Promising Characteristics of Bacteriophage Endolysins in Food Preservation

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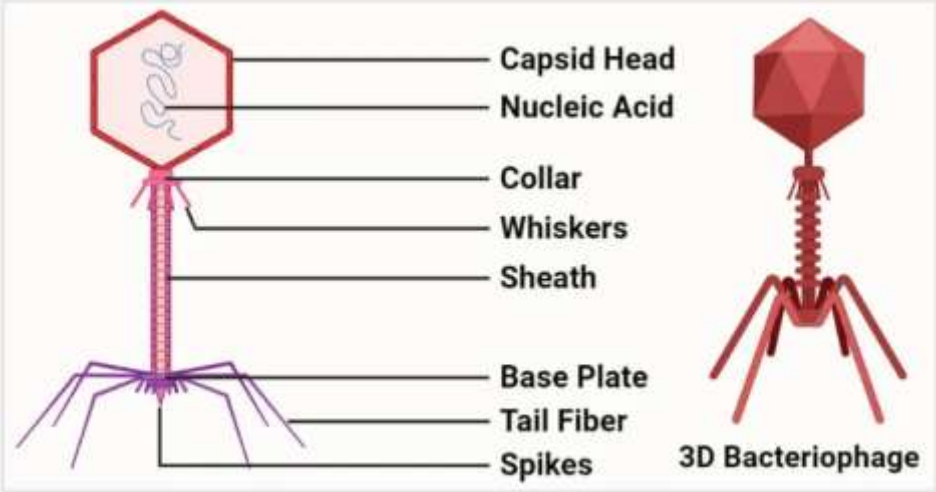
According to the World Health Organization (WHO) in 2015, there were 33 million foodborne outbreaks annually, leading to approximately 420,000 deaths worldwide. This highlights the significant impact of foodborne illnesses on public health. Furthermore, the emergence of antibiotic-resistant bacteria, such as Methicillin-resistant *Staphylococcus aureus* (MRSA), poses a global threat. In the United States alone, the Centers for Disease Control and Prevention (CDC) reported over 2.8 million antibiotic-resistant infections and more than 35,000 deaths each year in 2019 (Kadri, 2020). It is worth noting that antibiotic resistance also presents a food safety concern. The use of antibiotics in food animals, whether for treatment, disease prevention, or growth promotion, facilitates the spread of resistant bacteria and resistance genes from animals to humans through the food chain.

Thus ensuring food safety remains an ongoing and significant challenge for the food industry and healthcare systems worldwide. In recent years, bacteriophage-based biocontrol methods have garnered attention as natural and environmentally friendly technologies that effectively target bacterial pathogens in different food products. In this context, this discussion focuses on the potential of endolysins, cell wall lysis proteins derived from bacteriophages, as a promising class of antibacterial agents for controlling foodborne pathogens.

Bacteriophages

Bacteriophages, commonly known as "bacteria eaters," are a diverse group of viruses that exclusively infect bacterial cells. In nature, they are abundant and exhibit remarkable diversity, surpassing the number of bacterial species. Bacteriophages are considered obligate intracellular organisms, relying on host bacteria for their replication and survival. Structurally, bacteriophages are complex entities, consisting of distinct components such as a head, tails, collar, tail fibers, and base plate (Fig 1).

Fig 1. Structure of bacteriophage



The head of a bacteriophage houses its nucleic acid genome, which can be either RNA or DNA, but not both. This genetic material is encased within a protective protein coat called a capsid or can be surrounded by a lipid membrane known as an envelope. The capsid, composed of repeating protein subunits called protomers, plays a crucial role in packaging the phage genome and facilitating its transfer into a host bacterial cell. These structural intricacies enable bacteriophages to effectively target

and infect specific bacterial species, perpetuating their life cycle as viral predators in the microbial world (Nobrega *et al*., 2018).

