Title: Bacteriophage virion-associated peptidoglycan hydrolases: potential new enzybiotics

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Keywords: phage lytic proteins, phage therapy, biocontrol

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Abstract

Virion-associated peptidoglycan hydrolases (VAPGH) are phage-encoded lytic enzymes that locally degrade the peptidoglycan (PG) of the bacterial cell wall during infection. In contrast to endolysins, PGHs that mediate lysis of the host bacteria at the end of the lytic cycle to release of phage progeny, the action of VAPGHs generates a small hole through which the phage tail tube crosses the cell envelope to eject the phage genetic material at the beginning to the infection cycle. The antimicrobial activity of VAPGHs was first discovered through the observation of the phenomenon of ‘lysis from without’, in which the disruption of the bacterial cell wall occurs prior to phage production and is caused by a high number of phages adsorbed onto the cell surface. Based on a unique combination of properties of VAPGHs such as high specificity, remarkable thermostability, and a modular organization, these proteins are potential candidates as new antibacterial agents, e. g. against antibiotic resistant bacteria in human therapy and veterinary as well as biopreservatives in food safety, and as biocontrol agents of harmful bacteria in agriculture. This review provides an overview of the different VAPGHs discovered to date and their potential as novel antimicrobials.

Introduction

Bacteriophages (phages) are viruses that exclusively infect bacteria. They are widespread throughout nature and during their lytic life cycle kill their hosts. Interest in using bacteriophages as antimicrobial agents has increased in recent years due to the high incidence of antibiotic-resistant bacteria. Several reports have indicated the effectiveness of bacteriophages against pathogenic bacteria causing infections in animals or humans via phage therapy (Lu and Koeris, 2011). More recently, the potential applications of bacteriophages have been extended to other fields such as food safety which seeks to prevent contamination of food (biocontrol) (García et al., 2008; Mahony et al., 2011). Bacteriophages can also be used as tools for detecting pathogens in foods or the environment, for disinfection of medical tools and devices, to prevent formation of bacterial biofilms on industrial surfaces or as drug delivery vehicles (for a recent review see Courchesne et al., 2009).

In addition to bacteriophages, phage lytic proteins also provide successful elimination of pathogenic bacteria (Fischetti, 2010). There are two general classes of phage lytic proteins, which mediate the enzymatic cleavage of PG: endolysins and VAPGHs. These proteins exert their action at different steps of the infection cycle. VAPGHs act in the first step of the phage lytic cycle (Fig. 1) degrading the PG to allow the phage infection. Their action occurs prior to the transport of phage genetic material across the bacterial cell envelope (Letellier et al. 1999). They seem to be quite common in bacteriophages infecting both Gram-negative and Gram-positive bacteria as shown by zymograms of fully assembled virions and by homology analysis of sequenced phage/prophage genomes (Moak and Molineux, 2004). Endolysins are produced during the late phase of gene expression in the lytic cycle and are responsible for the progeny

phage release (Young et al., 2000). Their bacteriolytic activity when added exogenously to Gram positive bacteria has triggered intense study in the recent years (for review see Fischetti, 2010). Indeed, their potential to control pathogens on mucosal surfaces and systemic infections has been reviewed recently (Fenton et al., 2010), as well as in food control and as tools for pathogen detection (reviewed by Callewaert et al., 2011).

The presence of virion-associated muralytic activities has been detected in numerous bacteriophages (Table 1), but only a few labs have further sub-cloned, purified, and demonstrated the antimicrobial activity of the responsible proteins *in vitro*. Given the previous success of endolysins as antimicrobial agents and the steadily increasing prevalence of antibiotic resistant bacteria, it is worthwhile to study the antimicrobial potential of these proteins. In fact, both bacteriophages and bacteriophage-encoded proteins with antimicrobial activity represent a promising alternative to antibiotics, as potential new antimicrobials against infectious diseases (Matsuzaki et al., 2005; Fischetti et al., 2010).

Virion-associated muralytic activities are present in phages infecting Gram-negative and Gram-positive bacteria.

