PHAGE ENDOLYSIN: A WAY TO UNDERSTAND A BINDING FUNCTION OF C-TERMINAL DOMAINS A MINI REVIEW

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Abstract: Endolysins are bacteriophage-encoded peptidoglycan hydrolases, which are synthesized in the end of phage reproduction cycle, in an infected host cell. Usually, for endolysins from phages that infect Gram-positive bacteria, a modular structure is typical. Therefore, these are composed of at least two separate functional domains: an N-terminal catalytic domain (EAD) and a C-terminal cell wall binding domain (CBD). Specific ligand recognition of CBDs and following peptidoglycan (PG) binding mostly allows a rapid lytic activity of an EAD. Here we briefly characterize phage endolysin CBDs in conjuction with their domain architecture, (non)necessity for the following lytic activity and a high/low specificity of their ligands as well. Such an overall assessment of CBDs may help to find new ways to widen opportunities in their protein design to create ʻdesigner recombinant endolysinsʼ with diverse applications.

Key words: bacteriophage, endolysin, cell wall binding domain (CBD), ligand/receptor, peptidoglycan (PG)

1. Introduction

Bacteriophage (phage) is a virus that precisely infects bacterial hosts. After the completion of a replication inside the infected bacterial cell, newly formed phage particles need to be released outside the cell with the help of lytic enzymes (YOUNG, 1992; 2014). These lytic enzymes - endolysins, along with holins, are encoded by a phage and located in so called ʻlytic moduleʼ in the phage genome. Endolysins belong to a group of enzymes - peptidoglycan hydrolases (PGHs) that break down the peptidoglycan (PG) structure. Another efficacy of endolysin is ʻlysis from withoutʼ, by which PG scaffold could be cleaved from the outside of a cell (FISCHETTI, 2008; LOESSNER, 2005). Thorough studies of endolysin mechanism should bring a promising option for the various applications in practice (TIŠÁKOVÁ and GODÁNY, 2014). Theoretically, just a small amount of a recombinant endolysin added externally to Gram-positive cells could immediate a rapid and irreversible lysis of these cells. Conversely, for Gram-negative cells, this action is limited due to the presence of an outer membrane (OM) (SCHMELCHER *et al.*, 2012).

Depending on the origin, phage endolysins can be divided into several groups according to their molecular structure. Our focus is on the architecture of Gram-positive endolysins, known as modular structure, and is composed of at least two distinct functional domains: the enzymatically active domain (EAD) at the N-terminus and cell wall binding domain (CBD) at the C-terminus of an endolysin (NELSON *et al*., 2012).

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Here we evaluate key facts about phage endolysins, and especially about their binding domains - aimed at their diversity, variety of their ligands and possible manipulation (genetic and protein) with these domains. As it is getting to be favourable for such manipulations, there are some endolysin features outstanding, which place them in the category of irreversible inhibitors with diverse applications from without the bacterial cell, such as more or less specific binding of CBDs to the receptors, along with predominantly rapid lytic activity of EADs.

2. Endolysins as representatives of peptidoglycan hydrolases

In both Gram-positive and Gram-negative bacteria, peptidoglycan (PG) is an essential basic component of the cell wall that gives to bacteria: shape and physical integrity (SCHLEIFER and KANDLER, 1972; VOLLMER *et al.* 2008; HUMANN and LENZ, 2009). PG is a unique and covalent macromolecular structure (polymer) of linear glycan strands cross-linked by short peptides (ROGERS *et al.*, 1980). The glycan strands are formed by alternating N-acetylmuramic (MurNAc) and N-acetylglucosamine (GlcNAc), where (MurNAc) residues are linked by β(1→4) bonds. In general, peptidic chains differ in conformation through species (SCHLEIFER and KANDLER, 1972; CHAPOT-CHARTIER and KULAKAUSKAS, 2014). The stem peptides are cross-linked by an $(L\text{-}Ala)_2$ or L-Ala-L-Ser interpeptide bridge between the amino group of the L-Lys of one stem peptide and carboxylate of the D-Ala of another stem peptide (KARAKAWA and KRAUSE, 1966; REINSCHEID *et al.*, 2002; PRITCHARD *et al.*, 2004). Basically, the PG structure is moderately conserved, so there are limited types of covalent bonds (glycoside, amide and peptide bonds) which are accessible to be cleaved by endolysins (LOESSNER *et al.*, 1997).

2.1 Peptidoglycan hydrolases

Peptidoglycan hydrolases (PGHs) are lytic enzymes that target and degrade bonds found in the PG structure. The result of their catalytic activity is a disruption of the cell envelope and a subsequent cell lysis (SHOCKMAN and HÖLTJE, 1994). Advantage of PHGs is a huge diversity of sources including animals, insects, plants, prokaryotic organisms (bacteria) and non-cellular organisms (viruses) (PARISIEN *et al.*, 2008).

Firstly, bacteria possess a high number of PGHs and it appears that they have redundant roles (HÖLTJE and TUOMANEN, 1991; SMITH *et al.*, 2000). Secondly, PGHs may have more than one function – for instance *Escherichia coli* have five N-acetylmuramyl-L-alanine amidases, six membrane-bound lytic transglycolases and three peptidoglycan endopeptidases. All of them contribute at variable extent to cleave the bonds (HEIDRICH *et al.*, 2001, 2002; VOLLMER *et al.* 2008).

One of the essential functions is the ability of PGHs to inhibit the growth of other bacterial species. In this case, they are called **bacteriocins**, in this means PGHs would affect bacterial pathogenicity (they cause bacterial lysis). Generally, PGHs describe a wide range of lytic enzymes that can be classified into sundry groups, while their categorization is based on an individual author, for instance to: (i) **lysozymes**; (ii) **autolysins**; (iii) **exolysins** (bacteriocins); and (iv) **endolysins** (NELSON *et al.*, 2012).

2.2 Phage endolysins and their tasks

A lytic enzyme - endolysin - disrupts the host cell wall itself and new virions can be released into the external environment (THIEL, 2001). In bacteriophage genome, endolysins along with holins are part of the lytic module. Significantly, endolysins are phage-encoded PGHs produced in phage-infected bacterial cells in the end of their lytic cycle. They are accumulated in the cytoplasm of the infected cell while new phage particles are matured (YOUNG, 1992). Endolysins miss the signal peptide sequence, so they are not transferred through the cytoplasmic membrane. But this process is under control of holins (WANG *et al.*, 2000). Holins are expressed at a genetically predetermined time during the late phase of a phage infection and disrupt the inner membrane (bacterial cytoplasm). Subsequently, the PG structure is exposed and cleaved by an endolysin (LOESSNER *et al*., 1997). Their ability to lyse bacteria is often limited to specific bacterial species therefore endolysins mostly have a narrow host spectrum of the lytic activity (FISCHETTI, 2008).

2.3 Endolysin enzymatic and recognition tasks

The primary action of this enzyme is a lysis from within (from inside the infected cell), so it is referred as **endolysin**.

