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RESEARCH ARTICLE

Viral degradation of marine bacterial exopolysaccharides

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One sentence summary: Marine phages are able to passively degrade their hosts dissolved EPS.

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ABSTRACT

The identification of the mechanisms by which marine dissolved organic matter (DOM) is produced and regenerated is critical to develop robust prediction of ocean carbon cycling. Polysaccharides represent one of the main constituents of marine DOM and their degradation is mainly attributed to polysaccharidases derived from bacteria. Here, we report that marine viruses can depolymerize the exopolysaccharides (EPS) excreted by their hosts using five bacteriophages that infect the notable EPS producer, *Cobetia marina* DSMZ 4741. Degradation monitorings as assessed by gel electrophoresis and size exclusion chromatography showed that four out of five phages carry structural enzymes that depolymerize purified solution of *Cobetia marina* EPS. The depolymerization patterns suggest that these putative polysaccharidases are constitutive, endo-acting and functionally diverse. Viral adsorption kinetics indicate that the presence of these enzymes provides a significant advantage for phages to adsorb onto their hosts upon intense EPS production conditions. The experimental demonstration that marine phages can display polysaccharidases active on bacterial EPS lead us to question whether viruses could also contribute to the degradation of marine DOM and modify its bioavailability. Considering the prominence of phages in the ocean, such studies may unveil an important microbial process that affects the marine carbon cycle.

Keywords: marine phage; EPS; polysaccharidase; DOM; ocean

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INTRODUCTION

The dynamics of dissolved organic matter (DOM) in seawater plays a pivotal role in ocean biogeochemistry. Marine DOM is derived from different biological processes, including phytoplankton release particularly during bloom events (Carlson and Hansell 2014), excretion of capsular polysaccharides or exopolysaccharides (EPS) by heterotrophic bacteria (Stodereger and Herndl 1998, 1999, 2001), sloppy feeding by metazoan grazers (Møller 2005) or biodegradation of macroalgal biomass (Krause-Jensen and Duarte 2016). Viral-mediated lysis of microbes is also recognized as one of the largest source of DOM in the ocean (Middelboe, Jørgensen and Kroer 1996; Fuhrman 1999; Suttle 2005). Regardless of the source of DOM, extracellular enzymes encoded by microbial communities initiate the degradation of DOM in the sea (Arnosti 2011). These enzymes efficiently depolymerize high-molecular-weight organic matter into compounds of lower molecular weight. Part of these hydrolysis products is converted into bacterial biomass that supports higher trophic levels and inorganic nutrients that can be recycled by primary producers. Another part consists of refractory forms of organic matter that persist in the ocean for extended periods of time and contribute to carbon sequestration (Jiao et al. 2010; Krause-Jensen and Duarte 2016). Extracellular enzymes influence the fate of most of the organic matter and thereby, they play a pivotal role in marine carbon cycling (Arnosti 2011).

High molecular weight acidic polysaccharides represent one of the main constituents of marine DOM (McCarthy, Hedges and Benner 1993; Panagiotopoulos and Sempéré 2005). Despite their key-role in marine carbon cycling, the interactions between extracellular enzymes and marine DOM are not well understood. Studies, mostly conducted on terrestrial microorganisms, showed that Carbohydrate Active enZymes (CAZymes, Henrissat 1991; Davies and Henrissat 1995; Lombard, Golaconda and Drula 2014) are used to bind, modify and degrade these polymers for consumption. Among these CAZymes, the proteins that specifically catalyze the breakdown of polysaccharides are designated as polysaccharide depolymerases or polysaccharidases. These enzymes are either constitutive or induced; they act either on the terminal end of the polymers (exo-acting mode) or cleave the polymer mid-chain (endo-acting mode) (Sutherland 1995). They can be found attached to the cell or they can be released into the surrounding environments (Arnosti 2011). Studies of biological models from variable origins evidenced a notable diversity within these enzymes, which are currently classified among 304 protein families based on sequence homology in the CAZY database (Lombard, Golaconda and Drula 2014; <http://www.cazy.org/>). In marine environments, polysaccharidases are ubiquitous and there is no doubt that they degrade much of the DOM in ocean (Arnosti 2011a; Arnosti 2011b). It is commonly assumed that polysaccharidase activity derives mainly from bacteria. The recent advances in genomics clearly demonstrated that other microbes encode polysaccharidases. Among these, viruses, which usually outnumber bacteria by one order of magnitude in the ocean, can display such enzymes (Pires et al. 2016).

