Advances in the use of endolysins: general remarks, structure, applications, genetic modifications and perspectives

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The increase in the number of multirresistant bacteria to antibiotics makes necessary to find alternatives for the control and/or prevention of infectious diseases. Bacteriophages and their enzymes constitute one of such alternatives. The bacteriophages enzymes that cause death of bacteria by digestion of peptidoglycan are known as endolysins which can be used as enzybiotics. Endolysins have been used successfully as antimicrobial agents in areas like human medicine, veterinary medicine, agricultural sector and industry. Unlike conventional antibiotics, that are not cell selective, endolysins destroy only the target pathogenic bacterium for which the original bacteriophage was specific, without affecting the natural microflora of the organism, food or environment in which they are used. Generally endolysins structure consists of two functional domains, comprising the catalytic domain at the N-terminal end, and the cell wall recognition domain, at the C-terminal end. This allows endolysins to have enough plasticity to be the subject of genetic engineering modifications in such a way to increase its catalytic activity, stability and host range. All these features make endolysins a promising option for the biocontrol of pathogenic bacteria.

Keywords: endolysins; enzybiotics; bacteriophages

1. Introduction

Bacteriophages are viral particles that exclusively infect bacteria. They are the most abundant entity on earth [1] and are present in almost all kinds of environments and are particularly abundant in aquatic systems. Ernest Hanking in 1986 was the first to report the existence of an antimicrobial activity against the causal agent of cholera, the bacterium *Vibrio cholerae*. The antimicrobial was isolated from Ganges and Jumma rivers in India. Independently from each other, Twort in 1915 and D´Herelle in 1917 discover and coined the term "bacteriophage" for the viral particles that lysed bacteria [2]. As viruses, bacteriophages, also called phages, contain either DNA or RNA, single- or double-stranded as genetic material which is covered by the proteic capside. Some may present a proteic tail or a phospholipidic membrane. Bacteriophages may be classified with respect to their lytic properties as lytic or lysogenic. When bacteriophages reproduce they cause lysis of their host cells in order to be spreaded to other cells. This is known as the lytic cycle. When the bacteriophage genome is integrated into its host genome, the genetic information of the bacteriophage is replicated as part of it. No phage particles are produced and no lysis occurs. These are called atemperate phages and the reproductive cycle is known as lysogenic [3]. Filamentous phage reproduce without causing bacterial lysis [1] while non-filamentous phages produce endolysins (encoded in DNA double-stranded phages), bacterial enzymes that hydrolyse peptidoglycan, the major polysaccharide component of bacterial cell walls, by means of a holin-endolysin system or synthesize lysins (DNA or RNA single stranded phages) that interfere or inhibit peptidoglycan synthesis [4].

Peptidoglycan is the major component of the bacterial cell wall. While Gram negative bacteria present an internal and an external membrane in which a small layer of peptidoglycan is contained, Gram positive bacteria present only the cytoplasmic membrane followed by a large layer of peptidoglycan, without an external membrane. Peptidoglycan is a heteropolymer of alternate repeated residues of N-acetylglucosamine and N-acetyl muramic acid joint by glycosidic β1-4 linkages intercrossed by a peptidic bridge generally composed of L-alanine, D-alanine, D-glutamic, L-lysine and L-glycine. Tetrapeptides are linked to carbohydrate residues and are bridged by a penta-aminoacid chain (Figure 1). This confers the bidirectional rigidity to the bacterial cell wall. Disruption of the peptidoglycan structure causes osmotic lysis of the bacteria and hence it's dead. Most bacteriophages enzymatically disrupt the structure of the peptidoglycan by means of the endolysins. The term endolysin was coined until 1958 to refer to the phage component responsible of the bacterial lysis. Lytic phages present a genetic cassete encoding a holin-endolysin system. At the end of the reproductive cycle, once mature viral particles have been ensambled, holins are syntethized in critical concentrations and insert into the cell membrane, creating proteic pores through which endolysins, previously accumulated in the cytoplasm, are exported to reach the peptidoglycan structure [5], Electronic microscopy analysis showed that bacterial lysis indeed

occured by pore formation and disruption of peptidoglycan structure followed by hypotonic lysis of the cell and consequently, dead.