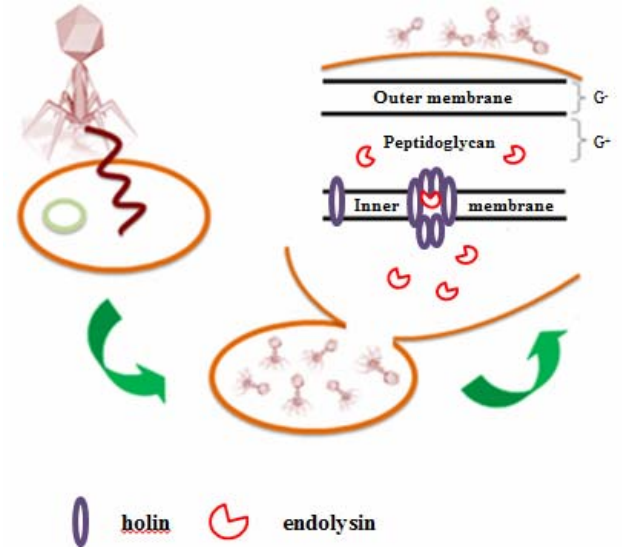

Fig. 1. Schematic representation of phage endolysin mechanism – how to access and breach the PG and release virions layer through the holin-endolysin lytic system.

In Gram-positive bacteria, the PG layer is thicker (up to 40 layers) thereby a surface for catalytic activity of an endolysin is increased (SCHLEIFER and KANDLER, 1972; BERNHARDT *et al.*, 2001). In contrast, Gram-negative PG lies in

periplasmic space protected by the outer membrane; it is relatively thin and not included surface proteins and carbohydrates. This outer membrane effectively prevents from lytic enzymes to directly access the PG structure. However, if the outer membrane is impaired (e.g. by EDTA, detergents), the cells become more sensitive to potential exogenous application of endolysin (LOESSNER *et al.*, 2005).

3. Variable modular structure of endolysins

As mentioned above, endolysins possess two basic functions: (i) binding with its specificity and (ii) enzymatic hydrolysis (KHOSLA and HARBURY, 2001). These two functions are allowed by domains (or modules) that form the structure of these lytic enzymes. Usually, endolysins of Gram-negative-infecting phages are globular proteins, most frequently composed of a single catalytic domain (15-20 kDa) (NELSON *et al.*, 2012). Nevertheless, KZ144 and EL188D, *Pseudomonas aureginosa* phage endolysins, were shown as two endolysins, containing also an EAD, in which first 83 amino acids were shown to be responsible for the proper binding to the cell wall (BRIERS *et al.*, 2009). The occurrence of modular structure was also confirmed in endolysins encoded by phages: 201, phiE202, phi52237, phiRSA1, phiE12-2, BCP3, Bcep781, Bcep43 and Bcep1 (BRIERS *et al.*, 2008; OLIVEIRA *et al.*, 2013). Despite of, the sequence analysis of available endolysins of Gram-negative-infecting phage has shown that their modular structure is really unique.

On the contrary, Gram-positive endolysins mostly use well-defined modular organization (DÍAZ *et al.*, 1990; OLIVEIRA *et al.*, 2012) with one or more enzymatically active domains (EAD) at the N-terminus and a cell wall binding domain (CBD) at the C-terminus, separated by a flexible interdomain linker sequence (KORNDÖRFER *et al.*, 2006).

For instance, endolysins of staphylococcal bacteriophages - phi11, Twort, 187, P68, phiWMY - have the typical modular structure, which consists of EAD (with enzymatic activity as D-alanyl-glycyl endopeptidase, L-muramyl-L-alanine amidase, N-acetyl-glucosaminidase) and CBD (LOESSNER *et al.,* 1998; NAVARRE *et al.*, 1999) (Fig. 2). In addition, EADs are also occasionally present in phage tail-associated PGHs; and both CBDs and EADs can be localized in some bacterial autolysins included in cell division and cell death (NELSON *et al*., 2012).

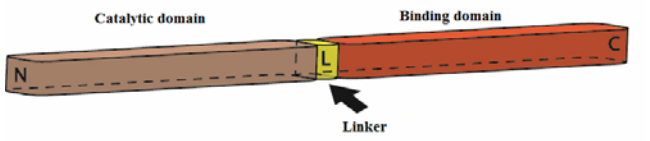

Fig. 2. Scheme of a typical two-domain modular organization of phage endolysin. N = amino terminus of the protein, C = carboxy-terminus of the protein, L = linker, linking the inter-domain region connecting the functional domains.

EAD, predominantly located at the N-terminus, is responsible for endolysin catalytic activity that is based upon the degradation of the PG by the (hydro)lysis of the layers in PG. More specifically, it is targeted directly to individual receptors in PG

(VOLLMER *et al.*, 2008). In the most cases of modular structure, EAD does not achieve sufficient catalytic activity of the cell wall without being connected to CBD, because of the weak affinity to the substrate. Proposed higher specificity and adequate affinity of the enzyme is typically mediated by its CBD (LOESSNER *et al.*, 2002).

Frequently, CBDs can be responsible for the rising strain- or species- specific binding (SCHMELCHER *et al.*, 2010). In general, the size of endolysins is between 25-45 kDa for DNA phages infecting Gram-positive bacteria (FISCHETTI, 2008). The exception is PlyC (114 kDa), a streptococcal endolysin that is unique because of its composition of two separate gene products (PlyCA, PlyCB) (NELSON *et al*., 2006).

Actually, the modular structure has been experimentally demonstrated by several means, e.g. deletion analyses or design of chimeric proteins (GARCÍA *et al.*, 1990; CROUX *et al.*, 1993; MORITA *et al*., 2001). Because of the modular structure, it seems to be plausible that different domains could be swapped and the result is an endolysin with altered enzymatic efficacy and specificity. This was accomplished for the first time by GARCIA *et al.* (1990), where an EAD of *Streptococcus pneumoniae* phage endolysin was swapped but its CBD was preserved so that the enzyme was able to cleave a different bond in PG structure.

The domain Cpl-7 of pneumococcal amidase is one of the earliest identified CBDs specifically in endolysins (GARCÍA *et al.*, 1990). A lot of conserved domains have already been described (HERMOSO *et al.*, 2007; TIŠÁKOVÁ *et al.*, 2014), including the domain LysM (GARVEY *et al.*, 1986; VISWESWARAN *et al.*, 2011) that belongs to the most frequently occurring domains of PGHs (OHNUMA *et al.*, 2008). Many authors have dealt with the characterization of endolysin properties through mutation analysis (CHENG and FISCHETTI, 2007; MAYER *et al.*, 2011; SCHMELCHER *et al.*, 2012). For instance, MAYER *et al.* (2011) described a method how to improve some enzymatic attributes – if the point mutation didn't affect the catalytic activity, changes would occur in the species specificity, in comparison with the original protein.

4. The fashion of endolysin CBDs

The cell wall of Gram-positive bacteria is characterized by the absence of the OM, which typically consists of a lipid complex, such as in the case of Gram-negative bacteria. In fact, CBD of any endolysin allows targeting and directly binding to a PG layer of the host bacterium (FISCHETTI, 2008).