Early evidence of muralytic enzymes associated with bacteriophages infecting Gram-negative bacteria were found in particles of the membrane encapsulated bacteriophage $\Phi 6$ which infects *Pseudomonas syringae*. It was demonstrated that the virion associated protein P5, digests the PG to allow penetration of the viral core into the host, and also serves as an endolysin to allow release of the phage from the host (Mindich and Lehman, 1979). Later it was postulated that gp5, a structural baseplate protein of phage T4, was required for 'lysis from without'. Mutations in this protein yielded phages with a reduced ability to cause 'lysis from without' following heat

treatment of the phage, while heating had no effect on the ability of the wild-type phages to lyse from without (Kao and McClain, 1980). Recently, it has been demonstrated that phage T4 penetrates the outer membrane of *E. coli* using a multifunctional device composed by the gp5 protein trimer that first ‘drills’ a hole in the outer membrane, and with its lysozyme domain further degrades the PG layer enlarging the initial hole, allowing the DNA to be injected (Nishima et al., 2011).

Other phages infecting *E. coli* such as phage T7, also displayed muralytic activity associated with their virions. Mutations in the catalytic domain of the internal phage capsid protein lytic transglycosylase, gp16, were associated with a failure in the translocation of DNA into the cell cytoplasm when the host growth conditions resulted in a highly cross-linked PG (Moak and Molineux, 2000). Moreover, zymograms of these virions containing mutations in gp16 did not reveal any muralytic activity (Moak and Molineux, 2004). Similarly, zymogram analysis and N-terminal sequencing revealed the presence of a PG hydrolase (PGH) (gp4) in phage P22 infecting *Salmonella typhimurium*, which is also an internal head protein. Viral particles lacking gp4 were unable to show PGH activity (Moak and Molineux, 2004). Furthermore, the straight fiber Pb2 from phage T5 also carries muralytic activity. It is involved in DNA transfer into the host and is able to cause both galactosidase release from *E. coli* cells and *in vitro* PG hydrolysis (Boulanger et al., 2008). The broad-host-range phage PRD1 also harbors a transglycosylase domain included in the amino-terminus of protein P7. This was demonstrated by zymogram analysis of wild-type PRD1 and various mutant phage particles. P7 protein is postulated to be involved in DNA entry as both DNA replication and host cell lysis were delayed in its absence (Rydman and Bamford, 2000). Phage PRD1 encodes another structural protein (P15) with glycosidase activity, which is a soluble 1,4-*N*-acetylmuramidase that causes phage-induced host cell lysis. The

incorporation of these two unique lytic activities enzymes into virions may in part be responsible for or support the broad host range of bacteriophage PRD1 (Rydman and Bramford, 2002).

Regarding phages infecting other species such as the *Pseudoalteromonas* PM2 phage, it was demonstrated that the P7 protein, one of the components of the internal viral lipid core particle, has bacteriolytic activity. This protein is involved in the PG penetration process and causes a limited depolarization of the cytoplasmic membrane (Kivela et al., 2004). A similar phenomenon was also observed in *P. syringae* phage Φ 13, which contains a thermosensitive lytic enzyme involved in PG penetration (Daugelavicious et al., 2005). Phages Φ KMV and Φ KZ infecting *Pseudomonas aeruginosa* strains also displayed structural proteins with PG hydrolase domains (Briers et al., 2006, 2008). Of note, the VAPGH gp36 from bacteriophage Φ KMV showed high thermoresistance. It has been postulated that this property is related to the location of this protein as part of the extensible tail from which it is ejected in an unfolded state during phage infection (Briers et al., 2006). Heat stability is not only a recurring property of structural lysins, but of any structural phage protein (Lavigne et al., 2004; Briers et al., 2006; Rodríguez et al., 2011).

From a limited screening of public data sets, PGH activities appear to be a frequent property of phages infecting Gram-positive bacteria (Nelson et al., 2012). Initially, VAPGH activities were described by zymogram analysis in phages sk1, r1t and c2 infecting *Lactococcus lactis*, and in phage phi11 infecting *Staphylococcus aureus* (Moak and Molineux, 2004). However, no further reports were published about the characterization of these proteins. In *Bacillus subtilis* SP- β prophage, zymogram analysis of purified particles showed a degradation band corresponding to the PGH CwIP (Moak and Molineux, 2004). The activity of this protein was later analyzed by

