



MINI-REVIEW

Bacteriophage-encoded depolymerases: their diversity and biotechnological applications

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Abstract Bacteriophages (phages), natural enemies of bacteria, can encode enzymes able to degrade polymeric substances. These substances can be found in the bacterial cell surface, such as polysaccharides, or are produced by bacteria when they are living in biofilm communities, the most common bacterial lifestyle. Consequently, phages with depolymerase activity have a facilitated access to the host receptors, by degrading the capsular polysaccharides, and are believed to have a better performance against bacterial biofilms, since the degradation of extracellular polymeric substances by depolymerases might facilitate the access of phages to the cells within different biofilm layers. Since the diversity of phage depolymerases is not yet fully explored, this is the first review gathering information about all the depolymerases encoded by fully sequenced phages. Overall, in this study, 160 putative depolymerases, including sialidases, levanases, xylosidases, dextranases, hyaluronidases, peptidases as well as pectate/pectin lyases, were found in 143 phages (43 *Myoviridae*, 47 *Siphoviridae*, 37 *Podoviridae*, and 16 unclassified) infecting 24 genera of bacteria. We further provide information about the main applications of phage depolymerases, which can comprise areas as diverse as medical, chemical, or food-processing industry.

Keywords Bacteriophages · Phage depolymerases · Capsular polysaccharides · Biofilms

Introduction

Phages are natural bacterial viruses and constitute the most abundant entities on Earth (Clokic et al. 2011). Phages have been used in clinical practice since the early 1920s (Kutter et al. 2010; Abedon et al. 2011), and although there was a period of declining interest on phage therapy after the antibiotics discovery (Kutter et al. 2010), the interest on phages re-emerged with the rise of antibiotic-resistant bacteria (Sulakvelidze 2005; Matsuzaki et al. 2005; Kutateladze and Adamia 2010). Therefore, many studies have reported the potential of phages as antimicrobial agents (McVay et al. 2007; Capparelli et al. 2007; Guenther et al. 2009; Oliveira et al. 2010; Morello et al. 2011; Trigo et al. 2013; Bertozzi Silva and Sauvageau 2014). However, the activity of phages is often hindered by the presence of polysaccharides at the bacterial surfaces and/or when bacteria are living in the biofilm form.

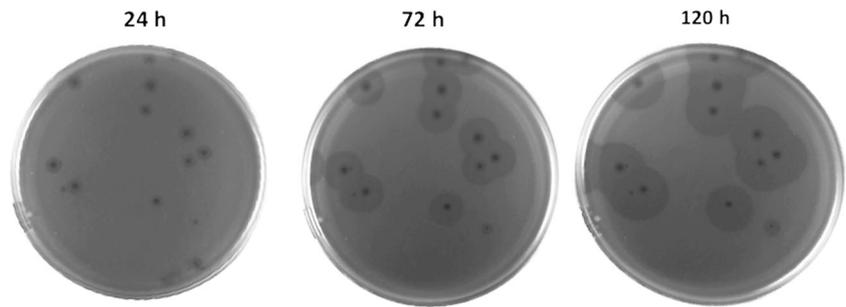
Biofilms are defined as microbial communities attached to a surface and encased in a matrix composed by extracellular polymeric substances (EPS) (Costerton et al. 1995; O'Toole et al. 2000), which represent over 90 % of the biofilm biomass (Flemming and Wingender 2010). The EPS matrix is constituted by a mixture of polysaccharides, proteins, nucleic acids, and lipids (Davey and O'toole 2000; Flemming et al. 2007; Flemming and Wingender 2010), and its composition varies with bacterial species, age of the biofilms, and environmental conditions such as temperature or nutrients availability (Flemming et al. 2007; Vu et al. 2009; Flemming and Wingender 2010; Harper et al. 2014). Biofilms are ubiquitous in nature (Davey and O'toole 2000; Donlan 2002), and on

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Fig. 1 Activity of phage-encoded depolymerases. Illustration of hazy rings (haloes) surrounding the phage plaques of the *Acinetobacter* phage vB_AbaP_CEB1 (unpublished) over time



account of their architecture that protects bacteria against external harsh conditions, they constitute an important survival strategy (Mah and O'Toole 2001; Flemming et al. 2007; Glonti et al. 2010). Furthermore, biofilms are frequently associated as a common source of contamination especially in healthcare (Reid 1999; Donlan 2001a, b) and food-processing settings (Brooks and Flint 2008; Shi and Zhu 2009). This happens as a consequence of their inherent tolerance to antimicrobial agents due to diffusion limitations, which can potentially lead to severe persistent infections, particularly difficult to treat (Costerton et al. 1999; Donlan 2001b; Parsek and Singh 2003; Hall-Stoodley et al. 2004).