4.1 Cell wall surface receptors

Endolysins have been evolving over millions of years and so their character has focused on recognizing many different ligands within the cell wall. A ligand (receptor) of a CBD could represent a variation of molecules on the cell wall of host organism and has a function as a specific ligand (LOESSNER, 2005). Moreover, binding ability of endolysin CBDs requires disparate type of ligands. Unfortunately, ligands recognized by CBDs of different endolysins have not been sufficiently identified yet. However, expected ligands predominantly belong to carbohydrates in the cell wall,

and their function in binding was confirmed by several studies (OHNUMA *et al.*, 2008; SCHMELCHER *et al.*, 2010, 2012). Ligands can represent different subunits of the PG, like carbohydrates, proteins, choline, as well as teichoic and lipoteichoic acids (OLIVEIRA *et al.*, 2013).

The knowledge of variability among CBDs brings new information that all the components of the bacterial cell wall (carbohydrates, protein, LTA, etc.) may serve as ligands (EUGSTER *et al.*, 2011). For example, the choline-binding sites anchoring of protein to choline group of teichoic acids were found for endolysin Cpl-1 of the pneumococcal phage Cp-1 (GARCÍA *et al.*, 1990; HERMOSO *et al.*, 2003, 2007). Further, endolysin Lyb5 of phage Φ PYB5 infecting *Lactobacillus fermentum* includes a LysM domain that is supposed to be largely responsible for binding to the PG (HU *et al.*, 2010). Several endolysins of phage infecting the genus *Listeria* have been characterized (LOESSNER *et al.*, 2002; KORNDÖRFER *et al.*, 2006; SCHMELCHER *et al.*, 2010), but the exact identity of ligands recognized by their CBDs is under cover. Such analyses will undoubtedly require additional complex and more detailed study.

4.2 (Non)Specificity of the CBDs

Endolysins have gained their substrate specificity in the process of evolution. This feature is based upon the selective interaction of the enzyme with a small group of substances or just with a particular substrate. The CBD is responsible for specificity and high affinity of the endolysin. CBD recognizes and binds to specific ligands on the cell wall of bacteria thereby affinity of the enzyme to the PG is increased. The obvious effect of substrate specificity was proved in two basic examples: for Ply118 and Ply500 - *Listeria monocytogenes* phage endolysins, wherein an irreversible system of binding to a ligand in the surface of the host bacteria was developed (LOESSNER *et al.*, 2002). Again, the CBD, located mostly at the C-terminus, targets and binds to specific ligands of a certain bacterial type of cell wall (it might be a part of PG or the other molecules of the cell wall) and these bonds have really important impact on range of the enzymatic activity (increasing affinity of enzyme to the PG substrate (LOESSNER *et al.*, 2002; SCHMELCHER *et al.*, 2010). Although CBDs are usually located at the C-terminus as well as they can be located at N-terminal end. In some cases their location might be marked as central domains, for instance LysM, Cpl-7 and PG-1 (e.g. streptococcal bacteriophage SMP with following structure - NLPC/Cpl-7/Cpl-7/GLUCO) (OLIVEIRA *et al.*, 2013).

Some molecular studies suggested that the molecular basis of interactions between CBDs and their ligands is in charge of their cell wall. Therefore it leads to the high affinity of the individual bacterial substrate (LOESSNER *et al.*, 2002; BRIERS *et al.*, 2009). For CBDs of endolysin expressed by phage infecting the genus *Listeria,* the equilibrium of constants of binding affinity (in pico- and nano- range) was established. Those values are comparable or higher than affinity of antibody-antigen complex (LOPEZ and GARCÍA, 2004; SCHMELCHER *et al.*, 2010). In some cases, an increase of the lytic activity was observed in the exact moment when CBD was absent (MAYER *et al.*, 2011). The same or similar features were observed while CBD was removed; thereby the higher lytic efficacy of endolysin was achieved (LOESSNER

et al., 1998; GAENG *et al.*, 2000; LOW *et al.*, 2005; CHENG and FISCHETTI, 2007). In other words, CBD likely helps endolysin with binding to the cell wall of the host bacterium (SANZ *et al.*, 1992). Conversely, CBD with its peptide structure may sometimes hinder the action of EAD, as soon as endolysin connects with the cell wall. Efficiency of endolysins also depends on the binding kinetics of CBD. But, on the other hand, there is an evidence of endolysins in which their lytic activity was destroyed after removing CBD from their structural sequence (SANZ *et al.*, 1992; LOESSNER *et al.*, 2002; KIKKAWA *et al.*, 2007; PORTER *et al.*, 2007; SASS and BIERBAUM, 2007). These exact cases confirm that CBD is required for EAD´s activity and, in most cases, these two domains together initiate the disruption of the bacterial cell wall (MAYER *et al.*, 2001).

4.3 Diversity of the CBDs

Endolysin CBDs can often show recognition affinity to more types of strains of the single bacterial species. Consequently, the spectrum of the binding specificity of bacterial cell wall receptors is higher than the host range for phage particle itself, encoding a definite endolysin (SCHMELCHER *et al.*, 2012). In comparison, endolysin PlyV12, encoded by enterococcal phage phi1 (infecting *Enterococcus faecalis,* strain V12), has a wider range of the catalytic activity as phi1 itself. Endolysin PlyV12 exhibits the lytic activity against 14 *E. faecalis* strains and *E. faecium* and against pathogenic streptococci, including *Streptococcus pyogenes* (group A, B, C) as well, and its catalytic activity was also demonstrated against *Staphylococcus aureus* (YOONG *et al.*, 2004). Endolysin Lyb5, encoded by phage phiPYB5 infecting *Lactobacillus fermentum*, is also known to exhibit lytic activity against Gram-positive bacteria (*S. aureus*, *Bacillus subtilis* and few species of the lactic acid bacteria) and against Gram-negative bacteria (*Escherichia coli* and *Salmonella typhimurium*) (WANG *et al*., 2008).

The distribution of CBDs of phages infecting Gram-positive bacteria was also analyzed by *in silico* approaches (Fig. 3).

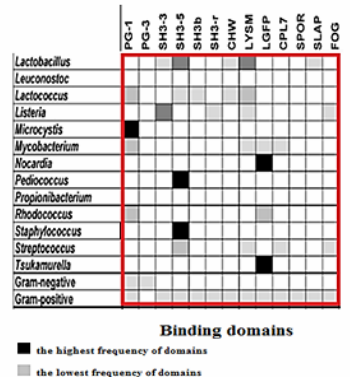

Fig. 3. Frequency of domain distribution of modular phage endolysins. The focus is on the diversity of different types of CBDs from endolysins targeting various Gram-positive and Gram-negative species (adapted from OLIVEIRA *et al.*, 2013).

It is necessary to note that there is increasing evidence about the multiple-domain architecture, important for binding specificity of endolysins. Interestingly, modular endolysins of phages infecting *Lactobacillus*, *Lactococcus* and *Bacillus* strains represent the broadest diversity of their CBDs (PG-1, SH3-3, SH3-5, SH3b, CHW, LYSM a SPOR). An example of endolysin with three CBDs is the endolysin of phage A2 infecting *Lactobacillus* (domain arrangement is: AMI- 2/CHW/CHW/CHW) or the endolysin of phage phiAT3 infecting the same bacterial genus, including three different CBDs (domain arrangement is: GH25/SH3-5/LYSM) (KHOSLA and HARBURY, 2001).

