Application of Bacteriophage - Derived Endolysins as Potent Biocontrol Agents for Enhancing Food Safety

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ABSTRACT
Endolysins, bacteriophage-encoded catalysts, have arisen as antibacterial specialists that can be effectively applied in food handling frameworks as food additives to control microorganisms and eventually improve sanitation. Endolysins separate bacterial peptidoglycan structures at the terminal advance of the phage generation cycle to empower phage offspring discharge. Specifically, endolysin treatment is a novel system for controlling anti-toxin safe microorganisms, which are an extreme and progressively continuous issue in the food business. What's more, endolysins can take out biofilms on the surfaces of utensils. Besides, the cell divider restricting area of endolysins can be utilized as an apparatus for quickly recognizing microorganisms. Exploration to expand the utilization of endolysins toward Gram-negative microbes is presently being broadly led. This survey sums up the patterns in endolysin research until this point and examines the future uses of these proteins as novel food safeguarding instruments in the field of sanitation.

Keywords: Food Safety, Biocontrol, Endolysins, Bacteriophage, Disinfection.

INTRODUCTION
Food borne pathogens result in the contamination of food which is a severe problem in the industry of food. For instance, Staphylococcus aureus, Escherichia coli, Salmonella spp, Clostridium spp. And Listeria monocytogenes causes contamination during the processing of food which can result in serious health issues in humans and lead to major economic losses (Loessner, 2005). Hence, it is known that the new strategies in order to control the pathogenic bacteria in the food are required as soon as possible.

The endolysins are the bacteriophage encoded peptidoglycan hydrolases which are synthesized in the conclusion of the multiplication cycle of phage; they lyse cell wall of the host bacterial and release the afresh accumulated bacteriophage virions (Loessner, 2005). To be specific endolysins targets the bonds in the peptidoglycan structure of the bacterial cell wall (Oliveira et al., 2013).

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The holing proteins aid the entry in the cytoplasmic membrane in the process where the endolysins lyse bacterial cell wall (Young et al., 1995). The endolysins in general can also act as the exolysins in Gram+ layer in bacterial peptidoglycan layer. Nevertheless, it cannot decrease outer bacterial membrane around the bacterial cells of the Gram- (Schmelcher & Loessner, 2016). Certainly, the access of endolysin is prevented well by outer membrane of the Gram- bacteria efficiently. Hence, the researches tried to establish the novel methods for making use of endolysins against the pathogens of the Gram.

The enzymatically active domains or the EADs and cell wall-binding domain or CBD composes a modular structure of the endolysins from the Gram+ phages (Rodriguez et al., 2016). Actual enzymatic activity is provided by the EADs which cleaves peptidoglycan structure. On the other hand, the CBD identifies the endolysin to specific cell wall that is associated with ligand molecules with higher specificity and leads the same.

The uses of endolysin are believed to be safe enough so that they do not aid an transduction of gene issues or ass to the developing issues of the resistant bacteria. Even though there are issues about phages application like the development of the gene transduction phage-resistant bacteria, the endolysins don’t initiate such issues (Bakhshinejad et al., 2014). Hence, the endolysins are useful biocontrol agents which could be used in the area of the food safety. Even though there are researches on medical applications of the endolysins has been made public, the studies that lists their uses in the industry of food around the usage of the endolysins are yet to be vigorously directed (Callewaert et al., 2011; Oliveira et al., 2012). Thus, this review aids to provide a current overview of the usage of endolysins and the ideas for the uses as the agents of control against the pathogens that are food borne. Both the potential and the fundamental queries about the endolysins in the food application are discussed.

1. Endolysins: The Structure, Substrate Recognition and Enzymatic Function

The latest phase of the gene expression expresses the endolysins in double stranded DNA phage lytic cycle (Oliveira et al., 2013). Further the replication of phage inside bacterial host, the phage progeny should be released by the degradation of the cell wall. Endolysins becomes the part in this step by wearying the bacterial cell wall and the hydrolyzing peptidoglycan of the host.