In the late 1990s, Hughes et al. partially purified enzymes capable of degrading bacterial polysaccharides (also known as depolymerases) from phage solutions (Hughes et al. 1998a). More recently, several works evidenced that phages can encode depolymerases to degrade polymers, either associated with the cell surface (capsule polysaccharides, e.g., K30 antigens) to facilitate phage adsorption, or EPS involved in biofilm matrix in order to promote phage diffusion through the bacterial slime (Glonti et al. 2010; Cornelissen et al. 2011, 2012; Hsu et al. 2013). The depolymerase activity is commonly identified by a constantly increasing halo surrounding the phage plaques (Cornelissen et al. 2011, 2012; Harper et al. 2014), as depicted in Fig. 1. Nonetheless, the formation of

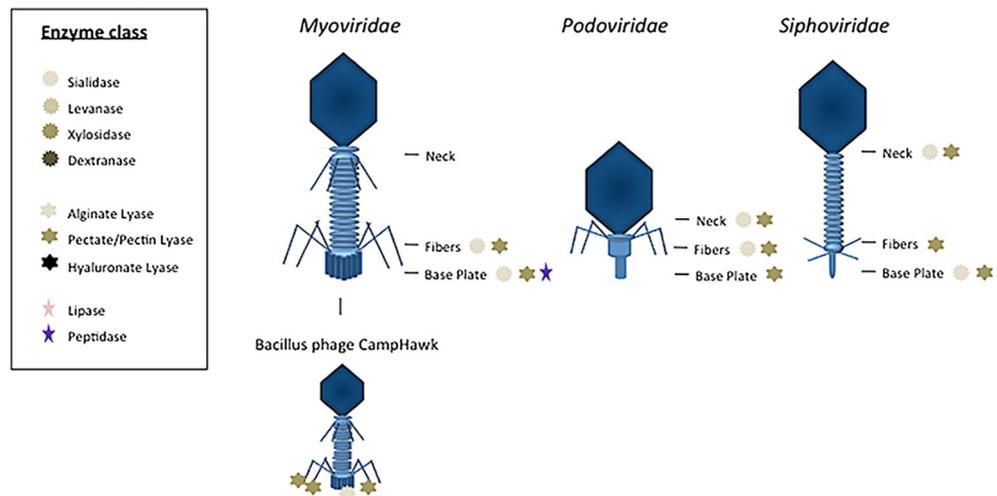
these halo zones is not so clear in some phages encoding depolymerases, such as the *Salmonella* phage phi PVP-SE1 that encodes a pectate-lyase in the gene 99 (Santos et al. 2009). It is believed that phages encoding depolymerases might have a better performance against bacterial biofilms (Hughes et al. 1998b; Yan et al. 2014). This is not completely true since there are phages deprived of depolymerases that have excellent anti-biofilm properties, which is the case of the *Pseudomonas fluorescens* phage phiIBB-PF7A (Sillankorva et al. 2008). Nonetheless, depolymerases may confer phages a great advantage in biofilm interaction (Lu and Collins 2007; Cornelissen et al. 2011, 2012).

As the presence and the distribution of depolymerases in the phage structures is not well understood, here, we report for the first time a complete study about EPS-degrading enzymes from all the phages completely sequenced to date.

Overview of phage-encoded depolymerases

The presence of depolymerases in phage genomes may act as an adjuvant for phage infection, since they degrade polymers either present in the bacterial surface, such as structural or capsular polysaccharides, or EPSs present in bacterial biofilms (Cornelissen et al. 2011; Yan et al. 2014). To get a better

Fig. 2 Distribution of the different depolymerase classes through the structure of tailed bacteriophages. Levanases, xylosidases, hyaluronidases, dextranases, and lipases represent rare depolymerases domains found in phages, and therefore, the location was not possible to identify. The *Bacillus* phage CampHawk represents the only phage to encode four depolymerases in different genes (one sialidase and three pectate lyases)



understanding of the diversity, structure, and function of phage depolymerases, we compiled information about all depolymerases present in fully sequenced dsDNA phages deposited in NCBI (Table S1).