A wealth of biomedical studies reports that viruses of bacteria (bacteriophages or phages) use polysaccharidases to depolymerize their host capsule/ slime/ biofilms in order to gain access to their primary receptors (Bayer et al. 1979; Sutherland 1999; Sutherland et al. 2004). These enzymes, typically located on

phage tail, display a high level of functional and molecular diversity (Sutherland 1999; Scholl, Adhya and Merrill 2005; Cornelissen et al. 2011, 2012; Yele et al. 2012) and can influence biological traits of viruses such as host specificities (Leiman et al. 2007). To the best of our knowledge, there is no experimental evidence of polysaccharidase activity in marine phages. Yet, marine bacteria are active producers of polysaccharides or EPS that are either associated with the bacterial cell surface or excreted into the environment (Heissenberger, Leppard and Herndl 1996; Bier-smith and Benner 1998; Casillo et al. 2018). This led us to question as to whether marine phages also carry polysaccharidases to infect their host. The finding of polysaccharidases associated to marine viruses could provide novel fundamental knowledge about the ecology of these prominent microbes. Because polysaccharidases initiate the infection cycle, we could expect that their intrinsic properties (life-time, substrate specificity, activity velocity) and their ability to withstand environmental changes (salinity, temperature, pH, pressure) directly influence the rates of infection and subsequent viral induced mortality or even the life-time of a viral particle in the ocean. Importantly, the passive degradation of dissolved bacterial EPS by viruses could have important biogeochemical implications, such as affecting the size spectrum, the reactivity and the bioavailability of marine DOM.

In this study, we investigated the capacity of marine phages to depolymerize their host EPS and attempted to relate this to important biological traits of marine viruses. Therefore, we used five phages (*CobetiA marINa* phages: Carin-1 to 5) that infect the globally distributed γ -Proteobacteria *Cobetia marina* (DSMZ 4741, hereafter *C. marina*) known for its ability to produce EPS (Arahal et al. 2002; Yumoto et al. 2004; Ivanova et al. 2005; Lechat et al. 2015b) and its potential contribution to the carbon export (Guidi et al. 2016). Our study revealed that marine phages do possess enzymes active on bacterial dissolved EPS, which may influence virus ecology and the recycling of bacterially derived DOM in the ocean.

MATERIAL AND METHODS

Bacterial host

The marine gram-negative Gammaproteobacteria *Cobetia marina* DSMZ 4741 was used as reference host to isolate phages from seawater. This strain belongs to a halotolerant mesophilic, heterotrophic and aerobic genus routinely grown on liquid and agar Zobell medium (Baumann, Bowditch and Baumann 1983; Romanenko et al. 2013).

Phage isolation

Cobetia marina phages were isolated from surface water of the bay of Brest at the long-term monitoring station SOMLIT (4° 33' 07.19 W, 48° 21' 32.13 N) from June to September 2011. Seawater samples (20 L) were filtered through 0.7 μ m glassfiber filter (Whatmann®) and concentrated (100-fold) using a 100 kDa ultrafiltration cartridge (Pellicon, Sartorius®). Once concentrated, samples were filtered through a PES syringe filter (0.2 μ m cut-off, Sartorius®) and directly used in a plaque assay by spreading a mixture of the reference host and viruses in sterile molten Zobell agar (noble agar 0.6%, 5 g.L⁻¹ peptone, 1 g.L⁻¹ yeast extract dissolved in seawater) heated at 37°C on a layer Zobell agar (5 g.L⁻¹ peptone, 1 g.L⁻¹ yeast extract, 15 g.L⁻¹

agar dissolved in seawater). The plate was incubated at 20°C (Swanstrom and Adams 1951; Kropinski et al. 2009). After 24 h incubation, well-resolved plaques (PFU—plaque forming unit) were picked from the lawn of host cells, eluted in sterilized ultrafiltered seawater, and combined with host culture in a new plaque assay. This procedure was repeated two more times to ensure isolation of a clonal population for each phage. Clonal lysates of each viral strain was stored in SM buffer (NaCl: 100 mM; MgSO₄, 7H₂O: 8 mM; TrisHCl: 50 mM) at 4°C until further study.