4.4 Categorization of the endolysin CBDs

In the large and diverse group of CBDs, some common domain motifs are comprised: LysM, PG-1, SH3, Cpl-7 (Tab. 1). All of them have also been described and characterized by *in vitro* experiments (BUIST *et al*., 2008; LOESSNER *et al.* 2002; HU *et al.*, 2010; WALMAGH *et al.*, 2012).

(i) LysM (**L**ysin **M**otif) (GARVEY *et al.*, 1986) is one of the most common domains that are present among PGHs (BUIST *et al.*, 2008; VISWESWARAN *et al.*, 2011). LysM polypeptide modules are considered to bind to the widest range of receptors; more than 4,000 proteins in prokaryotic and eukaryotic organisms have been identified. It is known that this domain may bind to GlcNAc residues in the sugar backbone of the PG (OHNUMA *et al.*, 2008). LysM often occurs in double-domain or three-domain architecture, an example of this type of organization in molecule is endolysin of phage Lb338-1 infecting *Lactobacillus* (GH25/LYSM/LYSM/LYSM) (OLIVEIRA *et al*., 2013).

(ii) PG binding (**P**eptidoglycan **B**inding **D**omain) targets specifically to D-Asn in PG cross-bridges. It is also a part of two different endolysins LcLys and LyLys2 that have been identified in prophages present in the genome of *Lactobacillus casei* BL23, targeting at D-Asn interpeptide bridge of the PG (REGULSKI *et al.*, 2013).

PG_binding_1 (**P**eptidoglycan **B**inding **D**omain **T**ype **O**ne) is mainly found in the structure of endolysin of Gram-positive-infecting phage (WALMAGH *et al.*, 2012; OLIVEIRA and MELO, 2013). Exceptions are endolysins of Gram-negative-infecting phages: phage PVP-SE1 infecting genus *Salmonella* and phages phiKZ, EL, 201phi21 and finally OBP infecting genus *Pseudomonas*. One or more CBDs may be uniquely placed at the N-terminus. PG_binding_1 consists of three alpha-helices and its binding activity has been examined f. e. in BRIERS *et al.* (2007), as well as in TIŠÁKOVÁ *et al.*, (2013).

PG_binding_3 type CBD is generally present in endolysins of Gram-negative-infecting phage. Interestingly, endolysin shows the modular structure, as it composed of two distinct polypeptide modules (separated functional domains - GH108/PG-3) (GRUNDLING *et al.*, 2006; OLIVEIRA and MELO, 2013).

(iii) FOG (**F**riend **o**f **G**ATA – zinc finger protein), **SLAP** (**SRC**-like a **D**apter **P**rotein), LGFP, SPOR (**S**porulation **R**elated **D**omain) belong to the group of uncommon CBDs motifs that may represent alternative types of domains for stronger binding and their catalytic activity can directly target against muropeptides or changes in species specificity (OLIVEIRA *et al.*, 2013).

(iv) Cpl-1 (**T**he **C**holine-**B**inding **M**odules) (GARCÍA *et al.*, 1988) recognizes the choline-containing teichoic acids in the cell wall of *Streptococcus pneumoniae* (HERMOSO *et al.*, 2007). Cpl-1 is a type of CBDs that binds to the pneumococcal cell wall and its binding activity is independent on the presence of choline (DIAZ *et al.*, 1991; BUSTAMANTE *et al.*, 2010).
(v) CHW (**C**lostridial **H**ydrophobic **w**ith **C**onserved **T**ryptophan **W**) domains belong to the protein family that targets to the cell wall of *Clostridium acetobutylicum* (SULLIVAN *et al.*, 2007). OLIVEIRA *et al.* (2013) demonstrated that nine domains of CHW are also present in three endolysins of phage infecting genus *Lactococcus* and six endolysins of bacteriophage infecting genus *Lactobacillus*.
(vi) SH3 (**SRC H**omology 3 – **D**omain **B**inds **t**o **P**roline-rich **L**igands) (MAYER *et al.*, 1988; STAHL *et al.*, 1988) is common in autolysins and phage endolysins, often as SH3b, SH3-3 or SH3-5 (GRUNDLING *et al.*, 2006; LU *et al.*, 2006). In the study by BUIST *et al.* (2008) it was shown that SH3 mostly occurs in the modular structure of endolysins encoded by phages infecting genus *Staphylococcus*.

Table 1. Representation of identified binding domains of Gram-positive and Gram-negative endolysins (adapted from OLIVEIRA *et al.*, 2013).

Binding domains	**Conserved domains**	**Phage example**
PG_binding_3	PF09374/IPR018537	*Vibrio* phage VP882
LysM	PF01476/IPR018392	*Enterococcus* phage phiEf11
SH3_3	PF08239/IPR013247	*Lactobacillus* phage Lv-1
SH3_5	PF08460/IPR013667	*Staphylococcus* phage phi2958PVL
PG_binding_1	PF01471/IPR002477	*Mycobacterium* phage Hertubise *Salmonella* phages phiKZ, EL, 201phi21
CHW	PF07538/IPR006637	*Lactococcus* phage 949
CPL-7	PF08230/IPR013168	*Streptococcus* phage SMP
LGFP	PF08310/IPR013207	*Nocardia* phage NBR1
SH3-r (related)	SUPFAM0051050	*Listeria* phage A500
FOG	COG5263	*Listeria* phage B054
SH3b	smart00287	*Lactococcus* phage P087
SPOR	PF05036/IPR007730	*Bacillus* phage AP50
SLAP	PF0321/IPR004903	*Bacillus* phage 0305phi8-36

4.5 Endolysin CBDs as enzymatic activity regulators

Binding activity of CBDs is their primary feature, but sometimes they can play determining roles in the enzymatic activity associated with the manner to allow substrate to achieve its catalytic site. Carbohydrate-cleaving hydrolases, such as xylanases and cellulases have a similar modular architecture as endolysins (KHOSLA and HARBURY, 2001) and their activity increase if there is enzyme-substrate proximity (BOLAM *et al.*, 1998). In the case of truncated version of CBD from *Clostridium* phage phi3626 endolysin, an entirely abolished activity has been achieved, accentuating the important role of CBD in the enzyme activity (ZIMMER

et al., 2002). Structural analysis of PlyL suggests that CBD not only targets the endolysin to the specific cell wall but also it may be significant for the lytic activity. It has been demonstrated that with the absence of CBD in the modular structure of endolysin, the activity has been inhibited (LOW *et al.*, 2005).

5. SH3 domain - universal though specific

Regularly, SH3 domain occurs in the modular structure of staphylococcal endolysins, but it is possible to be found in other endolysins, such as streptococcal. This unique CBD may be exploited for the enhancement of anti-staphylococcal efficacy (for instance in protein fusion constructs) (BECKER *et al.*, 2009). At first, SH3 domain (SRC Homology 3) was described in eukaryotic proteins involved in cell-cell communication and intracellular signalization from the surface to the nucleus. Moreover, this domain prefers to bind to the proline rich sequence (MAYER and ECK, 1995). Extensive description of its presence in phage endolysin was firstly presented by PONTING *et al.*, (1999), and WHISSTOCK and LESK (1999). SH3 domain is a small module of 50 to 60 amino acids (WENG *et al.*, 1995). In the Pfam domain database (http://www.sanger.ac.uk/Software/Pfam/), three groups of SH3b are possible to be found, respectively: SH3_3, SH3_4 and SH3_5 (LU *et al.*, 2006).

