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Bacteriophage endolysins and their applications

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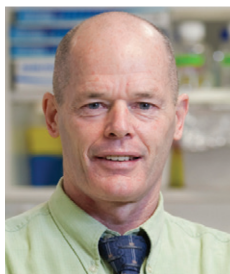

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ABSTRACT

Endolysins (lysins) are bacteriophage-encoded enzymes that have evolved to degrade specific bonds within the bacterial cell wall. These enzymes represent a novel class of antibacterial agents against infectious pathogens, especially in light of multidrug-resistant bacteria, which have made antibiotic therapy increasingly redundant. Lysins have been used successfully to eliminate/control bacterial pathogens in various anatomical locations in mouse and other animal models. Engineering tactics have also been successfully applied to improve lysin function. This review discusses the structure and function of lysins. It highlights protein-engineering tactics utilised to improve lysin activity. It also reviews the applications of lysins towards food biopreservation, therapeutics, biofilm elimination and diagnostics.

Keywords: *antibacterial, bacteriophage, detection, endolysin, lysis, protein engineering*

1. Introduction

Bacteriophages are viruses that can specifically target and infect bacterial cells without causing damage to cell lines from other organisms. These viruses have been employed in the treatment of bacterial infections for nearly a century[1]. Only recently did research into the use of phage encoded recombinant endolysins (lysins), as potential therapeutic candidates, begin[2].

During the phage lytic replication cycle, progeny phages (Figure 1) are released from their host by the action of the lysin enzyme that degrades the host's peptidoglycan cell wall layer, subsequently leading to cell death. Lysin accumulates in the cytoplasm of the host[3] but can also cross the cytoplasmic

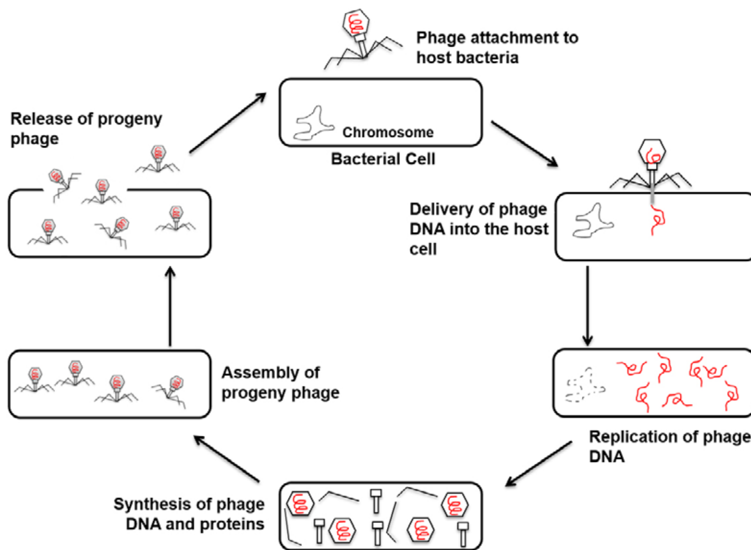

Figure 1 *Bacteriophage replication cycle (virulent phage).*

membrane due to the action of another phage-encoded protein, designated holin, at a specific time point. A holin-independent secretory lysin containing a signal sequence has also been reported[4]. It was suggested that this lysin crosses the cytoplasmic membrane to the cell wall compartment using a specific regulatory system preventing premature cell lysis[4].

Double-stranded DNA phages typically use lysin/holin for the lysis of host cells, whereas single-stranded RNA and DNA phages generally employ the expression of a single lysis gene encoding a small membrane protein[5]. An example of this is the ϕX174 phage lysis protein E, a 91 amino acid membrane protein, which causes lysis by inhibiting the MraY enzyme involved in lipid I synthesis[6]. Phage lysins possess a typical modular domain architecture, consisting of catalytic domain(s) and a cell wall binding domain (CWBD). Most lysins targeting the cell walls of Gram-negative bacteria do possess a single domain architecture[7]. The CWBD is thought to be responsible for targeting lysins to their bacterial cell wall substrate[8]. This binding property has been exploited in various applications ranging from pathogen detection to the isolation and differentiation of pathogenic bacteria from food sources[8]. The therapeutic application of recombinant lysins in eliminating bacterial infections has also been achieved[2]. Lysins are generally active against the bacterial genera associated with the phage, *i.e.* a lysin originating from a streptococcal phage will specifically target streptococci[3]. However, in some cases, phage lysins with broad lytic activity have been reported[9]. For example, the enterococcal lysin PlyV12 is capable of lysing streptococci and staphylococci in addition to *Enterococcus faecalis* and *Enterococcus faecium*[9]. In this case, it was suggested that the lysin recognises a common receptor across the different

