in the realm of food production, it is imperative to expedite the development of environmentally friendly alternatives that may effectively mitigate, eradicate, or manage infections within the food production process. Utilizing bacteriophages as biocontrol agents during the pre-harvest, harvest, and post-harvest stages presents numerous benefits in enhancing food safety and sustainability, aligning with the Sustainable Development Goals.

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