Endolysins are able to degrade Gram-positive cell walls when expressed as recombinant proteins [6]. This supports the potential of application of endolysins as antimicrobial agents. Endolysins are more effective when added externally to Gram-positive bacteria, since they have a fully exposed the peptidoglycan, while in Gram-negative bacteria the external membrane imposes a limitation to the access of the endolysin to the peptidoglycan. However, recent reports that include the use of an antimicrobial peptide that disrupts the integrity of the outer membrane in combination with endolysins are able to lyse this kind of bacteria [6]. Endolysins have been used successfully to control multi-drug-resistant bacterial infections in animal experimental models in surface mucoses and soft tissues [7]. Endolysins use for the control of bacterial infections has several advantages over classical antibiotics. They are specific for the pathogenic bacteria and do not alter the native microbiota; to the best of our knowledge, there have been no reports on the acquisition of resistance to particular bacteriophages or its endolysins; bacteriophages and there endolysins elicit an immune response but this may be controlled by the dose of the endolysisn, so been innocuous for human and animal use [7].

2. Structure and mechanism of action

Endolysins are classified according to its enzymatic activity in: 1) N-acetylmuramoyl-alanine amidases, which hydrolyse the amide bond between the N-acetyl-muramic in the glycan chain and the L-alanil residues; 2) endo-β-N-acetylglucosaminidases, which hydrolises the N-acetylglucosaminil-β-1,4-N-acetylmuramine acid linkage; 3) N-acetyl-β-muramidases, which catalyse the hydrolysis of N-acetylmuramoil-β-1,4-N-acetilglucosamine bond; 4) transglycosylases, which disrupt β-1-4 glycosidic bonds by forming a 1-6 anhidre ring in the N-acetylmuramic residue; 5) endopeptidases, which may hydrolyse both the tetrapeptide linked to the glycosil moieties or the pentapeptide entrecrossing bridge [6, 8, 9];. Substrate bonds for endolysins are depicted in Figure 1. With the exception of transglycosylases, the rest of the endolysins present hydrolytic activity. Muramidases and aminidases are the most commonly reported activities of endolysins. Typically, endolysins present just one of these activities, but there have been reports of bifunctional endolysins. *Staphylcoccus aureus* bacteriophage Φ11 endolysin possess both endopeptidase and amidase domains [4]. Endolysins encoded by double-stranded DNA bacteriophages infecting Gram-positive bacteria have a molecular weight between 25 and 40 kDa, with the exception of *PlyC* endolysin which is 114 KDa in weight [7]. Most of endolysins are composed of at least two functional domains: one containing the catalytic activity located generally in the N-terminal domain and one responsible of the recognition of a specific substrate associated to the C-terminal domain (Figure 2). Catalytic domain is also specific for its substrate and its frequency depends directly on the presence or absence of target bonds. This is supposed to explain why amidases and muramidases are the most common activities among endolysins [8]. The recognition domain usually joins to specific molecules in the bacterial cell envelopes, such as monosaccharides, coline or teicoic acids [10]. Endolysin activity is usually species specific, although there have been reports of endolysins with a wider substrate range. Besides, the cell wall recognition domain is not always essential for endolysin activity [4]. It has been shown that truncation, deletion or disruption of the C-terminal domain results in loss of the specificity of endolysin . Mayer et al. [11] showed that elimination of residues 1-179 of the N-terminal domain of endolysin CD27L against *Clostridium difficile* showed activity against species for which CD27L was not originally active and an increase in its activity. The endolysin got a wider substrate range but it conserved certain specificity, since it was no active against all bacteria. Studies of crystallography and mutation analysis with endolysin *PlyL* against *Bacillus anthracis*, led to propose that the C-terminal domain of this endolysin inhibit the activity of the catalytic domain by particular intermolecular interactions. This inhibition is released when the C-terminal domain binds to its particular ligands in the target cell wall, thus acting as a regulatory domain [12]. Park et al. [13] reported that the endolysin of bacteriophage SPN1S against *Salmonella Typhimurium* is constituted of a large and a small domain with a concave groove between them. Crystallography analysis showed the presence of a catalytic diad in the vicinity of the groove with catalytic residues in the larger domain and the other in the junction of the two domains. This was proposed to be a common structure for Gram-negative endolysins. Sainz-Gaitero et al. [14] analysed the crystallography structure of endolysin LisK from *Staphylococcus aureus* bacteriophage K. This endolysin contains a cysteine-hystidine-dependent amido-hydrolase/peptidase domain ($CHAP_K$) which showed a papain-like topology with a hydrophobic groove near which a catalytic triad composed by Cys54, Hys117 and Glu134 was identified. A calcium ion was also found that was bound to the protein interacting with residues Asp45, Asp47, Asp56 and the Tyr49 and His51 of the N-terminal domain. Both of these reports point to a major groove as responsible of the catalytic activity in endolysins.