Overall, 160 putative depolymerases were identified in 143 phages (43 *Myoviridae*, 47 *Siphoviridae*, 37 *Podoviridae*, and 16 unclassified) that infect 24 genera of bacteria, with lengths ranging from 197 to 2417 amino acids (Table S1). According to Drulis-Kawa et al., phage depolymerases appear in two forms: (i) as integral components of the phage particles or (ii) as soluble proteins generated during host cell lysis (Drulis-Kawa et al. 2015). Based on our search, the huge majority of phage depolymerases (126 proteins) are encoded in the same open reading frame of phage structural proteins (mostly on tail fibers, base plates, but sometimes also in the neck) or in close proximity to those genes (Fig. 2), and were thus considered as structural proteins (Table S1). Twenty other depolymerases found in this work might be soluble proteins since they are distant from any structural gene (Table S1). We were not able to classify 14 phage depolymerases, and thus, we considered them as unknown (Table S1).

According to their mode of action, the putative phage depolymerases were distributed in two main classes: hydrolases and lyases, which were further divided into different subclasses (Table 1). Exolysins (structural lysins) or endolysins, enzymes that hydrolase the structural LPS and peptidoglycan encoded by all *Caudovirales* phages, were already previously identified and described (Oliveira et al. 2013). Therefore, in this review, we focus on less explored enzymes that degrade EPS.

Sixteen different domain combinations were found throughout this analysis, and one representative of each architecture is depicted in Fig. 3. Although most of domains were found individually represented in a phage protein, combinations of domains were also found. For instance, Pectate_lyase_3 was found alone (*Shigella* phage Sf6) and combined with Peptidase_S74 (*Bacillus* phage CampHawk), End_tail_spike-Peptidase_S74 complex (Enterobacteria phage K5), and with Pectin_lyase (*Streptomyces* phage phiSASD1).

Most phages encode only one or two depolymerase motifs in the same gene (Table S1). Nevertheless, some phages can encode depolymerases in different genes. For example, phage ϕ K1-5 is able to infect and replicate on both K1 and K5 *Escherichia coli* strains because it encodes two different enzymatic tail fiber proteins: an endosialidase that enables the phage to attach to and degrade K1 polysaccharide capsule, and a K5 lyase that cleaves the K5 capsular polysaccharides (Scholl et al. 2001). Another case is the *Salmonella enterica* serovar Enteritidis phage Marshall that encodes two tail proteins with beta helix/pectin lyase domains (Luna et al. 2013). A very interesting and rare example is the *Bacillus subtilis* phage CampHawk which encodes a putative peptidase and

three pectate lyase domains in four different tail proteins (Table S1) (Ritz et al. 2013).

Diversity of phage depolymerases

Hydrolases

O-glycosyl hydrolases (EC 3.2.1.X) are enzymes that catalyze the hydrolysis of glycosidic bonds (Davies and Henrissat 1995). In this class of enzymes, six different groups of phage depolymerases were found: sialidases, levanases, xylosidases, dextranases, rhamnosidases, and peptidases (Table 1).