Generally, endolysins is present in the modular structure that is composed of two different functional domains (Fig. 1). The endolysins of the gram+ possess some EADs at N terminal end along with the CBD at the end of C Terminal and they are also connected with a small liner (Oliveira et al., 2013). As they have both CBD and EADs, endolysins possess both host bacteria substrate along with enzymatic hydrolysis factors identification functions respectively. To be specific the participates of EAD in cleavage of several bonds in peptidoglycan of bacterial cell wall, while CBD identifies and fixes to bacterial cell wall with the higher specificity. The Gram- endolysins commonly possess a globular structure which possess EADs; unlike the Gram+ it also rarely shows any modular structure (Oliveira et al., 2012). The few Gram- endolysins which possess a modular structure all have inverted molecular structure related to the Gram+ endolysins. EADs are located in the end of the C terminal and the CBD is placed in the N terminal end. For example: *Pseudomonas* endolysin KZ144 (Briers et al., 2009). One such endolysins known as OBPgp279 from *Pseudomonas putida* phage OBP which has been predicted to have double CBDs (Cornelissen et al., 2012). Remarkably, CBDs in the Gram+ endolysins displays the specificity of high post and betters the substrate affinity of enzymes, while CBDs from the Gram- endolysins displays a wide binding spectrum Oliveira et al. 2012.
Various types of EAD are present namely glycosidases, amidases and endopeptidases/carboxy. Glycosidases bout the bonds of amino sugar moieties, while peptidases and amidases cleave amide of the peptide linkages of cross-linking peptides and the interpeptide bridges Oliveira et al. 2013. Endolysins displays the high specificity since they possess a CBD which identifies and bonds to substrate (Eugster et al., 2011). Thus, EAD demonstrates much effectively when it coincides with CBD as it leads endolysin to host cell membrane with the high affinity (Becker et al., 2015). Subsequently, endolysin in specific can target the bacteria as CBD precisely links to host.

2. Mechanisms of the Action of endolysins against the Gram+ pathogens

Few applications of the endolysins on the fields of food science are disclosed in the Fig. 2. As per the linkages that endolysins attacks, it can be categorized into five distinctive classes (Oliveira et al., 2013). The N-acetylmuramidases or lysozymes, transglycosylases and N-acetylglucosaminidases attacks sugar backbone moiety of the peptidoglycan; endopeptidases target peptide moiety and the N-acetylmuramoyl-L-alanine amidases, that are foretold to lead one of the strongest damages in peptidoglycan, hewing the amide link between the N-acetylmuramic acid and the L-alanine. Amongst the endolysins, muramidase, that are found in Pseudomonas aeruginosa phage phiKZ gp144 lysin (Miroshnikov et al., 2006)., is very rare kind, contrary to the amidases that hydrolyze most conserved links in peptidoglycan are widely dispersed (Chang et al., 2017; & Loesser et al., 1998). The researches about endopeptidases showcased that Listeria endolysin Ply500, Ply118 (Guo et al., 2016) and few od Bacillus cereus endolysins (Son et al., 2012; & Swift et al., 2019) have L-alanyl-D-glutamate endopeptidases. Additional, staphylococcal endolysins phi11 have D-alanyl-glycylendopeptidase and hew within peptides that cross-linkage the cell wall (Donovan et al., 2006).

Endolysins may act proficiently when they are present together with holing (Young et al., 1995). Holin aids in leading endolysins to move towards their substrate (Young et al., 1995). During the phage maturation in the infected bacteria, the endolysins contributes in cytoplasm. Consequently, the protein of holing penetrates cytoplasmic membrane and develop holes which allow endolysins to get close and target, the peptidoglycan results in the cell lysis and releases the progeny phases (Young et al., 1995). Until bacterial cell loses the rigidity, the endolysins damages peptidoglycan sheet and interrupt internal pressure that is osmotic.

Generally, the Gram+ phage possesses a system that is holing endolysins in which the
holing gives endolysins access to cytoplasmic membrane which destroys bacterial cell wall (Wang et al., 2000). A number of phage contains signal peptides which leads to secretory pathways from proteins (Thammawong et al., 2006). Significantly, when the gram+ endolysins are put externally to the bacterial cell, it can access cell wall carbohydrates directly and also the peptidoglycan membrane from the outside of cells, thus behaving as antibacterial agents (Oliveira et al., 2012). Additionally, a little amount of purified endolysin is enough to speedy lyse gram+ bacterial cells within few minutes and seconds (Son et al., 2012). Thus, researchers tent to target the gram+ endolysins as the biocontrol agents against the few pathogens that includes Streptococcus pneumonia, S. aureus, L. monocytogenes, Enterococcus faecalis and Clostridium perfringens (Bai et al., 2016; & Ha et al., 2018).

3. Application of the endolysins against the Gram- pathogens
Contrary to the gram+ bacteria the gram- cells are resistant to treatment of external endolysin treatment as they have an outer membrane on the cell wall which refrains the interaction between, the peptidoglycan layer and endolysins (Oliveira et al., 2012). Even though the gram+ endolysins have been put as the biocontrol agents, the current research has displayed the methods in order to overcome outer membrane barrier to lyse and destroy the gram- bacteria (Bai et al., 2019; & Oliveira et al., 2014).