Endolysins active against Gram-negative bacteria are usually shorter, 25 to 20 kDa in weight, with a globular structure and a single domain, without an evident recognition domain [15]. When a recognition domain is present, the modular structure of the endolysin is inverted with respect to Gram-positive endolysins, this is with the catalytic domain in the C-terminal and recognition domain at the N-terminal [16]. An exception is endolysisn OBPgp279 active against *Pseudomonas putida* for which to cell wall recognition domains were predicted [17]. Crystal structure analysis has been reported for several endolysins: *PlyC* against *Streptococcus*, which is codified by two genes [18], *Ply PSA* active

against *Listeria* sp. with amidase activity [19], and an endolysin *Clp1* active against pneumococci and which present muramidase activity N-terminal domain and a recognition domain composed of six tandem repeat sequences [20]. All of these show that modular domains are connected by a linker or groove for which its role in catalytic activity has been described above. Besides X-ray crystallography, several other methods have been applied to understand the structure of endolysins, such as magnetic nuclear resonance, tridimensional modelling and homology studies [21].

Endolysins are stable and active in environmental conditions similar to those for the bacteria against which they act. So, optimal temperatures for must endolysins range from 20 to 37°C and optimal pH for maximum activity ranges between 6.0 and 7.0. Bacteriophage ΦH5 endolysin, when cloned and expressed in *Escherichia coli* showed enzymatic activity at pH from 4.0 to 6.0 in acetate buffer and from 7.0 to 8.0 in phosphate buffer [22]. Endolysins active in extreme conditions have also been reported. *PlyPH* active against *Bacillus cereus* and *B. anthracis* showed activity between a pH range of 4.0 to 10.5 [23]. *LysH5* endolysin showed higher activities at 35°C ot 45°C [22] but lisozyme from phage *phiKMV* from *P. aeruginosa* may resist temperatures above 100°C [24], and *LysZ5* endolysin is able to reduce contamination by *Listeria* in soybean milk at 4°C [25].

3. Applications

3.1 Human medicine

Several animal colonization models have been used to colonize mucoses with bacterial pathogens in which the efectivity of endolysins have been tested. This have been the case for vaginal colonization of Group B *Streptococcus [15]*, nasal colonization of *Streptococcus pneumoniae [26]*, oral colonization with *S. pyogenes* [27]. In all of these cases, a single dosis of endolysin reduced the number of bacterial pathogens in several logarithmic units [28]. Typically, endolysins administred intravenously showed a mean life of 15 to 20 minutes. These seems to be enough since endolysins are very active and its catalytic action is very rapid [29]. Experiments with mice infected with *S. pneumoniae* showed 100% of survivor if endolysin *Cpl-1* was added one hour after the inoculation with the bacteria while for the control group without endolysin only 20% of mice survived [28] Similar results were observed for animals after intraperitoneal inoculation with the endolysin. Endolysins have been also successfully tested in animal models for the treatment of meningitis, endocarditis [30] sepsis and influenza. Covalent modification of lisostafin with polyethylene-glycol (PEGylation) increases its half-life for near 24 horas, while unmodified lisostafin showed a half-life of less than one hour [31].

3.2 Use in food

Endolysins have a high potential in alimentary industry, as food preservatives. For example, endolysin *Ply511* was cloned, produced and secreted in *Lactococcus lactis* to create bacterial culture with properties against *Lysteria monocytogenes*. The advantage of the use of lactic acid bacteria as vectors for the expression of endolysins is that they are used for milk fermentation, so its activity may be also observed during fresh cheese elaboration [32]. *Clostridium perfringens* is a common cause of food poisoning. Endolysin *Ply3626* have been successfully used as preservative or biocontrol agent in food for this pathogen [33].

3.3 Veterinary uses

Endolysins may contribute in the treatment and prevention of zoonotic infectious diseases and avoid pathogen transmition through food. *LysH5* and *Ply700* are endolysins specific against *Staphylococcus aureus* and *Streptococcus* sp. which are causal agents of bovine mastitis [10, 34]. *PlyC* and *LysMP* are endolysins active against *Streptococcus equi* and *Streptococcus suis*, both pathogens of the horses and the swines [30].