bacterial targets[9]. Due to antibiotic resistance in many key pathogens, there is increased pressure for novel antimicrobials to replace the increasingly redundant traditional antibiotics. Lysins possess the potential to satisfy this role. Unlike antibiotics, there is limited to no possibility of bacterial resistance[10–12], making these agents interesting therapeutic candidates for biocontrol of pathogenic bacteria. Lysins targeting many well-known infectious bacteria have been reported to date including *Streptococcus*[2], *Staphylococcus*[13], *Listeria*[14], *Clostridium*[15] and *Bacillus*[12].

This review will focus on the current knowledge gained from the study of phage lysins, which includes: their structure and function; engineering tactics adopted to improve enzymatic function; lysin applications; and other phage encoded proteins associated with lysin activity.

2. Structure and function of phage lysins

Phage lysins in Gram-positive bacteria are generally comprised of multiple domains: typically one or more N-terminal catalytic domains and a C-terminal cell wall binding domain (CWBD). In contrast, the majority of lysins acting against Gram-negative bacteria usually have a globular structure, comprising of just the catalytic domain[7]. Although lysins of Gram-negative origin have been identified with more than one domain, this is uncommon[16].

The CWBD of lysins serves as a binding function to specialised ligands within the bacterial cell wall, and is often linked with substrate recognition. The catalytic domain is responsible for the enzymatic hydrolysis of the peptidoglycan after recognition. Lysins with multiple domains are known to display linker, which bridge the catalytic and cell wall binding domains[20–22]. This linker contains an amino acid cleavage residue allowing for autoproteolytic cleavage of the C-terminal CWBD[23] as reported for the clostridia lysin CTP1L[24] and CD27L[15].

2.1 Cell wall binding domain (CWBD)

The CWBD is responsible for recognising and binding to conserved modules within the bacterial cell wall, conferring specificity towards the lysin target. These targets include molecular structures like N-acetylglucosamine[25], choline[26] and polyrhamnose[27] as well as many other bacterial cell wall subunits. These components attach noncovalently to CWBDs with high affinity and specificity[22].

Further demonstrating CWBD specificity, the C-terminal of the *Lactobacillus casei* lysin Lc-Lys could specifically target bacterial strains of peptidoglycan containing an amidated D-Asn cross bridge, eventually leading to cell lysis but when tested against cell mutants with modified cell wall, lytic activity was completely abolished[28]. This supports the suggestion that the lytic activity of lysins acting against strains of related species is due to binding of CWBD to a specifically-conserved epitope in the cell wall[21]. As such, lysins without a CWBD tend to have a broad antibacterial host range in contrast to

those containing a CWBD, which exhibit a narrow host range[29]. In some cases, the CWBD is crucial for full enzymatic activity against the lysin substrate[30,31], as removal of such domain resulted in loss of lysin catalytic activity as reported with the *Bacillus anthracis* lysin PlyG[30].

X-ray crystallography has been used to determine the 3D structure of several CWBDs. These included the *Listeria monocytogenes* lysin PlyPSA, whose CWBD revealed a unique fold with its structural motif displaying a pronounced hydrophobic cleft consisting of aromatic side chain residues at the interface of the lysin's two subdomains, which was suggested to be involved in substrate recognition[31]. A similar structure based determination of the CWBD of pneumococcal lysin Cpl-1 revealed a choline binding motif that facilitates anchoring onto choline-containing teichoic acid of the pneumococcal cell wall[26].