The usage of the outer membrane permeabilizing the agents such as the chelators is most common strategy in order to increase the effectiveness of gram- endolysins as the biocontrol agents. For instance, the chelators like enthelynediamine tetra acetic acid or ETA and the organic acids (malic and citric acids) have commonly been used as the permeabilizers for outer membrane (Bai et al., 2019; & Briers et al., 2015). A proper instance comes from endolysins OBPgp279, that was stated to report to possess bactericidal activity against the Salmonella Typhimurium cell when it is used with EDTA as a combination (Walmagh et al., 2012). Additionally, Oliveira et al. showcased that Salmonella endolysins Lys68 destroys the Gram- cells such as Acinetobacter, Salmonella, Shigella, E. coli O157:H7, Pseudomonas, Pantoea, Cronobacter sakazakii, Proteus and Enterobacter when mixed with malic and citric acid. In the research (Oliveira et al., 2014), organic treatment of acid led to a better efficiency than the EDTA treatment. authors
display a - 5-log CFU/mL bacterial cell wall decrement within 2 hours when endolysin was put externally in the conditions that were slightly acidic. Nevertheless, usage of both the EDTA and the organic acids with the endolysins is problematic as the EDTA is said to harm the human cells and the organic acids can deactivate endolysins in the acidic pH conditions (Bai et al., 2019). In distinct researches, the combination treatment of the physical stressors and endolysins such as the high hydrostatic pressure that produced the endolysins antibacterial effects (Briers et al., 2015). In specific terms, high hydrostatic pressures led to transient permeabilization of outer membrane and allowed gram- endolysin to admittance the substrate (Briers et al., 2015). In a distinct case, Cronobacter sakazakii endolysins LySs1 showcased antibacterial action against gram- bacterial cell were pretreated with the chloroform or heat to destabilize in the integrity of outer membrane (Endersen et al., 2015).

4. Food Safety Application of the Endolysins as Biocontrol Agents

There are number of outbreaks of the food borne diseases is growing, and the development of the antibiotic-resistant bacteria is problematic. According to many research groups an interest in the endolysins as the alternative bacterial agents to the synthetic antimicrobials, together with antibiotics.

Even though phages are better biocontrol candidates some issues are present when considering the phages as antimicrobial agents for the usage in food industry; these also includes requirements to select the virulent phage to ignore transduction and the capable development of bacteria which are resistant to phages (Shannon et al., 2019).

5. Applications of Endolysins against the Biofilms for the Surface Disinfection

Biofilms are the stalk less communities of the microorganism which grows on the exterior and embedded in the self- made extracellular matrix. They are made up of a number of bacterial cells that are attached on the exterior and are surrounded by extracellular matrix which contains a combination of polysaccharides, extracellular DNA and proteins. The bacterial biofilms control is considered vital in the food industry because their existence on the exteriors of utensils might cause grave harm on human health. More intriguingly, the bacteria rooted in biofilms are extremely resistant to disinfectants or antibiotics in comparison to planktonic cells.
Endolysins are a good substitute of antibiotics as they are promising agents of antibiofilm which remove biofilms from the environment of food production. Several staphylococcal endolysins along with their derived proteins showed sturdy biofilm elimination activities against S. aureus and Staphylococcus epidermidis. According to various studies, endolysins are capable anti-biofilm agent which can be used for reducing the formation of biofilms in food business. The activities of biofilm removal by endolysin must be scrutinized under realistic conditions; precisely, flow on the basis of cell models (Purevdorj-Gage et al., 2007; & Snel et al., 2014), multispecies biofilm matrixes (Elias et al., 2012; & Rickard et al., 2012) moreover surface substrates or coating encountered in the facilities of food processing should be investigated (Chorianopoulos et al., 2011).

**CONCLUSION**

As this survey shows, endolysins are promising new specialists for the resistor of food borne microorganisms, especially in food handling and protection applications. Given their high host particularity, they can control just the focused-on microbes as opposed to the useful microscopic organisms, for example, probiotics, in nourishments. In any case, the use of endolysins should be painstakingly considered as their enzymatic properties can be changed under different physicochemical conditions, for example, temperature, pH, and NaCl concentrations focuses. Endolysins can likewise deny the spread of anti-toxin safe microscopic organisms, which is a significant issue around the world. Since endolysins additionally have biofilm evacuation capacities, they could be applied to the surfaces of food-creating amenities. Despite the fact that issues with endolysin application have already existed, particularly toward Gram- microorganisms, different examinations have now presented novel systems that use endolysins as control specialists against Gram-negative microorganisms. In this manner, endolysins are conceivably incredible proteins that could forestall foodborne diseases and upgrade wellbeing in the area of food science.

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