Phage purification

Bacteriophages were purified according a protocol adapted from Bachrach and Friedmann (1971). Phages solution was purified on sucrose linear gradients (10% to 40% w/v). First, 500 µL of freshly prepared viral lysate was treated with 50% (v/v) chloroform for 1 h to remove organic contaminants such as vesicles which can contain enzymes including polysaccharidases (Li, Azam and Zhang 2016; Arntzen et al. 2017). Then, the aqueous phase was separated by low-speed centrifugation (3000 x g, 5 min at room temperature) and loaded on the top of the gradient before ultracentrifugation (134 000 g, 45 min, 4°C) using a SW 41 Ti rotor. The viral band was extracted using a sterile syringe needle. The viral particles were diluted in SM buffer or sterilized ultrafiltered seawater and they were pelleted by a second ultracentrifugation (2 h, 352 000 g, 4°C) using a 70.1 Ti rotor to separate them from sucrose. After resuspension of the viral pellet in SM buffer, bacteriophages were then filtered through a 0.2 µm PES filter (Sartorius®).

Transmission electronic microscopy

A drop (10 µL) of clonal phage suspension was applied to a copper EM grid (400 mesh size) with a nitrocellulose backed carbon surface (Ackermann 2007, 2009). The sample was adsorbed on the grid for 10 sec after which the grid was blotted with filter paper and stained with 2% (w/v) uranyl acetate for 45 sec, blotted again and allowed to air dry. Specimens were imaged using a JEOL® JEM 1400 transmission electron microscope operating at 100 keV at a magnification of 50 000X. Pictures were analysed using ImageJ software.

Host range

CobetiA marINA phages: Carin-1 to 5 host specificities were determined by plaque assay using three strains of *C. marina* and six strains of the related *Halomonas* genus. Dilution series of Carin-1 to 5 suspensions (10² to 10⁸ PFU.mL⁻¹) were incubated with the potential host cultures in exponential growth phase for 15 min. After incubation, samples were mixed with the molten agar, plated onto a lawn of Zobell agar and incubated at 20°C in darkness for 24 h.

EPS production

Cobetia marina EPS (hereafter referred to as EPS L₆) were produced as described previously (Lelchat et al. 2015b). Briefly, a pre-culture was grown in ZoBell medium (1 g.L⁻¹ yeast extract, 5 g.L⁻¹ peptone diluted in 80% filtered seawater and 20% MilliQ) supplemented with glucose (30 g.L⁻¹) at 25°C under agitation. After 10 h incubation, pre-culture was inoculated (10% v/v) in a 3 L fermenter (INFORS®) containing Zobell medium supplemented with Tris-base (1.5 g.L⁻¹) and glucose (30 g.L⁻¹). The pH

medium was adjusted to 7.6 and maintained at this value by addition of H₂SO₄ or NaOH. The culture was grown at 25°C for 33 h until the culture reached the late exponential phase. During the stationary growth phase, the temperature was decreased to 20°C for 48 h in order to enhance the EPS production. The oxygenation was monitored and regulated by stirring and air flux. The consumption of glucose was monitored with the enzymplus kit (St Gobain®, France). At the end of the fermentation, the culture medium was centrifuged (1 h, 14 000 g, 4°C) and the supernatant was filtered with a Buchner through a 0.45 µm glass filter (Whatman®). Sodium azide (NaN₃, 0.4 g.L⁻¹ final concentration) was added to the filtrate to prevent bacterial regrowth. The filtered culture supernatant containing the soluble EPS was then purified by tangential ultrafiltration with a 100 kDa cut-off cartridge (Millipore®) against MilliQ water. The purified EPS was then frozen, freeze dried and stored away from light and moisture.