3.4 Use in agriculture

Endolysins are also useful to eliminate phytopathogenic bacteria. Transgenic potato plants expressing the lysozyme from bacteriophage T4 were active agains *Erwinia carotovora.* Endolysin from phage ΦEa1h was cloned and expressed in *E. coli*. Crude enzyme preparations from *E. coli* were applied over the plant surface. These examples suggest that endolysins may be viable for its use in the agriculture [35, 36].

3.5 Desinfectants

Endolysins have been used as disinfictants in nursery, surgery equipment, surgery room surfaces and diverse materials in elaboration and package of foods. Endolysins have shown effectivity against methicillin resistant *Staphylococcus aureus*, *Listeria monocytogenes*, and *B. anthracis.* Endolysins are better than chemical disinfectants since they do not left toxical residues due to its proteic degradable nature. For example, endolysin *PlyC* active against Group B streptococci show greater capacity than commercial disinfectants [37]. One mg of *PlyC* may sterilize 10 UFC/ml of *S. equi* in 30 minutes. Besides, *PlyC* is still active in the presence of detergents, hard water and organic matter [38].

3.6 Bacterial ghosts

When provided by bacteriophage infection, exogenous addition or expressed from inducible expression systems, endolysins may destroy peptidoglycan integrity and cause cell lysis, leaving cell envelopes and its surface molecules intact (Figure 3). These structures called "bacterial ghosts" may be used as immunogens to elicit immune response, as a substitute of attenuated or inactivated vaccines. Modern engineered bacterial ghosts express the endolysin and the staphylococcal nuclease A (SNUC) a thermostable DNAse that may simultaneously eliminate DNA from the bacterial ghost preparation, making it easy to wash [39]. A typical bacterial ghost preparation procedure consist of growing the bacterial cells at high density, inducing the endolysin/SNUC expression system to allow lysis of the bacteria, and then rising temperature in order for SNUC to hydrolyse DNA. An example of successful bacterial ghost-mediated immunization has been reported for the case of *Salmonella gallinarum.* Bacterial ghosts were used for the immunization of chickens intramuscularly or subcutaneously, both rendering an increase of survivor of chickens treated with the pathogen [40].

3.7 Biofilm elimination

Many bacterial pathogens produce and associate in biofilms that protect them from antibiotics and increases their pathogenicity. Biofilms are composed of a matrix in which bacterial cells and their secretion products (exopolysaccharides, proteins and/or DNA) combinate to construct a microbial community in which bacteria establish interdependent relationships through an active communication and local regulation of gene expression. Treatment of biofilms with endolysins result in elimination of biofilm. Endolysin Φ11 has been successfully used for the elimination of *S. aureus* NCTC8325 biofilms [10], while lysostaphyn is also able to eliminate biofilms from *S. aureus* and *S. epidermidis* [41].

3.8 Biotechnological uses

Endolysins have been most frequently used as part of the protocols to recover nucleic acids and proteins in laboratory research or for diagnostic procedures. Endolysin *Ply118* was used for the extraction of genetic material and proteins from *Listeria monocytogenes*. When the endolysin was added to a concentration of 1 unit, it promoted the lysis of *L. monocytogenes* cells in as short as 8 minutes at room temperature. After lysis, conventional protocols to extract nucleic acids or proteins were performed [42]. *PlyG* endolysin-mediated lysis of *B. anthracis* reached full lysis in as short ase 15 minutes. This is particularly important because current diagnostic methods for *B. anthracis* may extend as long as 48 hours [29].

4. Specificity and innocuousness of endolysins

Since endolysins are highly specific, this confers them a great advantage over other antimicrobial agents that are not selective and may alter normal microbiota of the patient in which they are been used. Gram-positive endolysins are specific due to the cell wall recognition domain [43] and are also biodegradable and non-corrosive [38].

5. Bacterial resistance to endolysins

Coevolution of the bacteriophages with their host bacterial cells results in the recognition of highly conserved structural motifs in the cell envelopes. This interaction suggest that, as little or no variability is expected in the conserved structural molecule in the cell envelopes, the possibility for the bacteria to develop resistance to bacteriophages or their lysins is quite low [44]. Experiments similar to those performed with classical antibiotics to find spontaneous resistance strains have been unsuccessful in generating an endolysin resistant strain. Bacterial cells exposed in culture medium to sublethal concentrations of endolysins have not shown the selection of endolysin-resistant strains. This has been demonstrated for *Pal* endolysin with *S. pneunoniae* [26] and for *PlyG* endolysin with *B. anthracis* [29*]*. Resistant for another peptidoglycan hydrolases as human lysozymes has been reported. The resistence is due to secondary structural modifications to peptidoglycan structure as O-acetylations, N-deacetylations in peptidoglycan or D-alanilations on teicoic acids [45]. Resistance to another peptidoglycan hydrolase such as lysostaphin has been reported [46]. The resistance seem to be due to modifications to the pentaglycine bridge, such as a reduction in one glycine residue [47] or addition of a serine residue [48].