2.2 Endolysin catalytic domain

The catalytic domain brings about the hydrolytic degradation of the peptidoglycan cell wall, specifically targeting its conserved bonds[32]. Lysins can be classified into five major groups depending on the cell wall peptidoglycan bonds they cleave (Figure 2). These groups include: (a) N-acetyl-β-D-acetylmuramoyl-L-alanine amidases that cleaves the amide bonds between N-actylmuramic acid and the first L-alanine[32]; (b) N-acetyl-β-D-muramidases; (c) Lytic transglycosylases, both of which are involved in the cleavage of glycosidic linkage between N-acetylmuramic acid and N-acetyl-D-glucosamine[33]; (d) N-acetyl-glucosaminidases which cleaves the other glycosidic bonds[32]; and (e) endopeptidases, involved in the cleavage of peptide bonds at the D-alanyl-glycyl moieties[34].

Several lysins are known to contain two catalytic domains. An example is the staphylococcal phage lysin LysK whose catalytic domain harbours a cysteine, histidine-dependent amidohydrolase/peptidase (CHAP) domain as

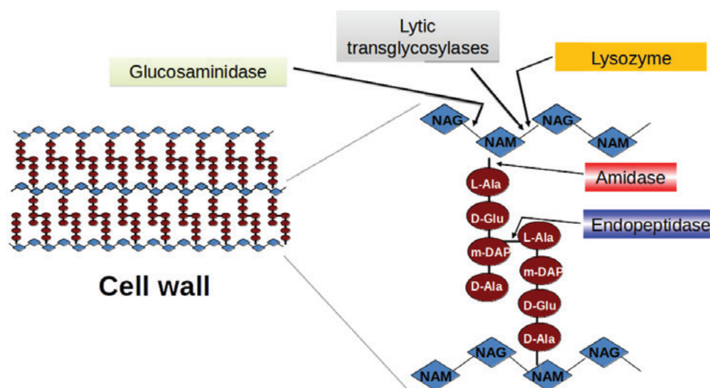

Figure 2 *Schematic representation of a Gram-positive bacterial cell wall structure depicting endolysin cleavage sites within the peptidoglycan.*

well as an amidase counterpart[13]. CHAP domains typically contain a cysteine and histidine residues at the active site[35,36]. Other examples with two catalytic domains include the mycobacteriophage lysins, which often contain a central catalytic domain, an N-terminal domain predicted to possess peptidase activity and an associate cell wall recognition motif [37].

2.3 Lysin activity

Lysins have the potential for use as therapeutic agents due to their antibacterial properties and this has been exploited in a variety of studies ranging from the elimination/control of drug resistant bacteria[19,38–40] to the elimination of bacterial biofilms[41–43]**.** Cell lysis by the exogenous application of lysins is more easily performed in the case of Gram-positive bacteria compared to Gram negatives (due to the presence of the outer membrane in Gram-negative bacteria). Pretreatment of Gram-negative cells with EDTA significantly increases the permeability of the outer membrane, thereby exposing the cell wall to the hydrolytic effect of lysins[44]**.** However, some lysins have been shown to possess lytic activity against Gram-negative cells without the need of an osmotic permeabiliser[45]. It is suggested that the C-terminal region of such lysins could be responsible, as it enhances the permeability of the bacterial outer membrane aiding the N-terminal enzymatic domain in reaching its peptidoglycan target[45]. The modified so called artilysins are other examples of lysins with the ability to penetrate the outer membrane of Gram-negative bacteria without the need of an osmotic permeabiliser[19]. These enzymes constitute a novel class of antibacterial enzyme[19].