Enzymatic screening

Enzyme assays were realized in triplicate. Purified viruses (10¹⁰ PFU.mL⁻¹) were mixed at 4°C with EPS L₆ (0.2% w/v final concentration) in incubation buffer (Tris-HCl 50 mM, NaCl 0.5 M, MgSO₄ 8 mM, pH 8 at 20°C), filtered through a 0.2 µm filter (Sartorius®) and incubated 24 h at 25°C under agitation (120 rpm). Controls containing viral suspension, *C. marina* lysate extract and EPS as well as EPS alone were processed and incubated under similar conditions to check for potential auto-hydrolysis phenomenon according Lelchat et al. (2015a).

The polysaccharide degradation was visualized using agarose gel electrophoresis according to the method of Lee and Cowman (1994). With L₆ being an acidic polysaccharide, runs were conducted at pH 8.5. Briefly, the agarose gel (1%) was prepared in Tris/acetic acid / EDTA (TAE) buffer (TAE 10X: Tris-Acetic acid 0.4 M, Na₂ EDTA 10 mM, pH 8.5). Samples (12 µL) were loaded on the gel and electrophoresis was run for 75 min at 100 V.

The gel was stained overnight in a Stains All solution (Lee and Cowman 1994; Volpi and Maccari 2002) (10 mL of 3,3'-diethyl-9-methyl-4,5,4',5'-dibenzothiacarbocyanine 0.1% (w/v) in *N,N*-dimethylformamide, 10 mL of *N,N*-dimethylformamide; 50 mL of isopropanol; 10 mL of 300 mM Tris HCl pH 8.8, completed to 200 mL with MilliQ water). The gel was then destained for 2 h under natural light in distilled water.

Polysaccharide degradation was also monitored using size exclusion chromatography (SEC) on an Akta Fast Protein Liquid Chromatography apparatus (GE Healthcare Life Sciences®) equipped with a Superdex 200 10/300 column (GE Healthcare 200®, optimum separation range: 1000–100 000 g.mol⁻¹ dextran equivalent) as described by (Lelchat et al. 2015a). Samples were recovered after incubation, then injected with a 200 µL loop, separated with the incubation buffer (Tris-HCl 50 mM, NaCl 0.5 M, MgSO₄ 8 mM, pH 8 at 20) at 0.5 mL.min⁻¹ and detected using UV and refractometry to discriminate signals that could be assigned to proteins or polypeptides, but not sugars.

Low molecular weight degradation (50kDa > x > 5 kDa) products were sampled, purified by centrifugal ultrafiltration (5 kDa cut-off, Amicon Ultra 15, Merckmillipore®) and analyzed by ¹H-NMR according (Lelchat et al. 2015b). About 10 mg of depolymerized L₆ fractions were analyzed by NMR after three exchanges/dehydration cycles in deuterated water (99.9%). EPS were resuspended in 700 µL D₂O. Spectra were recorded at room temperature in the Laboratory of Nuclear Magnetic Resonance

Spectroscopy (University of Western Brittany) on a 500 MHz Bruker® spectrometer.

Enzyme kinetics

The depolymerization kinetics of the EPS L_6 by Carin-1 and Carin-5 was monitored during a 32 h enzyme kinetic according Lelchat et al. 2015a. Purified bacteriophages (10^{10} PFU.mL⁻¹) were incubated with EPS L_6 (0.4% w/v) in incubation buffer (Tris-HCl 50 mM, NaCl 0.5 M, MgSO₄ 8 mM, pH 8 at 20°C) according the modified incubation conditions optimized previously but with addition of NaN₃ as bacteriostatic (6 mM final concentration). Samples were taken every hour from 1 to 17 h and at 23, 25, 29 and 32 h, flash frozen and stored at -80°C. Blanks containing virus solution alone, *C. marina* protein extract and EPS as well as EPS alone were incubated in the buffer to assess of the absence of auto-hydrolysis phenomenon. Depolymerization kinetics were visualized by 1% agarose gel electrophoresis and SEC through a superdex-200 column (GE Healthcare Life Sciences®) as described above.