reverse phase (RP)-HPLC and mass spectrometry revealing the presence of two domains, muramidase and endopeptidase. Both domains were able to hydrolyze PG and cell walls of *B. subtilis* (Sudiarta et al. 2010). Φ 29, another *B. subtilis* phage, showed PG hydrolytic activity of the DNA terminal protein gp3 by zymogram analysis (Moak and Molineux, 2004). An in-depth characterization of the tail-associated protein (Tal₂₀₀₉) from the *L. lactis* phage Tuc2009 was performed. Tal₂₀₀₉ belongs to the M37 protein family and it was determined to be located at the tail tip where it is essential to mediate the host infection (Kenny et al., 2004). Additional work was carried out on *S. aureus* phages P68, phiMR11 and phiIPLA88. Unlike other VAPGHs, protein 17 from phage P68 has a C-terminal region required for binding to the cell surface, while the N-terminus comprises the muralytic activity domain. A putative role in the infection process was deduced for this protein due to its ability to prematurely lyse *S. aureus* cells when added exogenously (Takac and Blasi, 2005). The presence of two active catalytic domains, CHAP (cysteine, histidine-dependent amidohydrolases/peptidase) (Bateman and Rawlings, 2003) and LYZ2 (lysozyme subfamily 2) (Rashel et al., 2008), was shown in gp61 from phiMR11. Gp61 has been localized to the tail tip of the phage by immuno-electron microscopy. Due the tail tip seems to be the first part of bacteriophages to contact directly the bacterial surface, this might indicate that this protein is involved in “lysis from without” (Rashel et al., 2008). A similar structure with those two catalytic domains was also identified in HydH5, the VAPGH from phiIPLA88. The high thermostability of HydH5 is remarkable, which retained activity after 5 min at 100°C (Rodríguez et al., 2011).

Putative role of the virion-associated muralytic enzymes in the phage infection cycle.

Although all the findings suggest a role of the VAPGH proteins in the first step of the infection cycle, there is still room for more definitive studies. Most authors speculate about the position of these proteins and the need to disrupt the PG layer to allow the tail tube to penetrate the cell wall. The most extensive work about tail structure and its involvement in the infection process is from the widely studied phage T4 (Leiman et al., 2010). This elegant work supports the hypothesis that the VAPGHs are involved in degrading the PG during infection, and explains how the conformational change of the baseplate and the tail contraction are related to the tail's host cell recognition and membrane penetration function. In this process, the full activity of gp5 lysozyme is not essential for infection because even partial lysozyme activity can digest the small (but sufficient) portion of the cell wall to permit the tail tube to penetrate (Kanamaru et al., 2005).

On the other hand, the contraction of the tail structure is not possible in *Siphoviridae* phages, and no reported data exists to explain the mechanism of DNA ejection into the cell. However, evidence of the role of these proteins was obtained from the siphovirus Tuc2009 phage where infection was reduced by 100-fold in the presence of antibodies against Tal₂₀₀₉ (Kenny et al., 2004). This phenomenon is further supported by the demonstration that the gp16 transglycosylase activity of *Podoviridae* bacteriophage T7 is not essential for phage growth, but was beneficial during infection of *E. coli* under conditions when the murein is more highly cross-linked (e.g. cells grown to high cell density or under low temperature conditions) (Moak and Molineux, 2000). These authors suggest that cells growing exponentially displayed a sufficiently porous PG to allow the phage tail tube proteins to pass through the mesh of glycan strands and peptide cross-links. In contrast, under conditions of high density or low temperature, the PG becomes multilayered and the porosity of the murein reduced,

necessitating a PG degrading activity to achieve a high infection efficiency. It should not be misconstrued that the phage must rely on VAPGHs to have a successful infection. In fact, it is not in the best interest of the phage that the VAPGHs degrade the PG too efficiently, because the cell has to be sufficiently stable and survive long enough to allow phage propagation. Along these same lines, it should also be mentioned that the host bacteria also initiates a cell wall restoration effort immediately after phage infection. Recent studies in *Lactococcus lactis* subsp. *lactis* IL1403 at an early stage of infection with the lytic phage c2 have shown a global molecular response strongly targeted to the cell wall. Induction of membrane stress proteins, D-alanylation of cell wall lipoteichoic acids (LTAs), and maintenance of the proton motive force (PMF) are all triggered after phage infection presumably to restore cell wall stability (Fallico et al., 2011).