The SH3 domain of lysostaphin was one of the first identified binding domains at all. A critical 92 amino acid region at the C-terminus of lysostaphin was proved as essential for a correct specificity of binding to the staphylococcal surface through fusion of this region to ligands (BABA and SCHNEEWIND, 1996). Successfully, this fusion strategy has also been used to verify SH3 for other PGHs: LytA (phi 11 endolysin) (BABA and SCHNEEWIND, 1996), Ba02 endolysin (LOW *et al.*, 2005), Ply500 and Ply118 endolysin (LOESSNER *et al.*, 2002). Becker *et al.* (2009) presented the first comprehensive comparison of all staphylococcal sequences of SH3_5 domains obtained from the publicly available databases.

The maintenance of SH3 binding domain in modular structure of endolysin has been optimized through evolution and gives the added value for these enzymes, although this fact has not been confirmed in every study (BECKER *et al.*, 2009). For some endolysins, SH3b domain is indispensable, especially for the binding of an endopeptidase domain to the PG of *Staphylococcus aureus* (BABA and SCHNEEWIND, 1996) and Ale-1 (LU *et al.*, 2006). Moreover, many studies have been directly focused on the phage endolysin infecting various staphylococci and streptococci. In this association, it has been shown that after the removal of the SH3 domain, the catalytic activity still carried on (DONOVAN *et al.*, 2006).

6. How to exploit the CBDs for the bacterial detection

Currently, detection methods of bacterial pathogens, causing a variety of human and animal infectious diseases, are often slow and limited. The same stands for a rapid identification of contaminating bacteria in food products as well. Endolysins may be used for specific detection of bacteria (KRETZER *et al.*, 2007; SCHMELCHER *et al.*, 2010). Using the enzyme features (as the specificity of the binding domain) may help in the selection and proper separation of the bacterial cell (KRETZER *et al.*, 2007).

For example, SCHUCH *et al.* (2002), focused on detection of *Bacillus anthracis* by endolysin PlyG with direct results in 15 minutes, based on the detection of substances that are produced during the bacterial lysis. In another study, magnetic balls were used for detection of selected bacterial hosts, where these special balls were coated with a layer of binding domain of Ply188 and Ply500 endolysins of phage infecting genus *Listeria*. By this strategy the effective separation of the cells *Listeria* in contaminated food was approached (KRETZER *et al.*, 2007). It was also proved by *in vitro* experiments that CBDs of bacteriophage endolysin infecting *Bacillus cereus* (LOESSNER *et al.*, 1997) and *Clostridium perfringens* (ZIMMER *et al.*, 2002) may also bind and mediate the immobilization of these bacteria (KRETZER *et al.*, 2007).

An efficient non-destructive monitoring of the bacterial host can be also mediated by the fusion of a target protein with GFP (green fluorescent protein) from jellyfish *Aequorea victoria* (CHALFIE *et al.*, 1994; ANDERSEN *et al.*, 1998). GFP can be expressed in a variety of organisms (bacteria, yeasts, insects, mammals) and its characteristic property - green fluorescence - is used in the studied system at the level of individual cells. According to some authors (LOESSNER *et al.*, 2002; SCHMELCHER *et al.*, 2010), fluorescent proteins are useful, for instance in the detection of *Listeria monocytogenes (*LOESSNER *et al*., 2002; SCHMELCHER *et al.*, 2010; TOLBA *et al*., 2012).

In recent years, bacteria causing serious infections have become resistant to a broad spectrum of antibiotics. Examples are strains of MRSA (*Methicillin*-R*esistant Staphylococcus aureus*) that are resistant to β-lactam antibiotics such as penicillin. Endolysin therapy is one way how to ameliorate bacterial resistance of *S. aureus* BORYSOWSKI *et al*., 2011; NELSON *et al*., 2012). LINDEN *et al*. (2015) identified endolysin PlyGRCS, which shows antimicrobial activity against MRSA *S. aureus* that are planktonic and active in biofilm formation. The occurrence of bacterial resistance to endolysins is highly unlike because the phage lytic system has been evolving for millions of years. The result of phage evolution is highly evolved enzyme - endolysin that focuses on specific molecules (ligands) in the PG layer of the bacterial cell wall, while these specific molecules are important for the viability of particular bacteria (YOUNG, 1992; FISCHETTI, 2008). For instance, choline like ligand is important for binding activity of endolysins of pneumococcal phages and for endolysins of phages infecting streptococci it is typical to bind to a ligand - polyrhamnose (YAMASHITA *et al.*, 1999; FISCHETTI, 2003). To sum up, the use of the feature of CBDs to distinguish specific ligands could without a doubt bring new detection methods (SCHMELCHER and LOESSNER, 2014) and opportunities to create novel ‘designer recombinant endolysins’.

7. Conclusion

According to reviewed information, phage-encoded endolysins represent a promising tool to control bacterial cells and especially those strains that cause infections, as well as reduce food quality by contamination, which has been showing a considerable health, social and economic importance. Endolysins from phages infecting Gram-positive bacteria show a well-defined modular organization, mostly comprising functional separate domains: N-terminal catalytic (EADs) and C-terminal

cell wall binding ones. Whereas CBDs target their endolysins to bind to the bacterial cell wall ligands, EADs contribute to peptidoglycan (hydro)lysis and so to cell wall degradation and bacterial death. To emphasize, recombinant endolysin-based strategies do not have to be convenient just in elimination of bacteria by the external application of EADs to the cells. As we have shown, a huge diversity of endolysin CBDs (e.g.) PG-1, SH3-3, SH3-5, SH3b, CHW, LYSM, SPOR) that determine endolysin specificity in terms of CBD ligand recognition might be effective agents themselves. So to understand the whole potential of endolysins, it is also important to study CBD binding specificity and ligands. It is prudent to stress out that CBDs can be favourable in various methods of bacterial detection. To sum up, using molecular engineering to design recombinant protein chimeras can improve and enhance both binding and lytic activities of bacteriophage endolysins for their potential applications.

References

ANDERSEN, J.B., STERNBERG, C., LARS, K.P., BJORN, S.P., GIVSKOV, M., MOLIN, S.: New unstable variants of green fluorescent protein for studies of transient gene expression in bacteria. Appl. Environ. Microbiol., 64, 1998, 2240-2246.

BABA, T., SCHNEEWIND, O.: Target cell specificity of a bacteriocin molecule: a C-terminal signal directs lysostaphin to the cell wall of *Staphylococcus aureus*. EMBO J., 15, 1996, 4789-4797.

BECKER, S.C., FOSTER-FREY, J., STODOLA, A.J., ANACKER, D., DONOVAN, D.M.: Differentially conserved staphylococcal SH3b_5 cell wall binding domains confer increased staphylolytic and streptolytic activity to a streptococcal prophage endolysin domain. Gene, 443, 2009, 32-41.