Sialidases or neuraminidases (EC 3.2.1.18) are a group of enzymes that hydrolyses the α -linkage of the terminal sialic acids of various glycans in diverse organisms (Kim et al. 2011b). Sialic acids represent a family of nine-carbon acidic sugars that are mostly found in the terminal position of oligosaccharide chains of glycan molecules present in cell surfaces (Traving and Schauer 1998; Kim et al. 2011b). Sialic acid plays an important role on bacterial pathogenesis since pathogenic bacteria such as *E. coli* K1, *Haemophilus influenzae*, *Pasteurella multocida*, *Neisseria* spp., *Campylobacter jejuni*, and *Streptococcus agalactiae* can use this molecule as a source of nutrients or to increase resistance to host's immune defenses by coating themselves in sialic acid (Severi et al. 2007). Nonetheless, it has also been shown that many bacterial species produce sialidases and that their presence has been associated with increased pathogenesis because they promote bacterial survival in mucosal niche environments and have important roles in the interaction with other organisms (Kim et al. 2011b; Lewis and Lewis 2012). So far, several phages have been reported to encode endosialidases as part of the tail structure, which enables the recognition and degradation of the capsular polysaccharides (Petter and Vimr 1993; Gerardy-Schahn et al. 1995; Long et al. 1995; Scholl et al. 2001; Scholl and Merrill 2005; Schwarzer et al. 2012). Here, we found sialidase domains in a wide range of phages targeting different bacterial species. However, the majority of phages carrying sialidases are specific for *Streptococcus* spp. (Table S1). Sialic acid is reported to be one of the most important carbohydrates for *Streptococcus pneumoniae* because it plays a significant role as source of carbon and energy, receptor of adhesion, and invasion and promotion of biofilm formation (Gualdi et al. 2012). However, among Gram-positive bacteria, only *S. agalactiae* and *Streptococcus suis* are reported to produce sialylated polysaccharides in the form of capsular polysaccharides (Chaffin et al. 2005; Severi et al. 2007). It was already demonstrated that the intraperitoneal administration of a phage-derived endosialidase in *E. coli*-infected mice successfully prevented bacteremia and death from systemic infection (Mushtaq et al. 2004, 2005). Although the enzyme had no effect on the viability of *E. coli* cells, the

Table 1 Bacteriophage-encoded depolymerases distributed according to their mode of action

Enzyme class	EC number	Predicted domains found	Polymer	Bacterium genus
Hydrolases				
Sialidases	3.2.1.18 or 3.2.1.129	Sialidases (SUPFAM0050026) Peptidase_G2 (PF11962) Peptidase_S74 (PF13884) End_tail_spike (PF12219) End_beta_propel (PF12217) End_N_terminal (PF12218)	Sialic acid	<i>Bacillus</i> <i>Enterobacter</i> <i>Escherichia</i> <i>Klebsiella</i> <i>Prochlorococcus</i> <i>Pseudomonas</i> <i>Serratia</i> <i>Staphylococcus</i> <i>Streptococcus</i> <i>Streptomyces</i>
Levanases	3.2.1.65	Glyco_hydro_32N (PF00251) Glyco_hydro_32C (PF08244)	Levan	<i>Bacillus</i>
Xylosidases	3.2.1.37	Glyco_hydro_39 (PF01229)	Xylan	<i>Caulobacter</i>
Dextranases	3.2.1.11	Glyco_hydro_66 (PF13199)	Dextran	<i>Lactobacillus</i>
Rhamnosidases	3.2.1.40	Activity detected ^a	Rhamnogalacturonan	<i>Escherichia</i> <i>Salmonella</i> <i>Shigella</i>
Peptidases	EC 3.4.19.9	DUF867	Poly- γ -glutamate	<i>Bacillus</i>
Lyases				
Hyaluronidases	(EC 4.2.2.1 or EC 4.2.99.1)	Hyaluronidase_1 (PF07212)	Hyaluronate	<i>Streptococcus</i>
Alginate lyases	4.2.2.3 or 4.2.2.11	Activity detected ^b	Alginate	<i>Azotobacter</i> <i>Pseudomonas</i>
Pectin/pectate lyases	4.2.2.10 or 4.2.2.2	Pec_lyase_C (PF00544) Pectin lyase-like (SUPFAM) Pectate_lyase_3 (PF12708)	Galacturonate	<i>Bacillus</i> <i>Brucella</i> <i>Burkholderia</i> <i>Cellulophaga</i> <i>Clostridium</i> <i>Cronobacter</i> <i>Enterobacter</i> <i>Escherichia</i> <i>Erwinia</i> <i>Klebsiella</i> <i>Leuconostoc</i> <i>Pantoea</i> <i>Shigella</i> <i>Staphylococcus</i> <i>Streptomyces</i> <i>Pseudomonas</i> <i>Rhodococcus</i> <i>Salmonella</i> <i>Serratia</i>
Others				
Lipases	3.1.1.3.	Lipase_GDSL_3	Triacylglycerols	<i>Cellulophaga</i> <i>Pseudomonas</i>

Different enzymes classes are sorted according to the predicted depolymerases domains found and corresponding EC numbers, which act on distinct polymers. Every bacterial hosts from which depolymerase activity was found in phages is also presented

^a Rhamnosidase activity detected in *Salmonella* phage P22 (Andres et al. 2010), *Escherichia* phage HK620 (Barbirz et al. 2008), and *Shigella* phage SF6 (Chua et al. 1999)

^b Alginate lyase activity detected in *Pseudomonas* phage PT-6 (Glonti et al. 2010) and *Azotobacter*-infecting phage (Davidson et al. 1977)

degradation of capsular polysaccharides sensitized bacteria to the bactericidal action of the complement system (Mushtaq et al. 2004) and also enhanced phagocytosis by macrophages (Mushtaq et al. 2005).