6. Endolysin immunogenicity

As must of the proteins, endolysins are able to elicit the immune system when they are applied systemically or the mucoses [49], which causes a decrease in the catalytic activity of the enzyme. Loeffler et al. [50] immunized rabbits against endolysin *Cpl-1* which is specific against *S. pneumonia*. The hiperimmune serum was able to decrease the

effectivity of *Cpl-1* to eliminate the pathogen, but does not inactivated the enzyme [50]. *In vivo* studies demonstrated that although endolysins activate the immune system, they are still capable of treating systemic or mucosal infections. After bacterial lysis, cellular debris elicit an inflammatory response through cytokines, which is directly proportional to the amount of endolysin used. So, inflammatory reaction may be avoided with the use of low and regulated doses of endolysins in systemic treatments.

7. Sinergism

Endolysins may act synergistically when used in combinations with antibiotics or other antimicrobial agents; endolysins reduce the amount of antibiotic needed and increase efficacy of the treatment. Synergism may also be observed when combinations of endolysins with different substrate specificity and/or enzymatic activities are simultaneously used. This may be possible because the hydrolysis of a first endolysin may facilitate the access of the second endolysin to its specific substrate. Examples of these are the use of endolysin *LysK* with lysostaphyn [51] or the use of endolysin *ClyS* against MRSA in combination with antibiotics vancomycin and oxacyllin. This combination was effective in *in vivo* studies in which infection of mice with MRSA and treatment with antibiotics led to a survivor of 30-35%, but when *ClyS* was added, survivor rised to 80-85% [52].

8. Recombinant Endolysins

8.1 Interchange of recognition domains

Chimeric endolysins with interchanged recognition domains have been described. An example of this are the LC7 and CL7 recombinant endolysins. They were constructed from *LytA* autolysin (LYTA amidase) from *S. pneumoniae*, and *cp17* that encodes the lysozyme CPL7 from cp-7 phage. Both genes encode for coline dependent enzymes. CL7 contains the N-terminal domain of CPL7 and the C-terminal domain of LYTA, and behaves as a lysozyme dependent on choline. LC7 was constructed with the N-terminal domain of LYTA and the C-terminal domain of CPL7, resulting in an amidase that degrades cell walls containing ethanolamine. LC7 showed autolytic activity when cloned and expressed in *S. pneumonia* [53]. Another example is the fusion of the N-terminal domain of the lactococcic phge Tuc2009 and the C-terminal domain of the mayor autilysin Tsl from neumococci. The enzyme shows glicosilase activity able to recognize cell walls containing choline residues [54]. These experiments support the idea of constructing chimeric tailor-maden endolysins with the desired hydrolytic activity, specificity, optimal pH or temperatures, etc.

Complete endolysins or truncated at the cell wall recognition domain from bacteriophage B30, specific for *S. agalactiae*, was fused with lysostaphin, a peptidoglycan hydrolase that is not from viral origin but by *S. simulans*. Specificity of the recombinant enzymes were combined and the resulting enzymes were both active against *S. agalactiae* and *S. aureus*. These enzymes are active in milk, also active against lactic acid bacteria, and may be destroyed during pasteurization of milk [55]. This same work demonstrated that the recombinant endolysin was expressed in mammary cells without damage of this cell type.