Recently, the staphylococcal lysin 2638A was reported with an unusual activity[46], as the amidase domain was more active than its peptidase counterpart. This was reported by Abaev and coworkers[46] to be in direct contrast with lysin possessing similar domain architecture such as the staphylococcal lysins LysK[38] and phi11[43], where both CHAP domains were reported to have higher lytic activities than their amidase counterparts[34,38,43,46]. A *Salmonella* phage lysin designated SPN1S with superior lytic activity to the non-phage-derived cell wall-degrading enzyme lysozyme has also been reported. This enzyme, containing a lysozyme-like catalytic domain, had a 30-fold increase in lytic activity over the chicken egg white lysozyme[47]. Interestingly, two individual lysins with only a 3 amino acid difference between their protein sequences exhibited a significant difference in their cell wall hydrolysing activities despite their high degree of similarities[48]. This indicates that certain amino acid residues play a key role in the overall catalytic function of lysins[49]. Some lysins can have broad spectrum lytic activity, as demonstrated by Lai and coworkers[50] who reported that the *Acinetobacter baumannii* phage lysin LysAB2 was capable of effectively lysing seven different bacterial genera including *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus sanguis*, *Acinetobacter baumannii*, *Escherichia coli*, *Citrobacter freundii* and *Salmonella enterica*[50]. Lysins with

different cleavage specificities have been shown to demonstrate synergy with each other against infectious bacteria, both *in vitro* and in mouse models[51,52]. Synergistic effects have also been demonstrated with other antibacterial agents such as nisin[53], lysostaphin[54] and antibiotics[55,56]. It is believed that such synergy arises from the cleavage of peptidoglycan at two different recognition sites leading to an increased overall activity[57]. Microscopic visualisation of lysin lytic activity has been demonstrated to portray physical changes experienced by bacterial cells in response to the lysin treatment. Treating a streptomycin-resistant *B. cereus* strain RSVF1 with the *B. anthracis* phage lysin PlyG resulted in normal filamentous RSVF1 being converted to short rod and mini-cell like forms, after 30 seconds of exposure[12].

2.4 Resistance to endolysins

Most bacteria have the capacity to develop resistance mechanisms to protect themselves against the action of antibacterial agents. These mechanisms include the modification of cell wall components, efflux pump overexpression, enzyme modification and porins[58,59]. However, no resistance mechanism has been reported for phage lysins to date. Repeated exposure of *Streptococcus pneumononiae* grown on agar plates containing low concentrations of the pneumococcal lysin Pal did not lead to resistant strains[12]. Neither did the successive exposure of *S. aureus* to subinhibitory concentrations of the staphylococcal lysin LysH5[11]. However, similar exposures to other antibacterial agents resulted in the generation of mutants resistant to lysostaphin, novobiocin and streptomycin[11,12]. This suggests that bacteriophage evolved their lysins over the millennia by targeting highly conserved, central modules in the bacterial cell wall, thereby making bacterial resistance to lysins a very rare event[3.]

3. Phage-encoded proteins associated with lysins

The principal phage-encoded proteins associated with lysins include the holins, signal peptides and spanins.

3.1 Holins

During phage replication, lysins move across the cytoplasmic membrane to degrade the peptidoglycan target aided by a membrane protein designated holin. These proteins accumulate in the cytoplasmic membrane of the host bacteria leading to lesion formation in the cytoplasmic membrane, thereby controlling lysins' access to the peptidoglycan[60]. Depending on their membrane topology, holins fall into one of three different classifications based on the number of transmembrane domains (TMD) they possess[61].

The most studied holin is the λ holin gene designated S, which encodes two distinct proteins termed S105 and S107, differing in their protein sequence by the first two amino acids[62]. The C-terminal domain of the λ holin was reported

not to be necessary for formation of inner membrane lesions but rather has a regulatory role in the proper scheduling of hole-forming events[63].

The first *in vitro* study of a phage encoded holin was reported for the λ holin and this involved using purified holin to form lesions in artificial liposome[64]. It was reported that the λ holin also directly interacted with the antiholin in the bacterial membrane by forming heterodimer under oxidative condition[65]. Green fluorescence protein (GFP) fusion study revealed that these proteins accumulate uniformly in the cytoplasmic membrane forming aggregates or rafts in the membrane[66]. Antiholins were also revealed to block lysis by preventing this raft formation[66].