Levanases (EC 3.2.1.65) are enzymes found in some microorganisms (e.g., *Bacillus* and *Pseudomonas*) that catalyze the hydrolysis of the β -2,6-linked main chain of levan, a natural product from the group of fructans (Murakami et al. 1992;

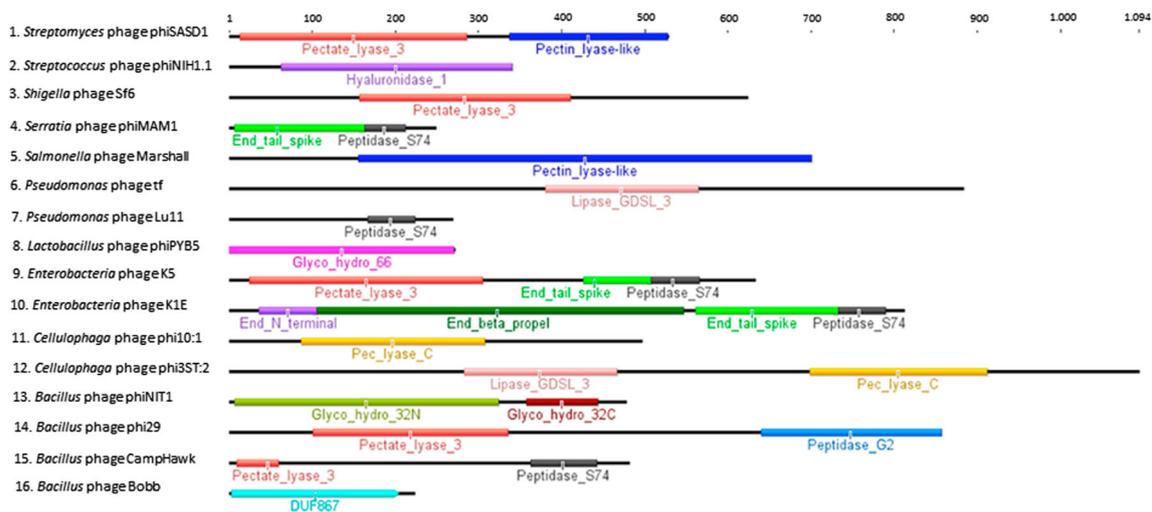


Fig. 3 Different phage depolymerase architectures. For the 16 different architectures, a phage representative was selected. Accession numbers of phage proteins: (1) YP_003714746, (2) AAL15086, (3) AAQ12204, (4) AFX93507, (5) AGY47556, (6) CCE60816, (7) AFH14728, (8)

ADA79897, (9) YP_654147, (10) CAJ29458, (11) YP_008242013, (12) AGO47740, (13) YP_008318429, (14) ACE96035, (15) AGY46940, and (16) YP_009056510

Miasnikov 1997). Levan polysaccharide is known to play an ecological role on *Bacillus* biofilm formation and maintenance (Marvasi et al. 2010), while on some strains of *Pseudomonas* are suggested to be part of a protective capsule against phages (PATON 1960). Therefore, with no surprise, the only phage depolymerase domain with levan degradation (Glyco_hydro_32) activity found belongs to the *Bacillus* phage SP10 (Table S1).

