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DETECTION OF FOODBORNE BACTERIAL PATHOGENS USING BACTERIOPHAGE

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ABSTRACT

Foodborne infections continue to pose a hazard to public health, necessitating the development of innovative technologies for their detection. Bacteriophages are great tools for bacterial detection due to their high target cell selectivity, intrinsic signal-amplifying capabilities, simple and affordable manufacturing. This overview outlines the ways in which bacteriophages have been used to accomplish and facilitate precise bacterial identification.

INTRODUCTION

Food is the major mode of transmission for many illnesses, and bacterial contamination of food items creates a difficulty for the food business and represents a high risk to the consumer. Food-borne pathogenic bacteria are bacteria that infect food items and have the potential to cause disease when consumed.[1] Food-borne bacterial infections such as E. coli, Salmonella, Campylobacter and Listeria monocytogenes are important sources of sickness and mortality across the world, posing significant costs to both the food business and health-care systems. Significant costs are incurred as a result of the distribution and consumption of foods contaminated with these bacteria, both in terms of the monetary cost to the food industry and, more importantly, in terms of the cost to the well-being of the members of the public affected by such a disease outbreak.[2] Despite rising knowledge and improved hygienic standards in most western nations, the incidence of foodborne illness outbreaks has remained steady or even increased.[3] Rising demand, particularly for ready-to-eat items, creates extra problems for food production and processing systems, as well as increased hazards for consumers. As a result, there is a need for the development of innovative processes for detecting these viruses in food that are fast and reliable, allowing for the rapid deployment of appropriate management tactics.[4] Bacteriophages provide good tools for bacterial detection. These viruses have co-evolved with their bacterial hosts to recognize and infect their target cells with remarkable precision, which may be used for a variety of quick detection methods. The whole infection cycle of a virulent phage generally takes just 1-2 hours, and via multiplying inside the host cell, offers an inbuilt amplification step, which in many detection assays makes it feasible to minimise or fully dispense with long pre-enrichment processes.[5] There is a multitude of reports in the literature on different phage based detection techniques.[6] However, despite the multiple bacteriophage-based diagnostic methods created thus far, and the clear advantages of harnessing phage for bacterial detection, only a handful of these tests have been converted into commercial solutions.[7] This review gives an overview of the current state of bacteriophage-based detection of foodborne pathogens.

Potential of Bacteriophage Based Pathogen Detection

Bacteriophages (phage) and phage-derived proteins have fascinating features for exploitation in pathogen detection, providing an alternative to more traditional nucleic acid and antibody-based techniques.[8] Before investigating how bacteriophages might be used to monitor and diagnose bacterial illnesses, it is critical to understand how they recognize, infect and disseminate within their bacterial hosts. Phage has a host range, which specifies the types of bacteria that they may infect. Host ranges might be extensive, infecting across bacterial strains, species, and families, or severely limited, infecting just within one bacterial serotype.[9] Bacteriophages (phages) are ubiquitous viruses that infect certain bacterial hosts. During the recent decade, there has been a renewed interest in using phages as therapies (phage therapy) against antibiotic-resistant bacterial infections due to their ability to target and destroy bacteria down to the species or even strain level.[10] Phages are also used as biocontrol agents to tackle foodborne bacteria such as Listeria and Salmonella.[11] There are two types of phage: temperate and virulent. Although billions of bacteriophages infecting diverse types of bacteria exist in the environment, the most well-characterized bacteriophages are members of the Caudovirales, which are either virulent or temperate, depending on the type of infection: Lysogenic and lytic.[12] Phage binds to the bacterial host, specifically on a receptor present on the bacteria's surface, and injects its genetic material into the cell. The host cell provides the molecular building blocks and enzymes required to replicate the phage genetic material and create progeny phage. Phage-encoded proteins, like endolysin & holin, lyse the host cell from inside. Holins are small proteins that accumulate in the host's cytoplasmic membrane and allow endolysin to degrade the peptidoglycan, allowing the offspring phage to escape. As a result, in the outside environment, lytic phage can infect and destroy all neighbouring microbes. When lytic phage are used in phage therapy, their capacity to create a large number of offspring is beneficial.[13] Lytic phage, on the other hand, has a narrow host range and exclusively infects certain bacterial species. This lack of a diverse host range might be addressed by using a phage cocktail. Temperate phage do not lyse the host cell immediately during the lysogenic cycle; rather, their genome is integrated into the host chromosome at certain locations. This phage DNA in the host genome is known as a prophage, and the host cell that harbours the prophage is called a lysogen. The prophage replicates alongside the bacterial host genome, forming a persistent link. One disadvantage of using temperate phage in phage therapy is that portion of the phage population integrates its genome into the host chromosome, causing them to go dormant or change the host's phenotype. The lysogenic cycle can continue indefinitely unless the bacteria are exposed to stress or harsh environmental conditions.[14] The induction signals differ across bacteriophages, but prophage are typically generated when bacterial SOS responses are triggered owing to antibiotic treatment, oxidative stress, or DNA damage.[13] When the lysogenic cycle is completed, phage DNA is expressed, and the lytic cycle starts. Phages that infect Bacillus species were recently discovered to rely on tiny molecules known as arbitrium to communicate and execute lysis-lysogeny choices.[15]