3.2 Signal sequences

Evidence of a signal sequence in the N-terminal of lysins has been reported[4,67]. The first experimental evidence of these secretory lysins was from Sao-Jose and coworkers[4]. In this case, expression of the oenococcal lysin Lys44 resulted in the generation of two polypeptides revealed as precursor and mature forms of the enzyme[4]. Supporting evidence was also reported for the *Lactobacillus fermentum* phage lysin Lyb5[68]. Here, chimeric linkage between the N-terminal of the lysin and the nucB gene from *S. aureus* resulted in the export of NucB protein into the surrounding environment following gene expression in *L. lactis*[68]. Moreover, expression of Lyb5 secretory lysin in *E. coli* also resulted in morphological changes as the normal rod-shaped *E. coli* adopted a spherical shape 20 minutes post induction. It was thus suggested that the morphological change was due to export of lysin to the cell wall[68].

An experimental assay to examine the production of secretory lysin during phage infection was reported for the fOg44 phage[4]. Immunoblot analysis revealed that the mature lysin was first detected at 80 minutes post-infection. It was thus suggested that a regulatory mechanism must be operational to down-regulate lytic activity of secreted lysin during the latent period, which was determined in this case to be 150 minutes postinfection[4]. It was also suggested that maturation of the secretory lysin Lys44 was dependent on the SecA general secretion pathway[4].

3.3 Spanins

A third class of lysis proteins, designated spanins, was also identified[69]. These proteins were composed of an outer membrane lipoprotein with a C-terminal transmembrane domain capable of integrating into the inner membrane[69]. The best-characterised spanins are the lambda Rz and Rz1 proteins. These were suggested to interact forming a complex, which spans the entire periplasm[70]. It was also reported that the Rz protein was unstable *in vivo* in the absence of Rz1 and required complex formation with Rz1 to prevent proteolysis[70].

It was recently reported that the spanin complex was essential for lambda lysis, as expression of lysogens carrying the lambda holin and endolysin genes as well as a null mutant spanin did not result in cell lysis, but rather led to the

development of fragile spherical cells. It was thus suggested that spanins carried out an essential step in outer membrane disruption, in a manner regulated by the state of the peptidoglycan layer[71].

4. Protein engineering

Various protein-engineering techniques have been utilised on lysins to modify their activity. These include domain swapping and shuffling, lysin mutagenesis and other modifications leading to active translocation of lysins.

4.1 Domain swapping and shuffling

The modular structure of lysins endows them with the potential for domain swapping and shuffling, which has been exploited in engineering lysins in several reports. For example, an improved version of a pneumococcal lysin was developed, following chimeric linkage between the catalytic domain of an engineered variant of pneumococcal lysin[72] and the CWBD of another pneumococcal lysin[41]. The resulting chimeric lysin showed increased bactericidal activity over the parent enzyme. In contrast, another chimeric lysin resulting from the fusion of a *Clostridium sporogenes* catalytic domain and *Clostridium difficile* CWBD showed reduced lytic efficiency against *Clostridium tyrobutyricum* compared with the parent *C. sporogenes* lysin[73].

The chimeric linkage between the catalytic domain of phage lysin and the CWBD of the antibacterial peptidase enzyme lysostaphin has also been reported[74]. Here, the resulting chimeric enzyme was capable of controlling *S. aureus* mastitis and could also reduce the bacterial load in mouse models in addition to possessing a synergistic effect with the parent lysostaphin[74], thus demonstrating the potential of chimeric lysins as potential antimicrobials. Protein engineering studies have also been utilised to improve the thermostability of lysins. For example, by replacing the CWBD of a *Clostridium perfringens* lysin with that of another lysin originating from a thermophilic phage, an engineered lysin with improved thermostability was created[75]. Also the poor solubility of a staphylococcal phage lysin resulting in inadequate large-scale production and purification of such lysin was improved by protein engineering studies[76]. The resulting chimeric enzyme, composed of CWBD of a staphylococcal phage lysin together with the highly soluble catalytic domain of an enterococcal phage[21], did not only possess improved solubility but also had a broad lytic activity against a range of staphylococcal strains including streptococci and enterococci[76].

Not only does domain swapping improve the lytic activity of lysin catalytic domains, experimental evidence also suggests that lysin-binding properties can also be affected by domain shuffling[77]. Supporting data on domain shuffling showed that substituting the CWBD of the *Listeria* lysin Ply118 with that of PlyPSA resulted in an abolished lytic activity towards *Listeria* strains of serovar 1/2*,* while enhancing its lytic activity towards serovar 4[77]. This is an interesting

finding, as the native Ply118 lysin could only target the cell wall of *Listeria* serovar strains 1/2, while PlyPSA could also target those of serovar 4[77].