On the other hand, there are also examples of phage-induced mechanisms that favourably alter the PG to promote the phage infection success but they are induced late in the infection cycle. For instance, the *Streptococcus pneumoniae* autolysin LytA is activated at the end of the phage lytic cycle by the holin-induced membrane permeabilization. The activation of autolysin LytA, together with the phage endolysin, leads to greater phage progeny release (Frias et al., 2009).

Antimicrobial activity of purified VAPGHs.

”Lysis from without” could theoretically be observed for all bacteria infected by phages containing VAPGHs. However, to observe this phenomenon, a high multiplicity of infection is likely required [a phage/bacteria ratio >100 and a high phage titer ($\geq 10^9$ phages/ml) has been suggested by Stephen Abedon (Abedon, 2011) and L.M. Kasman (Kasman et al., 2002)] although not all phages have been demonstrated to be capable of

causing lysis from without and the numbers of phages required per cell to elicit this effect are not clear and likely highly variable depending on the host.

It should be noted that the rate at which phage adsorb to bacteria is a function of phage density, whereas the total number of phages adsorbed to a bacterium is a function of both the phage/bacteria ratio as well as the inherent capacity of bacterium to adsorb the phage (Abedon, 2011).

Evidence that lytic activity of virions can be due to specific virion-associated proteins was obtained after purification and subsequent exposure of viable bacteria to these proteins. HydH5 from phiIPLA88 showed the highest antimicrobial activity against early exponential *S. aureus* cells at 45°C and in the absence of divalent cations (Rodríguez et al., 2011). Similar challenge assays also showed lytic activity against *S. aureus* by both catalytic domains from phiMR11 gp61 (Rashel et al., 2008). Similarly, phage P68 protein 17 displayed activity against clinical *S. aureus* isolates, including methicillin-resistant strains (Takac and Blasi, 2005).

Regarding the antimicrobial activity of PGHs (endolysins and VAPGHs) against Gram negative bacteria, it is worth noting that the presence of the outer membrane is a barrier that these proteins must overcome to reach the PG substrate. Despite this hurdle, the lytic activity of VAPGHs against these bacteria has been confirmed after destabilization of the outer membrane by incubation of the cells in chloroform-saturated buffer at room temperature. Most of the VAPGH studies in Gram negative infecting phages have studied *Pseudomonas* phages. It was shown that the phage $\phi 6$ P5 protein is active against several Gram negative bacteria (*Pseudomonas phaseolicola*, *P. aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *E. coli*, *Salmonella typhimurium* and *Proteus vulgaris*) under these outer membrane destabilizing conditions, but no activity was detected against Gram positive species. This protein is a

thermosensitive endopeptidase which cleaves the peptide bridge formed between meso-diaminopimelic acid and D-alanine (Caldentey and Bamford, 1992). The VAPGH from phage Φ KZ, Gp181, also has a wide lytic spectrum including seven Gram-negative species (*P. aeruginosa*, *P. fluorescens*, *P. putida*, *E. coli*, *S. typhimurium*, *Burkholderia solanacearum*, and *Yersinia enterocolitica*). In addition, this protein tolerates a high ionic strength and is not very sensitive to long thermal treatments. Finally, the presence of a lysozyme activity in the Φ KMV phage particle, named gp36, was also verified on chloroform-treated *P. aeruginosa* PAO1 and *E. coli* WK6 cells. It is highly thermostable, retaining 26% activity after 2 h at 100°C and 21% after autoclaving (Lavigne et al., 2004). These properties are interesting for applications as enzybiotics and to be included as part of food safety technology (Briers et al., 2008).

Antimicrobial activity of VAPGHs can be improved by domain swapping or by synergy with other antimicrobials. Toward this end, a chimeric protein (P16-17) harbouring the endopeptidase domain of P68 endolysin (Lys16) and the C-terminal cell-wall binding domain of phage P68 protein 17 reduced the required effective dose of gentamicin towards clinical *S. aureus* strains (Manoharadas and Blasi, 2009). Similarly, a substantial increase in the lytic activity of some PGH domains was observed by fusion to staphylococcal cell-wall binding domains. The CHAP domain from a tail-associated muralytic enzyme from phage K was fused to the lysostaphin SH3 cell-wall binding domain generating protein P128 with a potent anti-staphylococcal activity. It was shown that concentrations ≥ 2.5 μ g/ml killed 99.9% of 10^8 CFU/ml in an *in vitro* challenge. Moreover, staphylococcal colonization of rat nares with $\sim 10^9$ CFU was reduced by two orders of magnitude with twice daily treatments of P128 (100 μ g) (Paul et al., 2011). Likewise, a P128 hydrogel formulation was bactericidal against staphylococci [including *S. aureus*] recovered from the nares of 31 healthy patients (Vipra et al.,