Phage adsorption kinetics

The adsorption constant was determined for each phage with the host which was either induced or not induced to produce EPS following a protocol adapted from Hyman and Abedon (2009). A bacterial culture in exponential growth (5.10^8 bacteria.mL⁻¹) was incubated with each strain of bacteriophages at a multiplicity of infection of 1 in a total volume of 40 mL. Samples (1 mL) were taken every 3 min for 30 min, and immediately centrifuged (7000 rpm, 1 min, 20°C). A supernatant aliquot containing the non-adsorbed phages was fixed with glutaraldehyde (final concentration 0.5%) at 4°C for 15 min and flash-frozen in liquid N₂. Non-adsorbed viruses were enumerated by flow cytometry (FACs CANTO II, Beckton Dickinson®) upon SYBR-Green I staining (10 min, 80°C) according to Brussaard (2004). Blanks containing only viruses or bacteria were taken in parallel and processed as described above. Cytograms were analyzed using Flowing Software®. Adsorption coefficients (Cd) were calculated by dividing the regression coefficient of the natural logarithm of the curve describing the variation of viral abundance in 30 min ($x_{[virus]}$) by the average bacterial abundance [Bacterial abundance]: $Cd = -x_{[virus]} / [Bacterial\ abundance]$.

RESULTS AND DISCUSSION

In this study, five phages lytic to the EPS-producing bacteria *C. marina* DSMZ 4741 were isolated (referred to as Carin-1 to 5) from the coastal Atlantic Ocean. The transmission electronic microscopy examination of clonal suspensions of these phages indicated that they belong to the *Caudovirales* order characterized by an icosahedral head connected to a tail of variable morphology (Fig. 1). The strain Carin-1 exhibited a short tail and it was assigned to the *Podoviridae* family (head: $59_{+/-3.2}$ nm, tail: $12.8_{+/-2.1}$ nm) Carin-2 (head: $113.1_{+/-15.4}$ nm, tail: $137.5_{+/-18.9}$ nm), Carin-4 (head: $80_{+/-3.4}$ nm, tail: $140.3_{+/-6.8}$ nm) and Carin-5 (head: $69.7_{+/-3}$ nm, tail: $130.7_{+/-11.3}$ nm) displayed a contractile tail characteristic of the *Myoviridae* family while Carin-3 (head: $59_{+/-3.2}$ nm, tail: $12.8_{+/-2.1}$ nm) belonged to the *Siphoviridae* family with a long and non-contractile tail.

Evidence of marine phage mediated EPS depolymerization

Two phenotypic assays, that are typically used to screen for phages with biofilm disrupting abilities (Bessler et al. 1975), suggested that Carin phages possessed polysaccharidases active on their host EPS. All five phage isolates maintained their ability to form plaques on *C. marina* lawn even when the host EPS production was over stimulated using a glucose-enriched culture medium for growth. Furthermore, the five Carin phages formed clear plaques surrounded by an opaque halo zone that expanded in diameter over time (Fig. S1, Supporting Information). The increasing halo diameter is usually generated by phage-associated enzymes that depolymerize bacterial EPS as the phages spread out of the lysis zone by diffusion (Miyake et al. 1997; Glonti, Chanishvili and Taylor 2010; Cornelissen et al. 2011, 2012; Pleneteva et al. 2011).

To confirm the hypothesis that Carin phages possess EPS polysaccharidases, we incubated *Cobetia marina* EPS L_6 (hereafter L_6) with and without viruses and visualized the depolymerization pattern on agarose gel electrophoresis. The migration of native L_6 showed 2 EPS populations with distinct molecular weight (Fig. 2). The incubation of L_6 with purified Carin particles resulted in 3 degradation patterns. Carin-1 and -5 induced a nearly complete depolymerization of L_6 into products of low molecular weight. Carin-2 and -4 induced a partial degradation of the L_6 , while Carin-3 did not seem to affect L_6 migration profile. The ¹H-NMR analysis of degradation products (50kDa > x > 5 kDa) showed no difference of spectra compared to native L_6 (Fig. 3, Lelchat et al. 2015b), indicating that Carin-1 and 5 generated L_6 oligosaccharides.