BERNHARDT, T.G., STRUCK, D.K., YOUNG, R.: The lysis protein E of phiX174 is a specific inhibitor of the MraY-catalyzed step in peptidoglycan synthesis. J. Biol. Chem., 276, 2001, 6093-6097.

BOLAM, D.N., CIRUELA, A., MC-QUEEN MANSON, S., SIMPSON, P., WILLIAMSON, M.P., RIXON, J.E. *Pseudomonas* cellulose-binding domains mediate their effects by in- creasing enzyme substrate proximity. Biochem. J., 331, 1998, 775-781.

BORYSOWSKI J., LOBOCKA M., MIEDZYBRODZKI R., WEBER-DABROWSKA B., GORSKI A.: Potential of bacteriophages and their lysins in the treatment of MRSA: current status and future perspectives. BioDrugs, 25, 2011, 347-355.

BRIERS, Y., CORNELISSEN, A., AERTSEN, A., HERTVELDT, K., LAVIGNE, R., MICHIELS, C.W., VOLCKAERT, G., LAVIGNE, R.: Analysis of outer membrane permeability of *Pseudomonas aeruginosa* and bactericidal activity of endolysins KZ144 and EL188 under high hydrostatic pressure. FEMS Microbiol. Lett., 280, 2008, 113-119.

BRIERS, Y., SCHMELCHER, M., LOESSNER, M.J., HENDRIX, J., ENGELBORGHS, Y., VOLCKAERT, G., LAVIGNE, R.: The high-affinity peptidoglycan binding domain of *Pseudomonas* phage endolysin KZ144. Biochem. Biophys. Res. Commun., 383, 2009, 187-191.

BRIERS, Y., VOLCKAERT, G., CORNELISSEN, A., LAGAERT, S., MICHIELS, C.W., HERTVELDT, K., LAVIGNE, R.: Muralytic activity and modular structure of the endolysins of *Pseudomonas aeruginosa* bacteriophages phiKZ and EL. Mol. Microbiol., 65, 2007, 1334-1344.

BUIST, G., STEEN, A., KOK, J., KUIPERS, O.P.: LysM, a widely distributed protein motif for binding to (peptido)glycans. Mol. Microbiol., 68, 2008, 838-847.

BUSTAMANTE, N., CAMPILLO, N.E., GARCÍA, E., GALLEGO, C., PERA, B., DIAKUN, G.P., SÁIZ, J.L., GARCÍA, P., DÍAZ, J.F., MENÉNDEZ, M.: Cpl-7, a lysozyme encoded by a pneumococcal bacteriophage with a novel cell wall-binding motif. J. Biol. Chem., 285, 2010, 33184-33196.

CHALFIE, M., TU, Y., EUSKIRCHEN, W., PRASHER, D.C.: Green fluorescent protein as a marker for gene expression. Science, 263, 1994, 802-805.

CHAPOT-CHARTIER, M.P., KULAKAUSKAS, S.: Cell wall structure and function in lactic acid bacteria. Microb. Cell Fact., 13, 2014, 1-23.

CHENG, Q., FISCHETTI, V.A.: Mutagenesis of a bacteriophage lytic enzyme PlyGBS significantly increases its antibacterial activity against group B streptococci. Appl. Microbiol. Biotechnol., 74, 2007, 1284-1291.

CROUX, C., RONDA, C., LOPEZ, R., GARCÍA, J. L.: Interchange of functional domains switches enzyme specificity: Construction of a chimeric pneumococcal-clostridial cell wall lytic enzyme. Mol. Microbiol., 9, 1993, 1019-1025.

DELBRUCK, M.: The growth of bacteriophage and lysis of the host. J. Gen. Physiol., 23, 1940, 643-660.

DÍAZ, E., LOPEZ, R., GARCIA, J.L.: Chimeric phage-bacterial enzymes: A clue to the modular evolution of genes. Proc. Natl. Acad. Sci. USA, 87, 1990, 8125-8129.

DONOVAN, D.M., DONG, S., GARRETT, W., ROUSSEAU, G.M., MOINEAU, S., PRITCHARD, D.G.: Peptidoglycan hydrolase fusions maintain their parental specificities. Appl. Environ. Microbiol., 72, 2006, 2988-2996.

EUGSTER, M.R., HAUG, M.C., HUWILER, S.G., LOESSNER, M.J.: The cell wall binding domain of *Listeria* bacteriophage endolysin PlyP35 recognizes terminal GlcNAc residues in cell wall teichoic acid. Mol. Microbiol., 81, 2011, 1419-1432.

FISCHETTI V.A.: Bacteriophage lysins as effective antibacterials. Curr. Opin. Microbiol., 11, 2008, 393-400.

FISCHETTI, V.A..: Novel method to control pathogenic bacteria on human mucous membranes. Ann. N Y Acad. Sci.. 987, 2003, 207-214.

GAENG, S., SCHERER, S., NEVE, H., LOESSNER, M. J.: Gene cloning and expression and secretion of *Listeria monocytogenes* bacteriophage-lytic enzymes in *Lactococcus lactis*. Appl. Environ. Microbiol., 66, 2000, 2951-2958.

GARCÍA, P., GARCIA, J. L., GARCIA, E., SANCHEZ-PUELLES, J. M., LOPEZ, R.: Modular organization of the lytic enzymes of *Streptococcus pneumoniae* and its bacteriophages. Gene, 86, 1990, 81-88.

GARVEY, K.J., SAEDI, M.S., ITO, J.: Nucleotide sequence of *Bacillus* phage phi 29 genes 14 and 15: homology of gene 15 with other phage lysozymes. Nucleic Acids Res., 14, 1986, 10001-10008.

GRUNDLING, A., SCHNEEWIND, O.: Cross-linked peptidoglycan mediates lysostaphin binding to the cell wall envelope of *Staphylococcus aureus*. J. Bacteriol., 188, 2006, 2463-2472.

HEIDRICH, C., TEMPLIN, M.F., URSINUS, A.: Involvement of N-acetylmuramyl-L-alanine amidases in cell separation and antibiotic-induced autolysis of *Escherichia coli*. Mol. Microbiol., 41, 2001, 167-178.

HEIDRICH, C., URSINUS, A., BERGER, J., SCHWARZ, H., HÖLTJE, J.V.: Effects of multiple deletions of murein hydrolases on viability, septum cleavage, and sensitivity to large toxic molecules in *Escherichia coli*. J. Bacteriol., 184, 2002, 6093-6099.

HERMOSO, J.A., GARCIA, J.L., GARCIA, P.: Taking aim on bacterial pathogens: from phage therapy to enzybiotics. Curr. Opin. Microbiol., 10, 2007, 461-472.

HERMOSO, J.A., MONTERROSO, B., ALBERT, A.: Structural basis for selective recognition of pneumococcal cell wall by modular endolysin from phage Cp-1. Structure, 11, 2003, 1239-1249.

HÖLTJE J.V., TUOMANEN, E.I.: The murein hydrolases of *Escherichia coli*: properties, functions and impact on the course of infections *in vivo*. J. Gen. Microbiol., 137, 1991, 441-454.