8.2 Mutagenesis of endolysins

The main goal of random mutagenesis is to generate aminoacid changes that alter the structure, conformation and/or activity of a protein. An example is the *PlyGBS* mutant endolysins which were cloned and transformed in *E. coli* mutator strain. Selected mutants showed increased activity, 18 to 20 times that of the wild type, those that lost the recognition domain with only a third part of the original weight of the protein, but still specific agains Group B streptococci. Another mutant from this endolysin is PlyGBS90-1 that reduces colonization by 5 logaritmic units, lowering streptococci counts to less than 50 UFC/ml, after 4 hours of treatment in the vaginal tract of mice. This endolysin may be used as an antibiotic prophylactic to reduce neonatal infections, as an alternative to current intra-partum antibiotic prophylaxis (IAP). CD27l is another endolysin, specific against clostridia, for which directed mutagenesis allowed improvements of its activity. A change of Leu98 for Trp, common in *Listeria* specific endolysins, resulted in an increase of endolytic activity against *L. monocytogenes* serotypes [11]. Lowering the size of peptidoglycan hydrolases resulted in increased catalytic activity, probably by the elimination of sequences that modulate the endolysin activity. The presence of the cell wall recognition domain increases lytic activity by directing the catalytic domain near the substrate [19]. Mutants in which the cell wall recognition domain is duplicated showed increased specifity for a substrate but a reduction in lytic activity. Probably the additional catalytic domain generates an sterical restriction that alters the spatial mobility of the endolysin thus obstructing the affinity of the catalytic domain for its substrate. Modification of net charge of the endolysin have been performed in order to allow the enzyme to approach to the Gram-positive cell wall that contains teicoic acids, which confer a negative charge to the cell wall. Mutants with a positive net charge resulted in increased affinity and catalytic activity [56].

8.3 Expression from inducible promoters

An alternative for the application of endolysins in the contamination site is the use of probiotic bacteria as expression vectors. Lactic acid bacteria are a good choice for this goal. Endolysins *Ply 118* and *Ply 511*, active against *L. monocytogenes* were cloned and expressed in *Lactococcus lactis* MG1363 using the plasmid TRKH2 under the control of constitutive promoter P32. To allow secretion, endolysin was fused to a signal peptide $_{SP}$slpA rendering an active extracellular *Ply 511* endolysin [32]. Endolysins may be cloned in pIL6erm vector under the control of *Pgad* promoter, which allows induction of the endolysin by 0.3M NaCl and 50 mM glutamate [57].

9. Concluding remarks

To date, a plenty of information is available on structure-function relationships for several endolysins. Genetic engineering and mutagenesis have helped to understand the mechanistics of its hydrolytic activity. Knowledge related to structural features has allowed to modify endolysins to improve its substrate specificity, affinity and activity. However, there are still many approaches to do. For example, there are no extensive ecological studies that relate the microbiota in a particular environment with the evolution of endolysins. This is important to understand how catalytic domains may be duplicated or interchanged in nature. Most of the knowledge of endolysin structure and activity are concentrated in a few endolysins, must of them amidases and muramidases, but little has been described on transglycosylases, for example. Metagenomic approaches to study the presence of endolysins in particular environments are also scarce or absent. This kind of approaches will provide with greater numbers of endolysin sequences to enrich evolutionary and structure-function studies, and also as a source for biotechnological applications.