2012). Similarly, the CHAP domain from HydH5 phiIPLA88 increased its lytic activity by fusion to lysostaphin SH3 domain and even displayed antimicrobial synergy with the endolysin LysH5 *in vitro* (Rodríguez-Rubio et al., 2012a). The domain swapping approach has also proven to be useful for the improvement of endolysin activity (Manoharadas and Blasi, 2009).

Evolutionary insights

As mentioned before, the dominant feature of phage lytic proteins, endolysins and VAPGHs, is their extraordinary similarity in amino acid sequence and protein structure. Previously, it was noted that pneumococcal phage endolysins showed a modular structure based in catalytic domains like bacterial autolysins (López and García, 2004). More recently, computer-based analysis of VAPGHs amino acids sequences from different phages infecting *S. aureus* revealed a high similarity between these proteins (Rodríguez et al., 2011). Moreover, endolysins encoded by staphylococcal phages, and particularly the CHAP domain, presented a conserved sequence also found in bacterial PG hydrolytic proteins (Zou and Hou, 2010). This would not be surprising because they all share the same PG substrate. In addition, the similarity between VAPGHs, phage endolysins, and bacterial autolysins supports the so-called ‘moron accretion hypothesis’ for the origin of phage suggesting that bacteriophages have evolved by recombinational reassortment of genes and by the acquisition of novel genetic elements (Hendrix et al., 2000).

In contrast to this similarity, there is a notorious difference in protein structure. VAPGHs described to date do not have an identifiable cell wall binding domain (CBD) as do endolysins. VAPGHs, in contrast to endolysins, might not require a CBD because they are “delivered” to the PG substrate in large part by the virion particle structure.

For some bacteriophages PG-hydrolysing domains have been observed in the tape measure proteins (TMPs) rather than VAPGHs (Fraser et al., 2007; Rodríguez-Rubio et al., 2012b), which suggests they might be involved in infection and DNA ejection into stationary phase cells (Piuri and Hatfull, 2006). In fact, a growing number of conserved domains are being recognized in other phage structural proteins that probably play roles in attachment to host cells through sugar binding or degradation of the polysaccharides on the cell surface (Fraser et al., 2007).

The abundance of PG-degrading activities associated with phage particles, either as individual proteins or as catalytic domains included in other proteins, raises an important question about whether or not they are essential to the infection process. To date, it has not been possible to demonstrate that these proteins are essential for phage infection except under certain culture conditions that result in high PG cross-linking (Moak and Molineux, 2004). Although these conditions (low temperature or high bacterial density) might not generate PG that is representative of host cells grown under optimized laboratory conditions, these conditions might generate PG that better reflects growth of the host in some naturally occurring environments, suggesting that phages might gain a selective advantage by adding these PG hydrolytic activities to their virion surfaces.

Prospects for the use of purified VAPGHs.

The knowledge of the molecular biology of bacteriophages has allowed us to develop new weapons to prevent and combat unwanted bacterial growth (Fischetti, 2010; Clark and March, 2006). Moreover, since bacteriophages are the most abundant biological entities on earth, they constitute a natural source of information to design new phages and proteins (Lu and Collins, 2007; Lukacik et al., 2012). Bacteriophage

lytic proteins and specifically VAPGHs have several characteristics which make them suitable for their application as antimicrobials:

a) **Mode of action:** Due to the high degree of catalytic domain similarity at the amino acid level, both endolysins and VAPGHs are believed to share a similar mode of action hydrolyzing specific bonds in PG structure. It is reported that during co-evolution of phage and host, these phage lytic proteins have found unique and highly conserved bonds, modification of which is rare, thus, resistance to these proteins is a very rare event. To date, bacterial resistance development to phage lytic proteins has only been tested with endolysins but no resistance has been reported (Fischetti, 2005). Therefore, a similar result is expected for VAPGHs.