HU, S., KONG, J., KONG, W., GUO, T., JI, M.: Characterization of a novel LysM domain from *Lactobacillus fermentum* bacteriophage endolysin and its use as an anchor to display heterologous proteins on the surfaces of lactic acid bacteria. Appl. Environ. Microbiol., 76, 2010, 2410-2418.

HUMANN, J., LENZ, L.L.: Bacterial peptidoglycan degrading enzymes and their impact on host muropeptide detection. J. Innate. Immun., 1, 2009, 88-97.

KARAKAWA,W.W., KRAUSE, R.M.: Studies on the immunochemistry of streptococcal mucopeptide. J. Expt. Med., 124, 1966, 155-171.

KHOSLA, C., HARBURY, P.B.: Modular enzymes. Nature, 409, 2001, 247-252.

KIKKAWA, H., FUJINAMI, Y., SUZUKI, S., YASUDA, J.: Identification of the amino acid residues critical for specific binding of the bacteriolytic enzyme of gamma-phage, PlyG, to *Bacillus anthracis*. Biochem. Biophys. Res. Commun., 3, 2007, 1531-1535.

KORNDÖRFER, I. P.: The crystal structure of the bacteriophage PSA endolysin reveals a unique fold responsible for specific recognition of *Listeria* cell walls. J. Mol. Biol., 4, 2006, 678-689.

KRETZER, J.W., LEHMANN, R., SCHMELCHER, M., BANZ, M., KIM, K., KORN, C., LOESSNER, M.J.: Use of high-affinity cell wall-binding domains of bacteriophage endolysins for immobilization and separation of bacterial cells. Appl. Environ. Microbiol., 73, 2007, 1992-2000.

LINDEN, S.B., ZHANG, H., HESELPOTH, R.D., SHEN, Y., SCHMELCHER, M., EICHENSEHER, F., NELSON, D.C.: Biochemical and biophysical characterization of PlyGRCS, a bacteriophage endolysin active against methicillin-resistant *Staphylococcus aureu.* Appl Microbiol Biotechnol, 99, 2015, 741-752.

LOESSNER, M. J., M. RUDOLF, S. SCHERER.: Evaluation of luciferase reporter bacteriophage A511:luxAB for detection of *Listeria monocytogenes* in contaminated foods. Appl. Environ. Microbiol., 63, 1997, 2961-2965.

LOESSNER, M.J., MAIER, S.K., DAUBEK-PUZA, H., G WENDLINGER, G., SCHERER, S.: Three Bacillus cereus bacteriophage endolysins are unrelated but reveal high homology to cell wall hydrolases from different bacilli. J. Bacteriol., 179, 1997, 2845-2851.

LOESSNER, M.J., GAENG, S., WENDLINGER, G., MAIER, S.K., SCHERER, S.: The two-component lysis systemof *Staphylococcus* aureus phage Twort: a large TTG-start holin and an associated amidase endolysin. FEMS Microbiol. Lett., 162, 1998, 265-274.

LOESSNER, M.J., KRAMER, K., EBEL, F., SCHERER, S.: C-terminal domains of *Listeria monocytogenes* bacteriophage murein hydrolases determine specific recognition and high-affinity binding to bacterial cell wall carbohydrates. Mol. Microbiol., 44, 2002, 335-349.

LOESSNER, M.J., REES, C.E.D.: *Listeria* Phages: Basics and Applications, *In: M.* WALDOR, D. FRIEDMAN, S. ADHYA (Eds.) Phages. ASM Press, Washington, DC, 2005, 362-380.

LOPEZ, R., GARCIA, E.: Recent trends on the molecular biology of pneumococcal capsules, lytic enzymes, and bacteriophage. FEMS Microbiol. Rev., 28, 2004, 553-580.

LOW, L.Y., YANG, C., PEREGO, M., OSTERMAN, A., LIDDINGTON, R.C.: Structure and lytic activity of a *Bacillus anthracis* prophage endolysin. J. Biol. Chem., 42, 2005, 35433-35439.

LU, J. Z., FUJIWARA, T., KOMATSUZAWA, H., SUGAI, M., SAKON, J.: Cell wall-targeting domain of glycylglycine endopeptidase discriminates peptidoglycan cross-bridge. J. Biol. Chem., 281, 2006. 549-558.

MAYER, B.J., ECK, M.J.: SH3 domains. Curr. Biol., 5, 1995, 364-367.

MAYER, B.J., HAMAGUCHI, M., HANAFUSA, H.: A novel viral oncogene with structural similarity to phospholipase C. Nature, 332, 1988, 272-275.

MAYER, B.J.: SH3 domains: complexity in moderation. J. Cell Sci., 114, 2001, 1253-1263.

MAYER, M.J., GAREFALAKI, V., SPOERL, R., NARBAD, A., MEIJERS, R.: Structure-based modification of a *Clostridium difficile*-targeting endolysin affects activity and host range. J. Bacteriol., 19, 2011, 5477-5486.

MORITA, M., TANJI, Y., ORITO, Y., MIZOGUCHI, K., SOEJIMA, A., UNNO, H.: Functional analysis of antibacterial activity of Bacillus amyloliquefaciens phage endolysin against Gram-negative bacteria. FEBS Lett., 500, 2001, 56-59.

NAVARRE, W.W., TON-THAT, H., FAULL, K.F., SCHNEEWIND, O.: Multiple enzymatic activities of the murein hydrolase from staphylococcal phage phi11: Identification of a D- alanyl-glycine endopeptidase activity. J. Biol. Chem., 274, 1999, 15847-15856.

NELSON, D.C., SCHMELCHER, M., RODRIGUEZ-RUBIO, L., KLUMPP, J., PRITCHARD, D.G., DONG, S., DONOVAN, D.M.: Endolysins as antimicrobials. Adv. Virus Res., 83, 2012, 297-356.

OHNUMA, T., ONAGA, S., MURATA, K., TAIRA, T., KATOH, E.: Domains from *Pterisryukyuensis* chitinase - A: a stability study and characterization of the chitin-binding site. J. Biol. Chem., 8, 2008, 5178-5187.

OLIVEIRA, H., AZEREDO, J., LAVIGNE, B., KLUSKENS, L.D.: Bacteriophage endolysins as a response to emerging foodborne pathogens. Trends Food Sci. Tech., 2, 2012, 103-115.

OLIVEIRA, H., MELO, L.D.R., SANTOS, S.B., NÓBREGA, F.L., FERREIRA, E.C., CERCA, N., AZEREDO, J., KLUSKENS, L.D.: Molecular aspects and

comparative genomics of bacteriophage endolysins. J. Virol., 87, 2013, 4558-4570.

PARISIEN, A., ALLAIN, B., ZHANG, J., MANDEVILLE, R., LAN, C.Q.: Novel alternatives to antibiotics: Bacteriophages, bacterial cell wall hydrolases, and antimicrobial peptides. J. Appl. Microbiol., 104, 2008, 1-13.

PONTING, C.P., ARAVIND, L., SCHULTZ, J., BORK, P., KOONIN, E.V.: Eukaryotic signalling domain homologues in archaea and bacteria. Ancient ancestry and horizontal gene transfer. J. Mol. Biol., 289, 1999, 729-745.