Life cycle of Bacteriophage

Bacteriophages exhibit two primary life cycles: the lytic (virulent) cycle and the lysogenic (temperate) cycle. During the lytic cycle, lytic phages attach to bacterial cells and release their genetic material into the host cell. Inside the host cell, the phage genetic material utilizes the host cell's machinery to replicate and assemble new phage particles. Once mature, these phages cause the host cell to lyse, releasing the newly formed phages to infect other host cells. In contrast, the lysogenic cycle does not result in immediate host cell lysis. Instead, after the phage genome enters the host cell, it integrates with the host's genomic DNA and replicates alongside it without causing harm. In some cases, the phage genome may become established as a plasmid within the host cell.

During the lytic cycle, the bacteriophage first attaches to specific receptor sites on the surface of the host cell, followed by irreversible attachment. Enzymatically degraded tail penetration enables the insertion of the phage DNA into the host cell's cytoplasm. Since bacteriophages lack an independent replication system, they hijack the host cell's DNA replication and protein synthesis machinery. Within the host cell, specific enzymes encoded by the phage genome redirect the host cell's DNA and protein synthesis processes to generate new phage particles. These enzymes include structural phage proteins and enzymes necessary for cell lysis and the release of progeny phages. The newly synthesized phage structural components assemble to form complete phage particles, while the newly replicated phage genomes are packaged into the phage heads. At a precise time in the phage cycle, phage-encoded holins create pores in the cell membrane, allowing phage-encoded endolysin to access the peptidoglycan layer of the host cell. This action leads to cell lysis and the release of progeny phages (Mahmoudabadi *et al*., 2017).

Bacteriophage Endolysins

Endolysins are enzymes produced by bacteriophages (viruses that infect bacteria) to degrade the bacterial cell wall and release progeny phages. These enzymes have the ability to specifically target and destroy bacterial cells, making them promising antimicrobial agents. Endolysins are proteins that can effectively target and destroy bacteria. They remain stable and active under environmental conditions similar to those of the bacteria they target. These proteins have an optimal temperature range of 20 to 37°C and require a pH level between 6.0 and 7.0 for maximum activity. Endolysins are soluble and are expressed as cytoplasmic proteins. Endolysins encoded by bacteriophages that infect Gram-positive bacteria typically have a molecular weight ranging from 25 to 40 kDa. On the other hand, those effective against Gram-negative bacteria are generally smaller, weighing between 25 to 20 kDa. Gram-positive endolysins usually have a modular structure consisting of distinct functional domains. They possess an N-terminal end with enzymatic activity domains (EADs) and a C-terminal end with a cell wall binding domain (CBD), which are connected by a short linker. The EADs are responsible for breaking various bonds in the peptidoglycan of the bacterial cell wall, while the CBD exhibits high specificity in recognizing and binding to the bacterial cell wall. An example of such an endolysin is LysH5 from Staphylococcus aureus phage ΦH5. In contrast, Gram-negative endolysins often have a globular structure and typically only possess enzymatic activity domains (EADs). They rarely exhibit a modular structure. (E.g. EL188 from Pseudomonas aeruginosa phage EL) (Barrera-Rivas *et al.*, 2015; Liu *et al.*, 2023)

Modular Structure of Endolysin

A feature of all Gram-positive phage endolysins is their two-domain structure (Fig 2). The N-terminal domain contains the catalytic activity of the enzyme (EAD). This activity may be an N-acetylglucosaminidase, N-acetylmuramidase, transglycosylase, an endopeptidase or an N-acetylmuramoyl-L-alanine amidase (or amidase). On the other hand, the C-terminal cell binding domain, also known as the CBD domain, binds to a particular substrate (often a carbohydrate) present in the host bacterium's cell wall. The binding domain of the enzyme must bind to its cell wall substrate in order for cleavage to occur efficiently. This imparts some specificity to the enzyme because these substrates are only present in bacteria that are enzyme-sensitive.