b) **High specificity:** Most bacteriophage lytic proteins demonstrate near-species or – genus specificity, to the host species/genus of the phage from which the lytic protein is derived (Zimmer et al., 2002; Rodriguez-Rubio et al., 2012). However, there are exceptions, for example, an enterococcal phage lysin PlyV12 can not only kill enterococci but also several other Gram-positive pathogens, such as *Streptococcus pyogenes*, group B streptococci and *Staphylococcus aureus* (Yoong et al., 2004). Similarly, a lysin from *Lactobacillus casei* bacteriophage A2, was described with a wide lytic spectrum (Ribelles et al., 2012). Regarding VAPGHs, those derived from Gram negative infecting phages seem to have wider lytic spectrum (see above). In contrast, VAPGHs from Gram positive infecting phages have thus far demonstrated specificity limited to species/genus of the host species. Although this near-species specificity is not optimal when considering mixed-pathogen infections, it is a property that provides a clear advantage over broad range antibiotics in the context of resistance development. Broad range antimicrobials lead to selection for resistant strains not just in the target pathogen, but also in co-resident commensal bacteria that are exposed to the

drug. The acquisition of antibiotic resistance is often accomplished by transfer of DNA sequences (genomic or plasmid sources) from a resistant strain to a susceptible strain. This transfer is not necessarily species or genus limited, and it can lead to commensal bacteria that are both antibiotic resistant and that can serve as carriers of these DNA elements for propagation to neighboring bacteria. Those neighboring strains (potential pathogens) with newly acquired resistance elements can emerge as antibiotic resistant strains during future treatment episodes. Thus, in order to reduce the spread of antibiotic resistance, it is recommended to avoid subjecting commensal bacterial communities to broad range antibiotics. Toward this end, FDA, USDA, and CDC promote the development of antimicrobials that reduce the risk of resistance development (CDC Action Plan: <http://www.cdc.gov/drugresistance/actionplan/html/product.htm>).

c) **Effective on drug-resistant strains:** VAPGHs are effective even against antibiotic resistant bacteria (Paul et al., 2011; Rodríguez-Rubio et al., 2012a), a major advantage taking into account the increasing frequency of multi-drug resistant strains.

d) **Thermostability:** A remarkable number of VAPGHs are highly thermostable, retaining activity after high temperature treatments similar to those used in food technology. This property might be exploited in the improvement of food safety by implementation of these proteins in combination with other preservation approaches (e.g. pasteurization), to ensure the microbial safety as well as the organoleptic and nutritional quality of food products (hurdle technology).

e) **Modular organization:** VAPGHs described to date have a modular organization with one or two catalytic domains, similar to endolysins (Hermoso et al., 2007), which allows engineered domain swapping in order to improve their lytic activity (Rodríguez-Rubio et al., 2012a), but more importantly, multiple lytic domains are expected to reduce the likelihood of resistant strain development (Fischetti, 2005)

The potential applications of VAPGHs include human therapy as well as food safety, veterinary and agriculture. In the food industry, VAPGHs might be used as biopreservatives to avoid the spoilage and food safety concerns related to pathogenic bacterial growth in food (García et al., 2010). VAPGHs application as surface disinfectants could help to sterilize food equipment surfaces where pathogens are able to form biofilms which are highly resistant to cleaning and disinfection routine (Sharma and Anand, 2002). It is well known that food handlers are an important focus of bacterial contamination in the food chain (Wattinger et al., 2012). In this regard, decontamination of asymptomatic food handlers by VAPGHs could be considered. Similarly, there is a need for non-antibiotic antimicrobials in animal health due to the phasing-out of antibiotics in farm animals for nonmedical purposes (http://ec.europa.eu/health/antimicrobial_resistance/policy/index_en.html). There was a recent US court order supporting a similar restriction on the use of some antibiotics such as penicillin and tetracycline to promote growth in animals (<http://www.nytimes.com/2012/03/24/health/fda-is-ordered-to-restrict-use-of-antibiotics-in-livestock.html>) that is still under debate at the US Food and Drug Administration. VAPGHs could represent an alternative in the treatment and prophylaxis of infectious diseases, similarly to that proposed for phage endolysins (Fenton et al., 2010). These lytic enzymes might also play a role in agriculture to exclude the use of synthetic chemicals in the control and elimination of phytopathogenic bacteria (During et al., 1993).