10. Figures

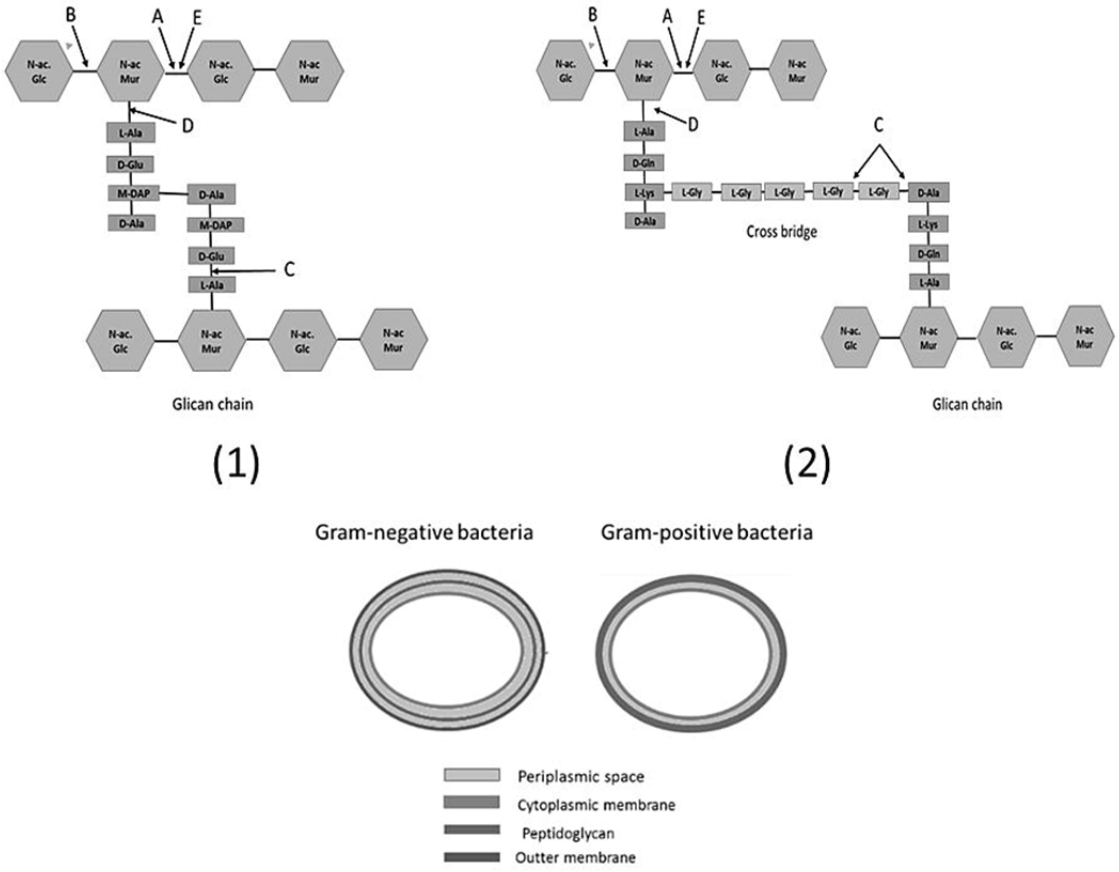

Fig. 1 Differences in peptidoglycan structure among Gram-negative (1) and Gram-positive (2) and cutting sites of endolysins. (A) N-acetil-β-muramidase, catalyzes the hydrolysis of N-acetylmuramoil-β-1,4-N-acetylglucosamine. (B) N-acetylglucosaminidase, catalyzes the hydrolysis of N-acetylglucosaminil-β-1,4-N-acetylmuramine. (C) Endopeptidase hydrolyze peptidic bonds on aminoacids chains linked to the glycan moiety or in the pentapeptidic bridge. (D) N-acetylmuramoil-L-alaninamidase, hydrolyzes the amide bond that connects the glycan with the aminoacids. (E) Transglycosylases, attach the glycosidic β-1,4 bonds resulting in the formation of a 1,6 anhidrous ring in N-acetylmuramic acid. Adapted from Oliveira et al., [58].

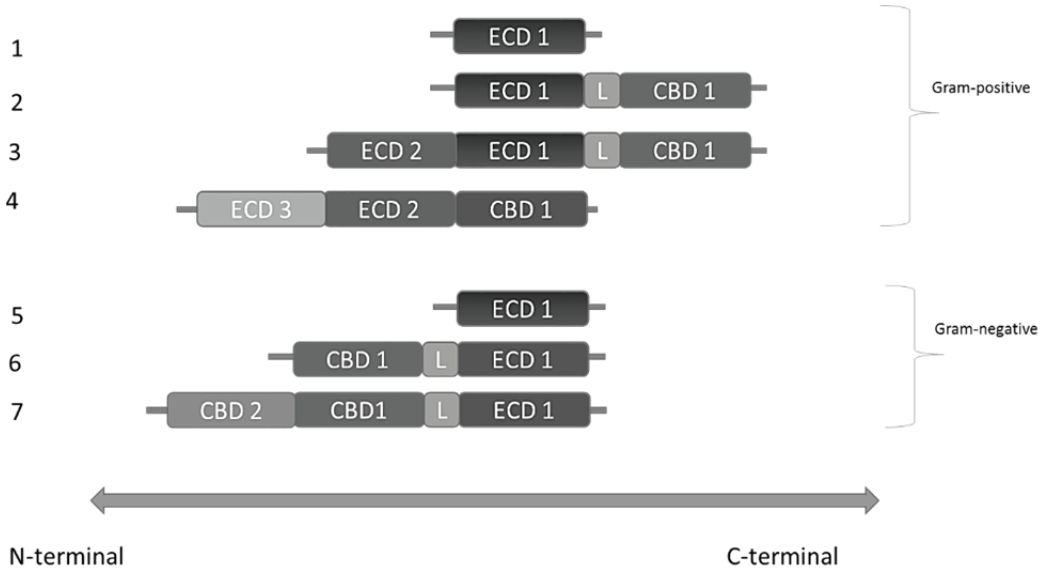

Fig. 2 General structure of endolysins ECD enzyme catalitic domain, CBD cell Wall binding domain, L linker. Adaptated from [16,59].