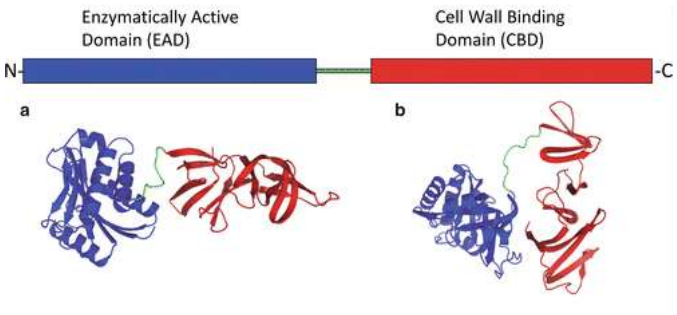


Fig 2. Modular structure of endolysin

Application of bacteriophage endolysins in food preservation

Bacteriophage endolysins have shown potential for various applications, including food preservation. Enzymatically active domain (EAD) has been mainly used in food preservation. Bacteriophage endolysins can be used to target and eliminate specific bacteria that may cause spoilage or foodborne illnesses. Endolysins possess several features that make them highly suitable for food preservation applications. They are:

Potent antimicrobial efficiency: Endolysins exhibit remarkable antimicrobial power, capable of eliminating bacteria in nanogram levels within seconds. This rapid and effective killing of microorganisms sets endolysins apart from other biological compounds.

Lack of bacterial resistance: Unlike traditional antibiotics, repetitive exposure to low concentrations of endolysins does not lead to the development of resistant bacterial strains. The unique mechanism of action of endolysins targets vital components of bacteria that cannot be easily modified, making bacterial resistance rare.

Host specificity: Endolysins selectively target bacterial cell walls by cleaving specific peptidoglycan linkages found exclusively in bacteria. This feature allows for the development of endolysins with a broad host range or a narrower, more specific range depending on the desired application.

pH tolerance: While the optimal pH for most endolysins is between 6 and 7, some endolysins retain significant antibacterial activity over a wide pH range. This flexibility in pH tolerance allows for effective action in various food environments.

Temperature tolerance: Endolysins have shown effectiveness across a wide range of temperatures. They can remain stable and active at elevated temperatures, with some endolysins exhibiting resistance up to 100°C. This thermal stability makes them suitable for food processing and storage conditions.

Long-term stability: Many endolysins demonstrate exceptional long-term stability, retaining their activity over extended periods. This characteristic is advantageous for food preservation, as it allows for prolonged storage without significant loss of efficacy.

Synergism with other antimicrobials: Endolysins can work in synergy with other bactericidal agents, such as antibiotics, organic acids, or high hydrostatic pressure (HHP). This synergistic effect enhances their antimicrobial activity, making them valuable in hurdle technology approaches for food preservation.

These features collectively make endolysins excellent candidates for food preservation applications, offering efficient and targeted antimicrobial action with minimal risk of bacterial resistance. Bacteriophage endolysins can be applied directly to food surfaces, incorporated into food packaging materials, or used as food washes or sprays. The specific application method depends on the food product and the desired outcome. Studies have demonstrated that endolysins show an excellent and efficient bacterial elimination activity from food matrix such as milk with a high content of lipids and proteins and a pH ranging from 6.4 to 6.8, green leafy vegetables, meat products etc.(Chang, 2020).

Advantages of using endolysins as antimicrobials

- No resistant bacteria to phage endolysins have been described to date
- No risks of transferring virulence genes
- Efficient on antibiotic resistant pathogens
- Rapid bactericidal effect
- Both specific and broad activity spectrum
- Target prokaryotic peptidoglycan and are harmless to humans, animals and plants
- Minimal interference with commensal flora
- Apparent safe status (non-toxic)
- Not self-replicating antimicrobial agent (better acceptance)

Future challenges

There are some challenges associated with using bacteriophage endolysins for food preservation. They are unable to inactivate bacterial spores, yeasts, and molds, which may restrict their efficacy in certain food preservation scenarios. Additionally, each endolysin needs to be thoroughly characterized before its application in a food system. This characterization involves evaluating its effectiveness under specific food matrices and conditions, such as varying ionic concentration, composition, pH, and temperature. Such characterization studies are crucial for gaining a better understanding of the potential of endolysins as effective antimicrobials in specific food environments.

Conclusion

It's worth noting that while bacteriophage endolysins hold promise for food preservation, more research and development are still needed to optimize their effectiveness, stability, and commercial viability. Regulatory bodies in different countries may have specific requirements and approvals for the use of these enzymes in food applications.

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