Detailed degradation kinetics as monitored by agarose gel electrophoresis (Fig. 4) and SEC (Fig. S2, Supporting Information) suggest that even Carin-1 and Carin-5, which generated a nearly complete L_6 degradation, possess different polysaccharidases. The kinetics show that both phages produced the formation of intermediate molecular weight species during the course of L_6 depolymerization, which suggests that they possess endo-active polysaccharidases as consistently reported in literature for phages of biomedical interest (Sutherland 1995; 1999). Endo-active enzymes can cleave glycosidic bonds all along the polysaccharidic chain and thereby enable phages to efficiently penetrate the EPS layer, even for polymers of high viscosity (Sutherland 1999). Despite similar endo-active modes of action, comparison of the migration (or elution) profiles suggest that Carin-1 and -5 polysaccharidases act differently on L_6 . Carin-1 was able to significantly depolymerize the population of HMW species within 2 h while products of high molecular weight were still apparent for Carin-5 after this incubation period. Depolymerization occurs within a few hours and seems to start very fast even when the incubation mixture is still at 4°C. At T0, the high molecular weight spot is fainter for incubation with Carin-1 compared to incubation with Carin-5 and despite a similar EPS initial concentration and all the possible precautions during the kinetic preparation. It is likely that Carin-1 can degrade the EPS of its host at cold temperature in order to adsorb and initiate its lytic cycle. *C. marina* DSMZ 4741 can indeed grow at 5°C (Yumoto et al. 2004).

The results described above provide unequivocal evidence that marine phages can have the ability to degrade dissolved polysaccharides derived from their marine host. Because these assays were conducted with purified phage suspension, free of contaminants from the viral lysate, it is very likely that the