4.2 Mutagenesis

Mutagenesis studies have also been employed in an effort to improve lysin activity. These studies usually employ amino acid substitution(s) and/or deletions. For example, an enhanced bactericidal activity of the pneumococcal phage lysin Cpl-7 was achieved following a 15 amino acid substitution in its CWBD[72]. This substitution also resulted in an inversion of the lysin's net charge at neutral pH from − 14.93 to +3. Using a similar approach, a CWBD-dependent catalytic domain was also converted to a CWBD-independent enzyme[78]. This study suggested that a positive net charge was a requirement for the lytic activity of lysins without its cognate CWBD. And it was also suggested that altering the net charge on the catalytic domain could bring about a refinement or increase in the host range of lysins[78].

The influence of deleting the CWBD on the lytic activity of lysin was studied. Interestingly, this was associated with variable effects. While CWBD deletion dramatically improved lysis in some cases, it either reduced or abolished activity in others. These effects are most likely due to the change in charge of the truncated lysin[78] as it is known that many Gram-positive bacteria do possess a negatively charged surface component, facilitating the action of small cationic antibacterials in the disruption of the bacterial cell[79].

4.3 Lysin translocation

Protein engineering studies involving the active translocation of lysins across the bacterial membrane have been undertaken. As protein secretion involves the attachment of a signal peptide (containing a positively charged N-terminal region, a hydrophobic core and a C-terminal cleavage site) to the protein under secretion[80]. This is vital for the active translocation of the attached protein through the cell membrane of the host following expression.

Gaeng and coworkers[14] revealed that by attaching the *Lactobacillus brevis* S-layer protein signal peptide to the *Listeria monocytogenes* phage lysin A511, active translocation of the lysin from the *Lactococcus lactis* host cells to the surrounding environment was possible[14]. This was demonstrated experimentally as the lysin-secreting *L. lactis* brought about a zone of inhibition around the recombinant *L. lactis* in agar medium embedded with heat-inactivated *L. monocytogenes*[14]. However, recombinant lysin-secreting lactic acid bacteria (LAB) showed poor antimicrobial activity against viable bacterial cells in an *in vitro* coculture assay. This was suggested to be related to the growth rate of the LAB strain affecting the production rate of the secreted lysin[81]. A similar approach in bringing about the active translocation of the *Clostridium perfringens* lysin CP25L to its surrounding environment has also been performed[82]. Here, the CP25L lysin was capable of lysing *C. perfringens* cells

in complex media designed to simulate the conditions of the gastrointestinal (GI) tract[82]. Given that the CP25L lysin did not lyse other members of the gut microflora tested, this suggested that lysins could have the potential to control specific pathogenic strains of bacteria residing in the gut, assuming secretion was adequate by the relevant recombinant bacterial delivery system. Codon optimisation could be an interesting avenue in bringing about increased secretion efficiency leading to higher bactericidal activity of secreted lysins[83]. This was demonstrated by Rodríguez-Rubio and coworkers[83], where codon optimisation of a gene encoding a signal peptide and lysin based on an *L. lactis* codon usage resulted in an increased activity of the secreted lysin[83].

5. Applications of lysins

The lytic capacity of phage lysins in the control of bacteria endows them with various potential applications. These applications ranging from food preservation to pathogen detection ultimately utilise either the lysin's peptidoglycan hydrolytic action or its (CWBD) binding function to achieve its end goal.

5.1 Food biopreservation

Numerous studies have demonstrated the ability of phage lysins to function as a preservative agent in the control of foodborne pathogens posing a major threat to the health and wellbeing of individuals, especially the elderly and the immunocompromised. Zhang and coworkers[84] provided experimental evidence for the ability of the *Listeria monocytogenes* phage lysin LysZ5 to successfully control *L. monocytogenes* to undetectable levels in soya milk. The lysin was also capable of controlling *L. monocytogenes* at refrigeration temperature. It was also reported that 45 U mL^{-1} of the staphylococcal lysin LysH5 was sufficient in eliminating *S. aureus* in milk at a contamination level of 10^3 CFU mL^{-1} [85]. The enzyme also exhibited synergy with the bacteriocin nisin at low concentrations resulting in complete elimination of *S. aureus* in milk[53]. This combination presents a potential food preservative in the control of food pathogens. Also, investigations of a lysin, formulated with silica nanoparticle with the ability to control bacterial growth in lettuce have been performed[86].