Xylosidases (EC 3.2.1.37), dextranases (EC 3.2.1.11), and rhamnosidases (EC 3.2.1.40) are the classes of enzymes representing the rarest depolymerase phage domains (Table S1). These enzymes catalyze the hydrolysis of sugars such as xylan (Sunna and Antranikian 1997), dextran (Jiménez 2009), and rhamnogalacturonan (Yadav et al. 2010), respectively. Such polymers are present in many bacterial species as described in Table 1. The xylosidase domain (Glyco_hydro_39) was found in the only *Caulobacter* phage present in Table S1, while the two dextranase domains (Glyco_hydro_66) were found in the only *Lactobacillus* phage. Although no rhamnosidases domains were found in our search of phage genomes, rhamnosidase activity was already reported in *Enterobacteria* phage P22 (Eriksson et al. 1979), *Shigella* phage SF6 (Chua et al. 1999), and *Escherichia* phage HK620 (Barbirz et al. 2008). The three *Podoviridae* phages cleave the respective host cell receptor polysaccharide by their homotrimeric tailspike proteins (Barbirz et al. 2008). Furthermore, after being identified, the endorhamnosidase of phage SF6 was cloned, and its activity was shown by the cleavage of O-antigen on LPS chain in endorhamnosidase-treated samples (Chua et al. 1999).

Among hydrolases, there are enzymes that act on peptide bonds known as peptidases (EC 3.4.X.X). In the present study, a poly- γ -glutamate (γ -PGA) hydrolase (EC 3.4.19.9)

encoding a peptidase domain DUF867 (PF05908) was found in four *Bacillus* phages, namely Bobb, phiAGATE, SPP1, and phiNIT1. Kimura et al. identified and purified a γ -PGA hydrolase from phage phiNIT1 and demonstrated its enzymatic activity against capsular polypeptides of glutamate with a gamma-linkage, called poly- γ -glutamate (Kimura and Itoh 2003). This polypeptide is a natural polymer exclusively synthesized by Gram-positive bacteria, including some strains of *Bacillus* (Kimura and Itoh 2003; Candela and Fouet 2006).

Lyases

Polysaccharide lyases (EC 4.2.2.X) are a class of enzymes that cleave (1,4) glycosidic bonds by a β -elimination mechanism (Sutherland 1995; Michaud et al. 2003). This class of enzymes comprises three groups of phage depolymerases: hyaluronate, alginate, and pectin/pectate lyases.

Hyaluronidases or hyaluronate lyases (EC 4.2.2.1 or 4.2.99.1) are a class of enzymes capable of, but not exclusively, digesting the hyaluronate, a linear unsulfated glycosaminoglycan polymer found in several organisms, including bacteria (*Staphylococcus*, *Clostridium*, streptococci groups A, B, C as well as *S. pneumoniae*) (Hynes and Walton 2000). Many extracellular bacterial hyaluronidases are able to cleave the β -1-4 glycosidic linkage by β -elimination and are often suggested to be a virulence factor, by increasing tissue permeability, facilitating the pathogen invasion (Hynes and Walton 2000). The same type of hyaluronidases has been found on some prophages from *Streptococcus pyogenes* and *Streptococcus equi* strains (Timoney et al. 1982; Hynes et al. 1995; Baker et al. 2002; Singh et al. 2014), and are thought to play a role in phage penetration of streptococcal hyaluronan capsules, facilitating bacterial lysogenization

(Singh et al. 2014). As no extracellular milieu hyaluronidase activity was detected, these enzymes were suggested to be part of the phage particle to degrade the host's hyaluronic acid capsules, in order to gain access to their surface where phage receptors are located. In our search, we have identified two novel temperate siphoviruses that contain hyaluronidase-degrading activities, namely the group A streptococcal phage phiNIH1 and *S. equi* phage P9.

Alginate lyases are mannuronate (EC 4.2.2.3) or guluronate lyases (EC 4.2.2.11) that catalyze the degradation of alginate, a linear polysaccharide of α -L-guluronate and its C5 epimer β -D-mannuronate (Wong et al. 2000; Kim et al. 2011a). Alginates are abundant in nature as a structural component of brown algae (*Phaeophyceae*) (Wong et al. 2000; Kim et al. 2011a), and they are also synthesized by two families of bacteria, the *Pseudomonadaceae* (Monday and Schiller 1996; Albrecht and Schiller 2005) and the *Azobacteriaceae* (Clementi 1997). Alginate lyases have been isolated from different sources including marine algae, marine molluscs, fungi, bacteria, and viruses (Wong et al. 2000), and can be used to enhance antibiotic killing efficacy against mucoid bacteria such as *P. aeruginosa* (Alkawash et al. 2006). To date, some *Azobacter* and *Pseudomonas* spp. phages were found to encode alginate lyases (Bartell et al. 1966; Davidson et al. 1977; Glonti et al. 2010), which help phages to penetrate through the acetylated poly(M)-rich EPSs produced by their bacterial hosts (Wong et al. 2000; Yan et al. 2014). An example of a phage with alginate-degrading activity is the *P. aeruginosa* phage PT-6 (Glonti et al. 2010). Glonti et al. showed that this phage was able to reduce the viscosity of four alginate polymers by 62 to 66 % within 15 min (Glonti et al. 2010).