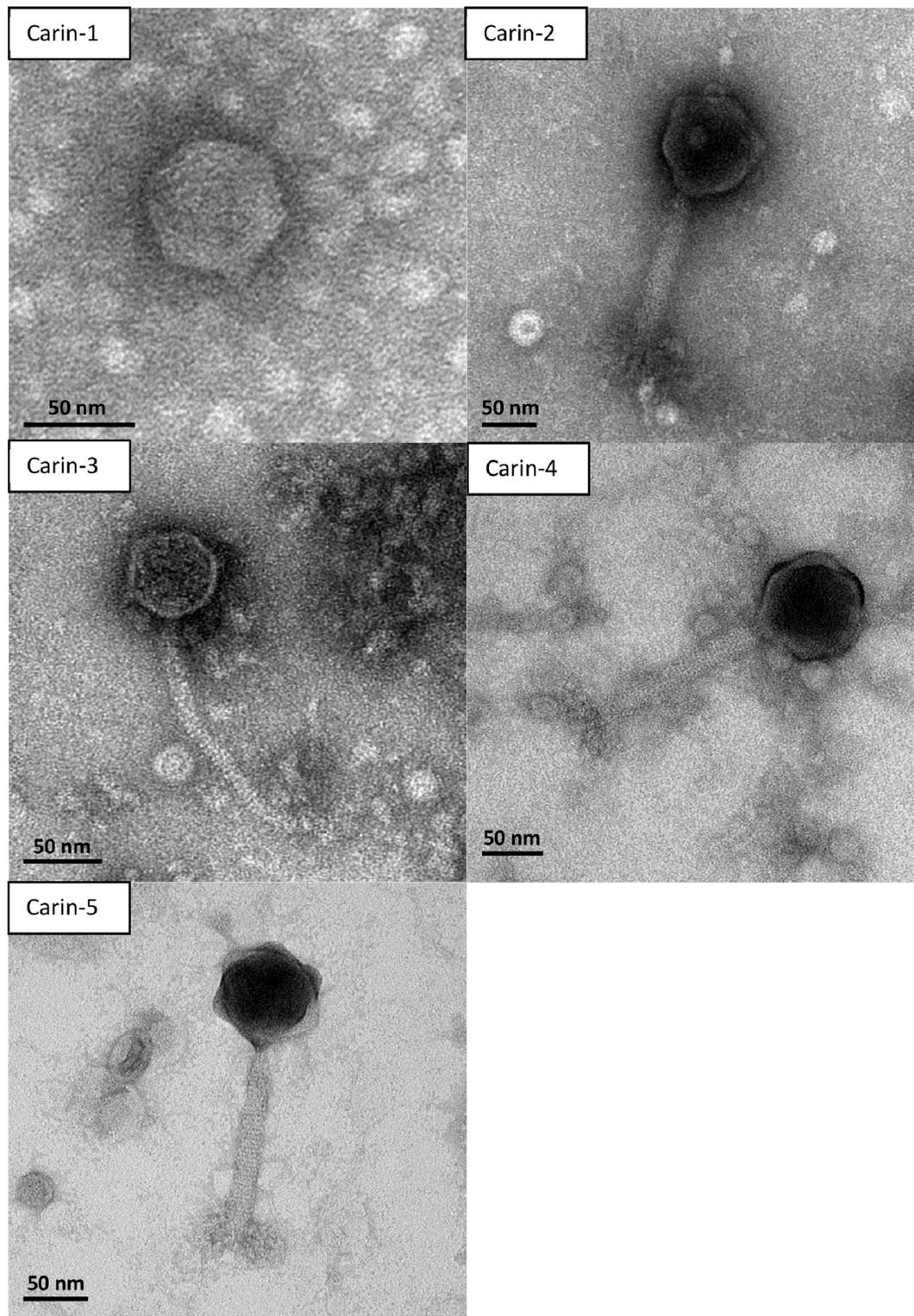


Figure 1. Transmission electron micrographs of the five bacteriophages that infect the marine bacterium *Cobetia marina* DSMZ 4741 (Carin phages).

polysaccharidases are bound to the virus particle. In the literature, the presence of structural polysaccharidases have often been described in *Podoviridae* (Miyake et al. 1997; Linnerborg et al. 2001; Jakobsson et al. 2007; Leiman et al. 2007; Shaburova et al. 2009; Glonti, Chanishvili and Taylor 2010; Pleneteva et al. 2011; Cornelissen et al. 2012; Roach et al. 2013) and *Myoviridae*

(Elsässer-Beile and Stirm 1981; Kwiatkowski et al. 1983; Nimnich et al. 1992; Shaburova et al. 2009; Cornelissen et al. 2011) that infect pathogenic bacteria but are also reported in *Siphoviridae* (Niemann et al. 1976, 1977; Rieger-Hug and Stirm 1981; Nimnich et al. 1992; Smith, Zamze and Hignett 1994; Miyake et al. 1997; Gutierrez et al. 2010, 2012; Chertkov et al. 2011; Roach et al.