5.2 Lysins as therapeutics

Lysin technology represents an alternative therapeutic approach for the control of pathogenic bacteria involved in a variety of animal and human infections. Lysins differ from antibiotics as there is little to no chance of the development of bacterial resistance. This is because lysins generally target conserved bonds within the peptidoglycan structure as mentioned earlier. Lysins' ability to combat pathogenic bacteria *in vitro* and *in vivo* (mouse models) has been demonstrated in several laboratories, with the first *in vivo* experiments reported

by the group of Fishetti[2]. Here, the streptococcal lysin PlyC was capable of providing protection against *Streptococcus pyogenes* colonisation following bacterial challenge in a mouse model[2]. Several other *in vivo* experiments utilising lysins in the control of infectious bacterial pathogens residing in the nasal cavity have also been reported. These include studies performed by Loeffler and coworkers[10], Rashel and coworkers[55], Daniel and coworkers[87] as well as Fenton and coworkers[88].

Studies involving *in vivo* applications of lysins in the control of infectious bacteria in other anatomical locations of mouse models have also been reported. One such study reports the treatment of *S. aureus* induced endophthalmitis by the lysin ply187 [89]. In this work, a single intravitreal injection of the enzyme at six hours post infection drastically reduced bacterial load in the mice's eyes. This also provided a protective effect on the retina at the tissue level[89]. In another study, an intraperitoneal injection of *Enterococcus faecalis* in mouse sepsis model also revealed that the lysin IME-EF1 was capable of providing better protection against infectious *E. faecalis* compared to its producing phage[90]. Topical skin application of lysin has also been reported, where a chimeric lysin ClyS[87] was found to be effective for bacterial decolonisation from mice infected skin. In this case, the lysin formulated in ointment had a better decolonisation effect compared to the standard topical antibacterial agent mupirocin[91]. For respiratory infections, it has also been shown that the pneumococcal lysins Cpl-1 could be delivered to the respiratory airway in aerosolised format to combat pneumococcal lung infections[92]. This was demonstrated in a mouse model, where aerosolised Cpl-1 significantly reduced bacterial load in the lung, thus protecting the mice from pneumococcal bacteraemia. Other *in vivo* studies focussed on the zebrafish embryo infection model[72]. Here, the engineered pneumococcal lysins Cpl-7s improved the survival rate of zebrafish embryo.

Applications of endolysins in animals or humans obviously necessitate the undertaking of safety studies. Accordingly, the first GLP-compliant toxicology and safety study of a phage lysin revealed no sign of toxicity or adverse effect in rats in a trial carried out by Jun and coworkers[93]. Although some side effects were recorded when lysin administration was continued for more than one week in dogs, these were resolved within an hour and were suggested to be due to immune response to the lysin[93]. Studies such as this will advance the use of lysins as therapeutic candidates in the control of pathogenic bacteria in animals and humans.

5.3 Biofilm elimination by lysins

An important feature of many pathogenic bacteria is their ability to form biofilms, resulting in their tolerance to many antimicrobial agents[94,95] and lysins possess the potential to eliminate these structures. The most frequently recognised causative agents of biofilm-associated infections are the staphylococci[94] and lysins with the ability to disrupt their associated biofilms

have been reported. Sass and Bierbaum[43] provided experimental evidence that the phi11 lysin was capable of eliminating *S. aureus* biofilms. The lysin was also suggested to destabilise biofilm structure by rapid lysis of sessile cells embedded within extracellular matrix[43]. Another phage-encoded lysin reported to eliminate staphylococcal biofilm was LysH5. This enzyme was capable of reducing bacterial population in biofilms formed by either *S. aureus* or *S. epidermidis* including persister cells (a bacterial subpopulation that show multidrug resistance)[42]. It was also reported that subinhibitory concentrations of this enzyme completely inhibited staphylococcal biofilm for some of the strains tested in this study[42]. Other lysins reported to eliminate staphylococcal biofilms include SAL-2[96], $CHAP_k$[97], SAL-1[40], PlyGRCS[57] and Ply187[89].

