Endolysins

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Endolysins are phage-encoded enzymes utilized by mature phage virions to hydrolyze the cell wall from within. There is significant evidence that proves the ability of endolysins to degrade the peptidoglycan externally without the assistance of phage. Thus, their incorporation in therapeutic strategies has opened new options for therapeutic application against bacterial infections in the human and veterinary sectors, as well as within the agricultural and biotechnology sectors. While endolysins show promising results within the laboratory, it is important to document their resistance, safety, and immunogenicity for in-vivo application.

Endolysins antibiotic resistance bacteriophage

1. Introduction

Endolysins are gaining importance in recent years due to their broad lytic activities against Gram-positive and Gram-negative bacterial cells [1][2]. Endolysins are bacteriophage-encoded enzymes, which act by hydrolyzing the host cell wall and subsequently allowing the release of bacteriophage progenies. Therefore, such enzymes are essential components of the lytic phage life cycle and are a promising alternative to antibiotics [1][2]. The lytic activity of endolysins is classified into different types, namely; (a) acetylmuramidases, (b) transglycosylases, (c) glucosaminidases, (d) amidases, and (e) endopeptidases [1][3]. These five different types are further explained in this review to emphasize their mode of action, as well as regulation of expression. As mentioned, endolysins are involved in peptidoglycan degradation during cell lysis, which is regulated by different phage lytic enzymes and holins [4]. Holins are small hydrophobic hole-forming proteins (>100 nm in diameter) [4][5][6][7][8]. Notably, besides a few exceptions such as lysozyme or lysostaphin, phage proteins rarely develop resistance among their bacterial hosts, mostly due to the horizontal gene transfer among phage–host systems over a long time [1]. However, bacterial hosts additionally develop resistance mechanisms against free endolysins such as development of the outer membrane (capsule), exopolysaccharides (biofilms), etc. In a similar realm, the host immune system such as pro-inflammatory cytokines and antibodies may have implications in the use of endolysin; both aspects will be discussed in further detail throughout this review.

The research surrounding phage lytic enzymes as an alternative for antibiotic resistance has rapidly increased. However, due to the lack of in-vivo studies and sufficient clinical trials, the use of bacteriophages and endolysins for the development of an effective phage therapy has been hindered [3].

2. The Effect of Endolysins on the Normal Microbiota

The human host is colonized with a large number of microbial cells as part of their normal microbiota, covering different parts of the human body such as the urogenital and gastrointestinal tracts, the nasal and oral cavities, and the skin surface [9]. It is estimated that more than 100 trillion symbiotic microorganisms colonize human beings and are of great significance to human health and illness [10]. The normal microbiota forms a physical barrier to protect its host from foreign pathogens, through competitive exclusion and antimicrobial production [11]. Any disturbance to the normal microbiota may cause serious disease. For example, intestinal microbiota dysbiosis influences the level of immune mediators' production leading to metabolic dysfunction and chronic inflammation [12]. Additionally, hepatitis B virus (HBV), human immunodeficiency virus (HIV), and other diseases are associated with microbiota disturbance [12].

Even though antibiotics act to improve human health and life expectancy, broad-spectrum antibiotics disrupt the existing microbiota, causing dysbiosis and leading to disease outcomes [9]. Figure 8 shows the effect of microbiota disturbance on different systems. In contrast to antibiotics, endolysins selectively treat specific species or subspecies of pathogenic bacteria without causing disturbance to the surrounding normal microbiota [13], as previously reported by experiments on Enterococcus faecalis and *E. faecium* using bacteriophage-induced lysin called PlyV12. Their results demonstrated that PlyV12 showed a great lytic effect on the vancomycin-resistant strains of E. faecalis and on multiple strains of *E. faecium*. Therefore, lysins show less disruption of the normal microbiota when they are used to treat various infections, as they do not transfer resistance genes or bacterial toxins destroying the colonizing bacteria of mucous membranes [14]. They can induce the response of the immune system without neutralizing or preventing antimicrobial activity. Hence, they can be used for systemic infections treatments [15]. Additionally, lysins treat the rapid lysis of Gram-positive bacteria through an exogenous application as recombinant enzymes [14].

Figure 1. Relationship of microbiota disturbance with developing diseases in different systems.

For instance, an enterococcal lysin is reported to kill enterococci and a number of other Gram-positive bacteria, including *S. aureus*, *S. pyogenes*, and group B streptococci [13]. Despite their low activity toward these pathogens, this enterococcal lysin has been recorded as one of the broadest acting lysins recognized [13]. Additionally, some naturally occurring lysins have potent activity toward Gram-negative bacteria despite the structural barrier and low permeability of their outer cell membrane to lysins. Ghose and Euler (2020) stated that the addition of some stimulants increases the intrinsic bactericidal activity of lysins significantly toward Gram-negative bacteria. For instance, the fusion protein called artilysin that is composed of a lysine fused to destabilizing peptide is active against both Gram-positive and Gram-negative bacteria. One example of artilysins is the broad-spectrum Art-175 that is composed of *P. aeruginosa* lysin KZ144 modified version [16][17][18]. Therefore, using lysins in infection control is recently of great importance for treating different specific infectious diseases without causing serious harm to the normal microbiota [15]. Imanishi and colleagues conducted experiments in-vivo and in vitro to examine the lethal effect of the Kayvirus-derived endolysin on staphylococcal impetigo [19]. The results showed that the enzyme could exhibit a bactericidal effect on S. aureus after 15 min incubation period and reduced intra-epidermal staphylococci number and pustules size in the impetigo mouse. Additionally, the treatment with lysin increased the skin microbiota diversity in the same animal model.

3. Conclusions and Future Perspectives

Due to the rise in multi-drug resistance bacterial infections across the globe, endolysins as a novel therapeutic approach has received significant attention. Endolysins are an attractive option as they show lytic potential against numerous bacterial species of concern within veterinary and human medicine and show advantages within the agricultural and biotechnology sector. Additionally, endolysins show promising advances against biofilm formation. Interestingly, the recent exploration of resistance, safety, immunogenicity, and the synergy with antibiotics has advanced the research of endolysins further.

Endolysins are the best alternative therapeutic approach to cure and treat multi-drug resistant bacteria. Up to now, many endolysins are reported which show good results in treating antibiotic-resistant bacteria. However, endolysin also has some challenges. Endolysins show good efficacy to treat Gram-positive bacteria, but due to Gram-negative bacterial outer membrane barrier, it shows less activity to treat Gram-negative bacteria [20][21]. Another limitation of endolysin is its short *in-vivo* half-life, due to the production of cytokines' inflammatory response, and the neutralizing antibodies against it. Endolysin provokes an immune response when it is used systematically, so due to immune response, it loses its enzymatic lytic activity *in-vivo* [15][22][23].

New strategies are needed to develop universal chimeric lysin, to cross the Gram-negative bacterial outer membrane barrier, and to overcome these immunological responses against endolysin. While endolysins are proving to be advantageous as novel therapeutics, further research is required to consider their formulation and engineerability towards clinical trials.

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