Use of Phages for Detection of Pathogens

Several phage-based food additives have been certified by administrative organisations for the biocontrol of bacteria, using the phage replication cycle for its bactericidal effects.[16] The Food and Drug Administration (FDA) has authorised

ListShieldTM (Intralytix Inc.), a phage preparation containing six bacteriophages known to infect and destroy L. monocytogenes cells, for use in the preservation of ready-to-eat meats. Listex P100 (Micreos Food Safety) is a formulation of a single broad spectrum bacteriophage active against several L. monocytogenes serotypes, which has been generally considered as safe by the FDA and is commercially accessible for use in cheese manufacturing as well as other areas of the food business.[17]. VIDAS (bioMérieux) is a commercially accessible enzyme-linked fluorescence test that uses phage proteins to identify a variety of infections.[18] These automated assays offer "next day detection", requiring a single enrichment step created a much narrower host range gfp reporter phage using the E. coli O157:H7-specific phage PP01 which was capable of discriminating between E. coli O157:H7 and E. coli K12 cells within 10 min based on fluorescence emission.[19] Sensitivity was further increased by once again inactivating the phage's lytic activity, yielding clearer and sharper epifluorescent pictures, translating into simpler and more straightforward detection of E. coli O157:H7 in mixed cultures. These experiments also distinguished between healthy and stressed cells. Healthy cells generated vivid green fluorescence, while metabolically stressed cells emitted fading fluorescent signals.[20] This allows for quick, simultaneous identification of healthy cells vs. cells in a viable but nonculturable (VBNC) state, which, when employing standard plating methods, cannot be achieved without additional and time-consuming stages.

Limitations of Using Bacteriophage

In the period of organic food production and increased awareness of healthy eating, non-chemical solutions for food protection are becoming increasingly popular. Phage cocktails satisfy all the requirements to be recognised as a green technique for treating food-borne pathogenic and spoilage microorganisms. The application of phages in the food chain also has several advantages as follows: [21]

- Bacteriophages are very specialised, typically infecting only one species or kind of bacteria. As a result, the natural commensal microbiota in people and animals' gastro-intestinal tracts is preserved.
- The usage of bacteriophages has not had any negative or harmful effects on eukaryotic cells.
- Phages do not affect the sensory qualities of food items.
- Bacteriophages are particularly resistant to stress caused by food preparation.

Bacteriophage for Biocontrol of Pathogen in Food

Bacteriophage-based biocontrol measures offer a considerable potential to promote microbiological safety, especially, their long history of safe usage, relatively easy handling, and their high and specific antimicrobial activity. The notion of treating viruses in food by using phages may be handled at all stages of production in the traditional 'farm to fork' manner along the whole food chain. Several examples of phage application throughout the food chain.[22]

The Shiga toxin-producing E. coli serotype O157:H7 can infect the human gastrointestinal system and cause sickness with symptoms including abdominal cramps & hemorrhagic diarrhoea. Recent studies have shown that an E. coli-specific phage preparation is efficient in suppressing this serotype.[23]

Salmonella may be isolated from a variety of animal species and is the leading cause of food poisoning. Phage cocktails decreased Salmonella enteritidis fecal levels by 0Æ3–1Æ3 log units. However, even with over 10^7 PFU of phage per gram of fecal material, the pathogen was not entirely eliminated.[24] More recently, artificially infected broilers treated orally with high numbers of bacteriophages (10^{11} PFU).[25] Although the germs were not eliminated from the birds, both experiments revealed that phage therapy would reduce levels of pathogen bacteria entering the chicken production line.

Campylobacter infections are among the most frequently encountered food-borne bacterial infections around the world. The primary mode of transmission for these diseases has been identified as the handling and ingestion of raw or undercooked chicken items. Studies have analysed the use of phages to target Campylobacter bacteria growing on the surface of chicken carcasses, raw chicken meat, raw and cooked beef.[26]

L. monocytogenes has been tested on a number of foods experimentally contaminated with L. monocytogenes, including lettuce, hard pasteurized cheese, smoked salmon, and Gala apple slices; application of this bacteriophage cocktail reduced L. monocytogenes levels in all these foods by ~0.7–1.1 logs.[27]

CONCLUSION

Future research into bacteriophage-based diagnostics has the potential to result in the commercialization of high throughput platforms capable of detecting food-related infections in food matrices in comparatively short turnaround times when compared to present technologies. The simplicity with which phage and phage-derived proteins may be integrated into detection systems makes them an appealing choice for disease detection in the food industry.

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