5.4 Diagnostic applications

Pioneering work in the laboratory of Loessner has shown that lysins also have a potential application in the detection and quantification of bacterial pathogens in food materials[32]. Essentially, the lysin's CWBD with its affinity for specific cell wall structures in the host bacterium has been exploited in a few bacterial genera, namely *Listeria*, *Bacillus* and *Clostridium*[98]. Indeed to date, a variety of bacterial detection technologies involving the CWBD have been reported. One involved the use of fluorescent protein attached to lysin's CWBD[99,8]. Another approach incorporated the development of CWBD-based surface plasmon resonance (SPR) technology[100]. Here, CWBD was genetically engineered by attaching glutathione S-transferase to its N-terminal. This allowed immobilisation of the engineered CWBD unto glutathione chips. The use of paramagnetic beads coated with endolysin-derived CWBD proteins in the development of immobilisation and magnetic separation technology has also been reported[98].

The detection technologies mentioned above have allowed for several practical applications in the use of CWBD for detection of bacterial pathogens. For example, CWBD immobilisation onto a glutathione chip allowed for specific and quantitative detection of *Bacillus cereus* and the SPR response intensity was significantly higher than that of antibody-based chip used in comparison[100]. Also, milk contaminated with *L. monocytogenes* was detected using paramagnetic beads coated with CWBD-derived proteins. The average recovery rates recorded for both plating and real time PCR based detection was 97.8% and 70.1% respectively[101].

5.5 Other applications of lysins

Lysins also have a potential application as narrow spectrum disinfectants and this has been investigated by Hoopes and coworkers[102]. The streptococcal lysin PlyC was reported as the first protein-based narrow-spectrum disinfectant against *Streptococcus equi*. The enzyme was also reported to be 1,000 times

more active than the commonly used disinfectant virkon-S as 1 μg of the enzyme sterilised 10^8 CFU mL^{-1} of *S. equi* culture in 30 minutes[102].

Lysins have also been reported as antimicrobial candidates for the control of lactic acid bacterial contaminations in fuel ethanol fermentation[103]. Here, the streptococcal lysin λSa2 was reported to exhibit lytic activity against majority of LAB tested. This enzyme was also capable of reducing *L. fermentum* in a mock fermentation of corn fiber hydrolysate[103].

6. Conclusion

Lysins have increased potential as effective antibacterial agents against infectious pathogens. Their specific nature makes these enzymes and/or their phages good candidates to complement increasingly redundant antibiotic therapy, but in an approach that is far more specific than antibiotics. The application of protein engineering has the potential to significantly improve lysin activity for various biotechnological applications.

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Table 1 *Typical applications of recombinant phage lysins*

Lysin name	Application	Reference
LysZ5	Controlled *Listeria monocytogenes* in soy milk	84
LysH5	Acted in Synergy with nisin to control *Staphylococcus aureus* in milk	53
Cpl-7S	Reduced population of *Streptococcus pneumoniae* in infected zebrafish model providing a 99% survival rate	72
Cpl-1	Protected mice model infected with *streptococcus pneumonia* in aerosolised form	92
$CHAP_k$	Completely eliminated *S. aureus* in nares of mice models as displayed in *In vivo* imaging system (IVIS)	88
SAL-1	Preformulated as SAL-200 with lysin as active pharmaceutical ingredient	40
PBC1	Utilised CWBD in the development of surface plasmon resonance (SPR) technology	100
Ply500 and Ply118	Utilised CWBD in development of magnetic separation technology for immobilisation and separation of bacterial cells	98
λSa2	Controlled *Lactobacillus fermentum* contaminate in a mock fermentation of corn fibre hydrolysate	103
Ply500	Covalent attachment to silica nano particles allowed for decontamination of *Listeria innocua* on iceberg lettuce	86

7. References

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