Pectin or pectate lyases (EC 4.2.2.10 and EC 4.2.2.2) are pectolytic enzymes that degrade galacturonic acid, a major constituent of bacterial polysaccharides and plant cell walls (Fridrich and Whitfield 2005; Garron and Cygler 2010). Although these enzymes are mainly produced by plant-associated organisms, they are also commonly produced by bacteria (Hugouvieux-Cotte-Pattat et al. 2014). During this search, we found that the majority of phage depolymerases belong to the class of pectolytic enzymes (Table S1). The *Pseudomonas putida* phages ϕ 15 and AF, which exhibit biofilm-degrading properties, encode both pectate lyases on their tailspike genes 17 (Cornelissen et al. 2011) and 19 (Cornelissen et al. 2012), respectively. The EPS-degrading activity of the tailspikes of phage ϕ 15 was further confirmed after dropping the recombinant expressed and purified protein on a bacterial lawn followed by the formation of opaque halo zones (Cornelissen et al. 2011). However, biofilm growth was not influenced after addition of UV-

inactivated ϕ 15 phage particles, which are non-infective but keep the enzymatic activity of their tailspikes (Cornelissen et al. 2011). The same was observed with phage AF (Cornelissen et al. 2012). Another example of phages encoding these enzymes are the *Staphylococcus epidermidis* phages vB_SepiS_phiIPLA5 and vB_SepiS_phiIPLA7 (Gutiérrez et al. 2012). The depolymerase domains, located in the pre-neck appendage proteins of those phages, are responsible for the good biofilm-degrading properties of both phages (Gutiérrez et al. 2012; Melo et al. 2014).

Others

Lipases (EC 3.1.1.3), or triacylglycerol hydrolases, are enzymes acting on the carboxyl ester bonds of triacylglycerols to liberate organic acids and glycerol (Jaeger et al. 1994; Gupta et al. 2004). Lipases are ubiquitous in nature, and they are produced by different types of organisms including bacteria. *Bacillus*, *Pseudomonas*, and *Burkholderia* are some of the most important lipase-producing bacterial genera (Gupta et al. 2004). In this work, only one phage depolymerase domain with lipids hydrolysis activity (Lipase_GDSL_3) was found in eight *Cellulophaga* phages and one *Pseudomonas* phage (Table S1).

Potential applications of phage depolymerases

Overall, phages with depolymerase activity have an improved performance against their target bacteria since these enzymes help phages in many important processes such as adsorption, invasion, and disintegration of host bacterial cell as well as biofilm disruption (Yan et al. 2014).

Phages encoding EPS depolymerases are expected to be more suitable for phage therapy purposes. In 2002, Tait et al. reported a complete eradication of *Enterobacter cloacae* biofilms after applying a cocktail of three phages with depolymerase activity (Tait et al. 2002), which revealed the potential of these enzymes to act as adjuvants for biofilm control. Nonetheless, most of the phages do not encode such enzymes, and therefore, phages can be engineered to express depolymerases during phage infection. For this purpose, Lu and Collins genetically engineered a T7 phage to express the biofilm-degrading enzyme dispersin B (Lu and Collins 2007). As a result, the enzymatic phage was able to efficiently kill biofilm cells and, at the same time, degrade the biofilm EPS matrix (Lu and Collins 2007).