Conclusion

Phage encoded PGH proteins, endolysins and VAPGHs, have a remarkable potential as novel bacteriolytic antimicrobials to combat antibiotic-resistant bacteria. This is

especially true when considering that an extracellular site of action avoids many of the intracellular resistance mechanisms that bacteria have evolved to combat conventional broad-range antibiotics (e.g. efflux pumps). Previous studies with phage endolysins, which share protein structure and mode of action with VAPGHs, support their potential use to combat infectious diseases in animal models and in the biocontrol of numerous pathogenic species. In this context, VAPGHs provide a new source of lytic enzymes. Noteworthy is the thermoresistance observed for some of this class of enzymes, which might find application in novel biotechnological settings (e.g. pasteurization). In light of the promising results reported from the limited number of VAPGHs that have been examined for their antimicrobial properties, this is an area of phage biology that deserves a much more concerted effort.

Declaration of Interest

This research study was supported by grants AGL2009-13144-C02-01 (Ministry of Science and Innovation, Spain), IB08-052 (Science, Technology and Innovation Programme, Principado de Asturias, Spain), PIE200970I090 (CSIC, Spain), bacteriophage network FAGOMA, National Institutes of Health, grant 1RO1AI075077-01A1 (DMD); National Research Initiative grant 2007-35204-18395 (DMD) and US State Dept funds (DMD). L. R-R. is a fellow of the Science, Technology and Innovation Programme (Principado de Asturias, Spain). Mentioning of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture. The USDA is an equal opportunity provider and employer.

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Tables

Table 1. Main characteristics of VAPGHs described to date.

Phage	Host	Protein	Location	Characteristics as antimicrobial	Reference
φ6	<i>Pseudomonas syringae</i>	P5	Viral membrane	Inactivated at temperatures above 20 °C	Caldentey & Bamford, 1992
φ13	<i>P. syringae</i>	-	Viral membrane	Thermosensitive	Daugelavicius <i>et al.</i> , 2005
φKMV	<i>Pseudomonas aeruginosa</i>	gp36	Extensible tail	Thermoresistant	Briers <i>et al.</i> , 2006
φKZ	<i>P. aeruginosa</i>	gp181	Cell-puncturing device	Tolerates high ionic strength	Briers <i>et al.</i> , 2008
T4	<i>Escherichia coli</i>	gp5	Cell-puncturing device	Lysozyme domain	Nishima <i>et al.</i> 2011
T5	<i>E. coli</i>	Pb2	Straight fiber	-	Boulanger <i>et al.</i> 2008
PRD1	<i>E. coli</i>	P7	Viral membrane	-	Rydman & Bamford, 2000
PRD1	<i>E. coli</i>	P15	Viral membrane	-	Rydman & Bamford, 2002
T7	<i>E. coli</i>	gp16	Head	-	Moak & Molineux, 2004
PM2	<i>Pseudoalteromonas</i>	P7	Lipid core	-	Kivela <i>et al.</i> , 2004
P22	<i>Salmonella typhimurium</i>	gp4	Head	-	Moak & Molineux, 2004
Tuc2009	<i>Lactococcus lactis</i>	Tal ₂₀₀₉	Tail tip	-	Kenny <i>et al.</i> , 2004
P68	<i>Staphylococcus aureus</i>	P17	Unknown	Activity against clinical <i>S. aureus</i> isolates	Takac & Blasi, 2005
phiMR11	<i>S. aureus</i>	gp61	Tail tip	CHAP and LY22 domains	Rashel <i>et al.</i> , 2008
phiIPLA88	<i>S. aureus</i>	HydH5	Unknown	Thermoresistant	Rodriguez <i>et al.</i> , 2011
SP-β	<i>Bacillus subtilis</i>	CwIP	Unknown	Muramidase and endopeptidase activity	Sudiarta <i>et al.</i> , 2010
φ29	<i>B. subtilis</i>	gp3	Associated to DNA	-	Moak & Molineux, 2004
Chimeric proteins					
P68	<i>S. aureus</i>	P16-17		Synergic with gentamicin	Manoharadas & Blasi, 2009
Phage K	<i>S. aureus</i>	P128		Anti-staphylococcal activity in decolonization of rat nares	Paul <i>et al.</i> , 2011
phiIPLA88	<i>S. aureus</i>	CHAPSH3b		Synergic with endolysin LysH5	Rodriguez-Rubio <i>et al.</i> , 2012

Figures

Figure 1. Mode of action of virion-associated PG hydrolases (VAPGHs).