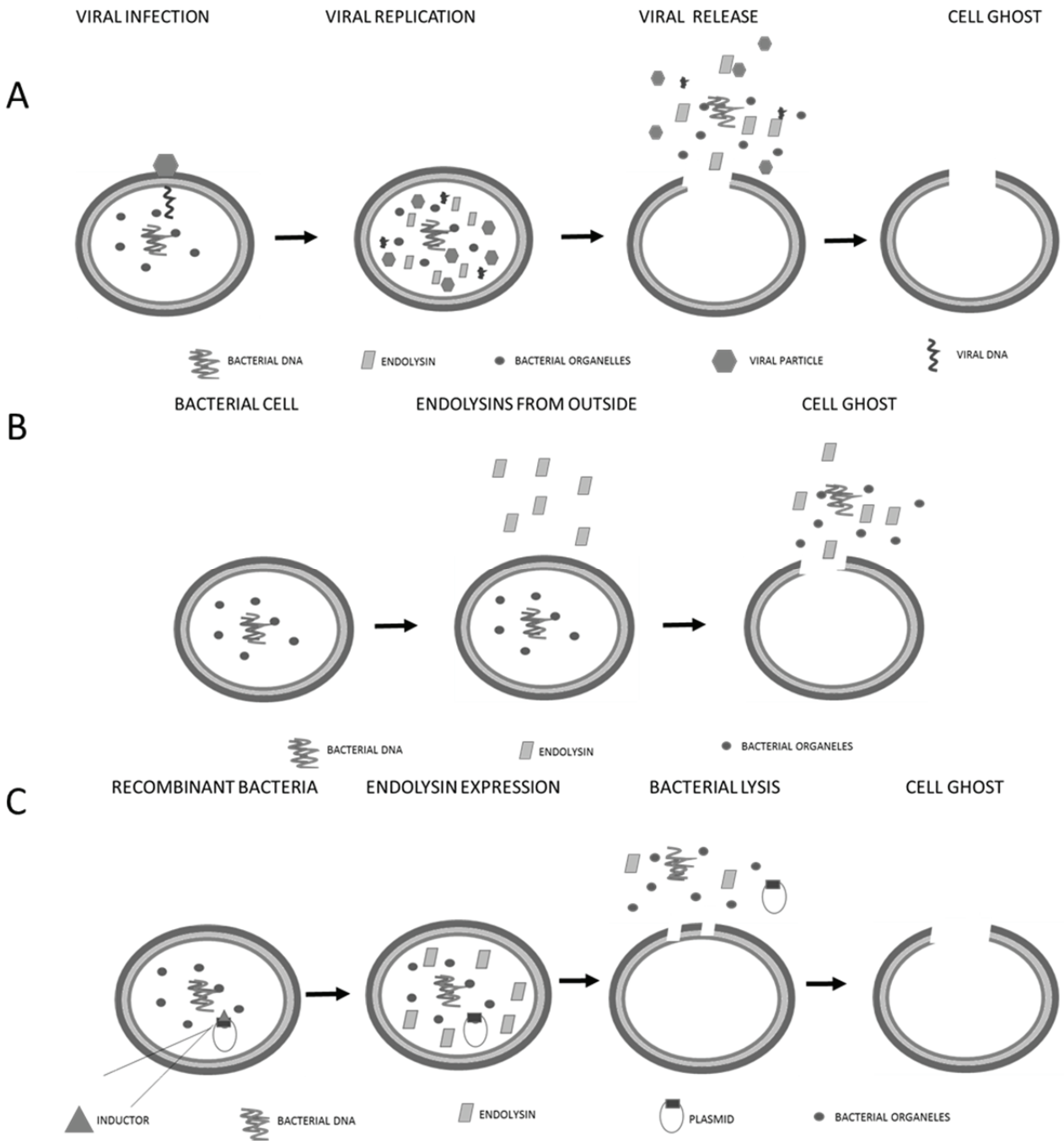

Fig. 3 Bacterial host technology. (A). Using bacteriophage infection. (B) By exogenous addition of an endolysin. (C) By expressing the endolysin from a recombinant plasmid. This may also contain the SNUC to eliminate DNA.

Acknowledgements This work was supported by the Consejo Nacional de Ciencia y Tecnología, México, with the project from Fondos Mixtos No. MICH-2012-C05-196879 to JJVA, and by the Coordinación de la Investigación Científica from Universidad Michoacana de San Nicolás de Hidalgo, México.

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