PORTER, C.J., SCHUCH, R., PELZEK, A.J., BUCKLE, A.M., MCGOWAN, S., WILCE, M.C., ROSSJOHN, J., RUSSELL, R., NELSON, D., FISCHETTI, V.A., WHISSTOCK, J.C.: The 1.6 A crystal structure of the catalytic domain of PlyB, a bacteriophage lysin active against *Bacillus anthracis*. J. Mol. Biol., 366, 2007, 540-550.

PRITCHARD, D.G., DONG, S., BAKER, J.R., ENGLER, J.A.: The bifunctional peptidoglycan lysin of *Streptococcus agalactiae* bacteriophage B30. Microbiology, 150, 2004, 2079-2087.

REGULSKI, K., COURTIN, P., KULAKAUSKAS, S., CHAPOT-CHARTIER, M.: A novel type of peptidoglycan-binding domain highly specific for amidated D-Asp cross-bridge, identified in *Lactobacillus casei* bacteriophage endolysins. J. Biol. Chem., 288, 2013, 20416-20426.

REINSCHEID, D. J., STOSSER, C., EHLERT, K., JACK, R. W., MOLLER, K., EIKMANNS, B. J., CHHATWAL, G. S.: Influence of proteins Bsp and FemH on cell shape and peptidoglycan composition in group B streptococcus. Microbiology, 148, 2002 3245-3254.

ROGERS, H.J., PERKINS, H.R., WARD, J.B.: Microbial Cell Walls and Membranes. Chapman and Hall, London, 1980.

SANZ, J.M., DÍAZ, E., GARCÍA, J.L.: Studies on the structure and function of the N-terminal domain of the pneumococcal murein hydrolases. Mol. Microbiol., 6, 1992, 921-931.

SASS, P., BIERBAUM, G.: Lytic activity of recombinant bacteriophage phi11 and phi12 endolysins on whole cells and biofilms of *Staphylococcus aureus*. Appl. Environ. Microbiol., 73, 2007, 347-352.

SCHLEIFER, K.H., KANDLER, O.: Peptidoglycan types of bacterial cell walls and their taxonomic implications. Clin. Microbiol. Rev., 36, 1972, 407-477.

SCHMELCHER M. and LOESSNER M.J.: Application of bacteriophages for detection of foodborne pathogens. Bacteriophage, 4, 2014, 28137-14.

SCHMELCHER, M., DONOVAN, D.M., LOESSNER, M.J.: Bacteriophage endolysins as novel antimicrobials. Future Microbiol., 7, 2012, 1147-1171.

SCHMELCHER, M., SHABAROVA, T., EUGSTER, M.R.: Rapid multiplex detection and differentiation of *Listeria* cells by use of fuorescent phage endolysin cell wall bindingdomains. Appl. Environ. Microbiol., 76, 2010, 5745-5756.

SCHUCH, R., NELSON, D., FISCHETTI, V.A.: A bacteriolytic agent that detects and kills *Bacillus anthracis*. Nat. Commun., 418, 2002, 884-889.

SHOCKMAN, G.D., HÖLTJE J.V.: Microbial peptidoglycan (murein) hydrolases. Ghuysen and Hakenbeck, Amsterdam, 1994, 131-166.

SMITH, T.J., BLACKMAN, S.A., FOSTER, S.J.: Autolysins of Bacillus subtilis: multiple enzymes with multiple functions. Microbiology, 146, 2000, 249-262.

STAHL, M. L., FERENZ, C. R., KELLEHER, K. L., KRIZ, R. W., KNOPF, J. L.: Sequence similarity of phospholipase C with the non-catalytic region of src. Nature, 332, 1988, 269-272.

SULLIVAN, L., PAREDES, C.J., PAPOUTSAKIS, E.T., BENNETT, G.N.: Analysis of the clostridial hydrophobic with a conserved tryptophan family (ChW) of protein in *Clostridium acetobutilicum* with emphasis on ChW14 and ChW16/17. J. Microbiol. Biotechnol. Food Sci., 42, 2007, 29-43.

THIEL, K. 2001. Old dogma, new tricks-21st century phage therapy. Nat. Biotechnol., 22, 2001, 31-36.

TIŠÁKOVÁ, L., GODÁNY, A.: Bacteriophage endolysins and their use in biotechnological processes. J. Microb. Biotech. Food Sci., 3, 2014, 164-170.

TIŠÁKOVÁ, L., VIDOVÁ, B., FARKAŠOVSKÁ, J., GODÁNY, A.: Bacteriophage endolysin Lyt μ1/6: characterization of the C-terminal binding domain. FEMS Microbiol. Lett., 2013, 1-10.

TOLBA, M., AHMED, M.U., TLILI, C., EICHENSEHER, F., LOESSNER, M.J., ZOUROB, M.: A bacteriophage endolysin-based electrochemical impedance biosensor for the rapid detection of *Listeria* cells. Analyst, 137, 2012, 5749-5756.

VISWESWARAN, G.R., DIJKSTRA, B.W., KOK, J.: Murein and pseudomurein cell wall binding domains of bacteria and archaea – a comparative view. Appl. Microbiol. Biotechnol., 92, 2011, 921-928.

VOLLMER W., BLANOT D., DE PEDRO M.A.: Peptidoglycan structure and architecture. Microbiol. Rev., 32, 2008, 149-167.

VOLLMER, W., JORIS, B., CHARLIER, P., FOSTER, S.: Bacterial peptidoglycan (murein) hydrolases. Microbiol. Rev., 32, 2008, 259-286.

WALMAGH, M., BRIERS, Y., SANTOS, S.B, AZEREDO J., LAVIGNE, R.: Characterization of modular bacteriophage endolysins from *Myoviridae* phages OBP, 201Q2-1 and PVP-SE1. PLOS One, 7, 2012, 1-10.

WANG, I.N., DEATON, J., YOUNG, R.: Holins: the rotein clocks of bacterial infection. Annu. Rev. Microbiol., 54, 2000, 799-825.

WANG, S., KONG, J., ZHANG, X.: Identification and characterization of the two-component cell lysis cassette encoded by temperate bacteriophage phiPYB5 of *Lactobacillus fermentum.* J. Appl. Microbiol., 105, 2008, 1939-1944.

WHISSTOCK, J.C., LESK, A.M.: SH3 domains in prokaryotes. Trends Biochem. Sci., 24, 1999, 132-133.

YOONG, P., SCHUCH, R., NELSON, D., FISCHETTI, V.A.: Identification of a broadly active phage lytic enzyme with lethal activity against antibiotic-resistant *Enterococcus faecalis* and *Enterococcus faecium.* J. Bacteriol., 186, 2004, 4808-4812.

YOUNG, R.: Bacteriophage lysis: mechanism and regulation. Microbiol. Rev., 56, 1992, 430-481.

YOUNG, R.Y: Phage lysis: three steps, three choices, one outcome. J Microbiol., 52, 2014, 243-258.

ZIMMER, M., VUKOV, N., SCHERER, S., LOESSNER, M. J.: The murein hydrolase of the bacteriophage phi3626 dual lysis system is active against all tested *Clostridium perfringens* strains. Appl. Environ. Microbiol., 68, 2002, 15311-15317.