Although not derived from phages, there are already EPS-degrading enzymes commercially available such as alginate lyase (Sigma-Aldrich) and dispersin B (Kane Biotech). This

Table 2 Other biotechnological applications of bacteriophage-encoded depolymerases

Enzyme class	Main applications	References
Hydrolases		
Sialidases	Medical industry (processing of clinically relevant asialoproteins)	(Kim et al. 2011b)
Levanases	Food industry (production of levanoligosaccharides, enzymes involved in the hydrolysis of polyfructans are of interest for industrial applications)	(Dahech et al. 2013)
Xylanases	Chemical industry (biobleaching of wood pulp and in the bioprocessing of textiles) Food industry (food additives to poultry and in wheat processing) Processing industry (bioconversion of xylan into higher value added products such as xylitol) Bioenergy industry (for more economic biorefining processes)	(Juturu and Wu 2012; Kulkarni et al. 1999; Beg et al. 2001)
Dextranases	Food industry (sugar beet processing)	(Jiménez 2009)
Rhamnosidases	Food industry (removal of bitterness from citrus fruit juices) Wine-making (aroma enhancement) Medical industry (drug preparation)	(Manzanares et al. 2007)
Lyases		
Hyaluronidases	Medical industry (to increase the effectiveness of local anesthesia in many fields)	(Clark and Mellette 1994)
Alginate lyases	Preparation of bio-functional alginate oligosaccharides (increase the bifidobacteria flora) Medical applications (co-administration with antibiotics to treat cystic fibrosis) Food industry (food additives, stabilizer, and gelling materials)	(Wong et al. 2000; Kim et al. 2011a)
Pectin/pectate lyases	Food and wine-making industry (fruit juice extraction and clarification)	(Sieiro et al. 2012)

suggests that phage-based depolymerases may also have commercial potential.

Furthermore, phage depolymerases can be heterologous expressed having a wide range of other potential applications in medical, chemical, or food-processing industry as suggested in Table 2. Phage depolymerases can also be used in combination with antibiotics to act as adjuvants. These enzymes play an important role, for example, against alginate-producing strains that use alginate as a barrier to block the diffusion of antibiotics (Bayer et al. 1991; Hatch and Schiller 1998; Abdi-Ali et al. 2006). The ability of phages to effectively reduce the viscosity of alginates and EPS produced by *P. aeruginosa* cells was already demonstrated (Hanlon et al. 2001), and it was also shown that the efficacy of antibiotics against mucoid *P. aeruginosa* biofilms is enhanced by the administration of alginate lyase (Alkawash et al. 2006).

Other works have reported the potential of using phage depolymerases for multiple applications. It was shown that a capsular depolymerase encoded by a *B. subtilis* phage improved phagocytic killing of encapsulated *Bacillus anthracis* both in vitro (Scorpio et al. 2007) and in vivo (Scorpio et al. 2008). Another work also revealed the potential of a phage endosialidase to treat systemic infections in mice caused by *E. coli* K1 (Mushtaq et al. 2005). The enzyme degraded the capsular polysaccharide of pathogen cells rendering them sensitive to phagocytosis (Mushtaq et al. 2005). It was also shown that a depolymerase from an *Erwinia amylovora* phage effectively reduced colonization of fire blight host plants by *E. amylovora* (Kim and Geider 2000).

Another application of phage depolymerases is the detection or diagnosis of bacteria. Lin et al. successfully cloned and expressed a capsule depolymerase from a *K. pneumoniae* phage with specificity for K1 capsule suggesting a potential tool for the diagnosis of K1 *K. pneumoniae* infections (Lin et al. 2014). Furthermore, a work reported by Hsu et al. described the characterization of a *K. pneumoniae* phage depolymerase with specificity to KN2 capsular polysaccharides which can be successfully used for efficient *Klebsiella* capsular typing (Hsu et al. 2013).

Conclusions

The advances in omics and *in silico* techniques opened the way to new knowledge about the diversity of phage-encoded proteins. Phages are natural toolboxes that can offer an arsenal of proteins with a multitude of potential biotechnological applications. In this review, we demonstrated that phage genomes encode a diverse group of enzymes, called depolymerases, which are used to facilitate early steps of the phage infection cycle (adsorption and viral DNA injection). Depolymerases acting on many different substrates (including sialic acid, levan, xylan, dextran, rhamnogalacturonan, poly- γ -glutamante, hyaluronate, alginate, and triacylglycerol) were found in phage genomes, illustrating the complex phage-host interaction system that was refined during millions of years. The ability of phage depolymerases to degrade EPS makes them very attractive for a broad range of applications especially in clinical and industrial settings.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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