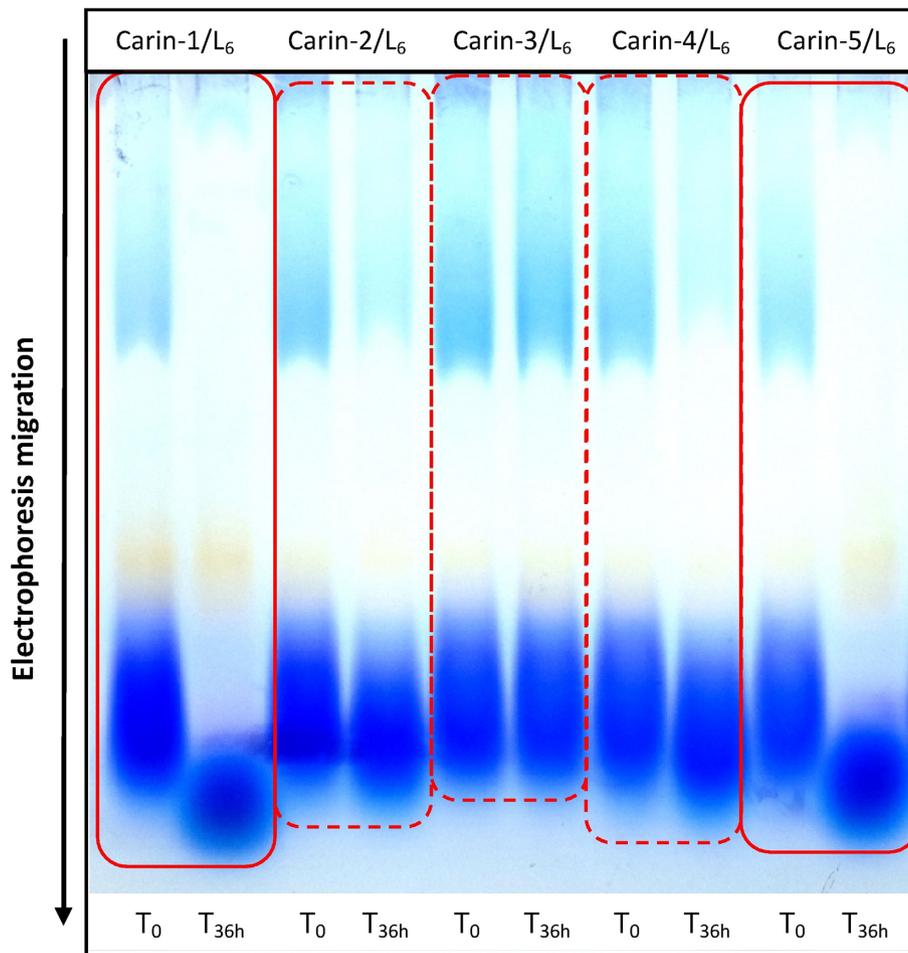


Figure 2. Migration pattern of *Cobetia marina* L₆ EPS (0.4 g.L⁻¹) with and without purified Carin phage suspensions (10⁹ PFU) as assessed by agarose gel electrophoresis after Stains all coloration (blue smear: polysaccharides, yellow smear: proteins). This assay indicate that Carin-1, -2, -4 and -5 were able to depolymerize, *C. marina* EPS into products of low molecular weight.

2013). Nevertheless, we cannot rule out that virion-free polysaccharidases are excreted during the course of Carin-3 infection cycle as suggested by the formation of plaques surrounded by halo zones. It is also possible that Carin-3 uses another type of enzyme, such as lysine murein hydrolase, which degrades the protein moieties of bacterial biofilm as reported previously (Fischetti 2008). The different degradation patterns observed for Carin phages suggest that their polysaccharidases are functionally diverse. This diversity does not appear to be related to the classification of the selected isolates. Indeed, Carin-2, -4 and -5 are all members of the *Myoviridae* family but display different L₆ degradation patterns. A detailed molecular and biochemical characterization would certainly help understanding whether this functional variability arises from the activity of different types of polysaccharidases, from a variable number of polysaccharidase copies or from a combination of different enzymes bound to phage particle as reported for the coliphage K1-5 (Leiman et al. 2007).

Implications of EPS polysaccharidase activity for phage ecology

In the literature, the characterization of phage-associated polysaccharidases is mostly dedicated to the discovery of novel enzymes active on the biofilm or polysaccharidic capsule formed

by pathogenic bacteria (Pires et al. 2016). By contrast, very few studies have investigated how EPS polysaccharidases influence phage biological traits and to a larger extent their ecology. The finding that four out of five of the Carin phages we isolated possessed EPS polysaccharidases suggests that this property may provide a competitive advantage in the natural environment.

Because the assumed primary role of phage-associated polysaccharidases is to degrade their host capsular/biofilm EPS in order to attain membrane receptors (Bayer, Thurow and Bayer 1979), it is reasonable to expect that the presence of these enzymes may affect the adsorption kinetics of Carin phages, particularly upon intense host EPS excretion. To address this question, we determined the adsorption constant (Cd) of Carin isolates (data missing for Carin-4) in culture conditions mimicking normal and stimulated EPS production (Table 1). The phage Cd in control conditions varied between 5.08×10^{-8} to 3.51×10^{-10} mL.min⁻¹ for Carin-2 and Carin-1, respectively, which ranged within the values reported in the literature (Fujimura and Kaesberg 1962; Olkkonen and Bamford 1989; Murray and Jackson 1992; Moldovan, Chapman-McQuiston and Wu 2007; Storms et al. 2010; Gallet, Kannoly and Wang 2011). The observed Cd variation between phage isolates may arise from differences in viral particle diameter, morphology, viral electric charge but

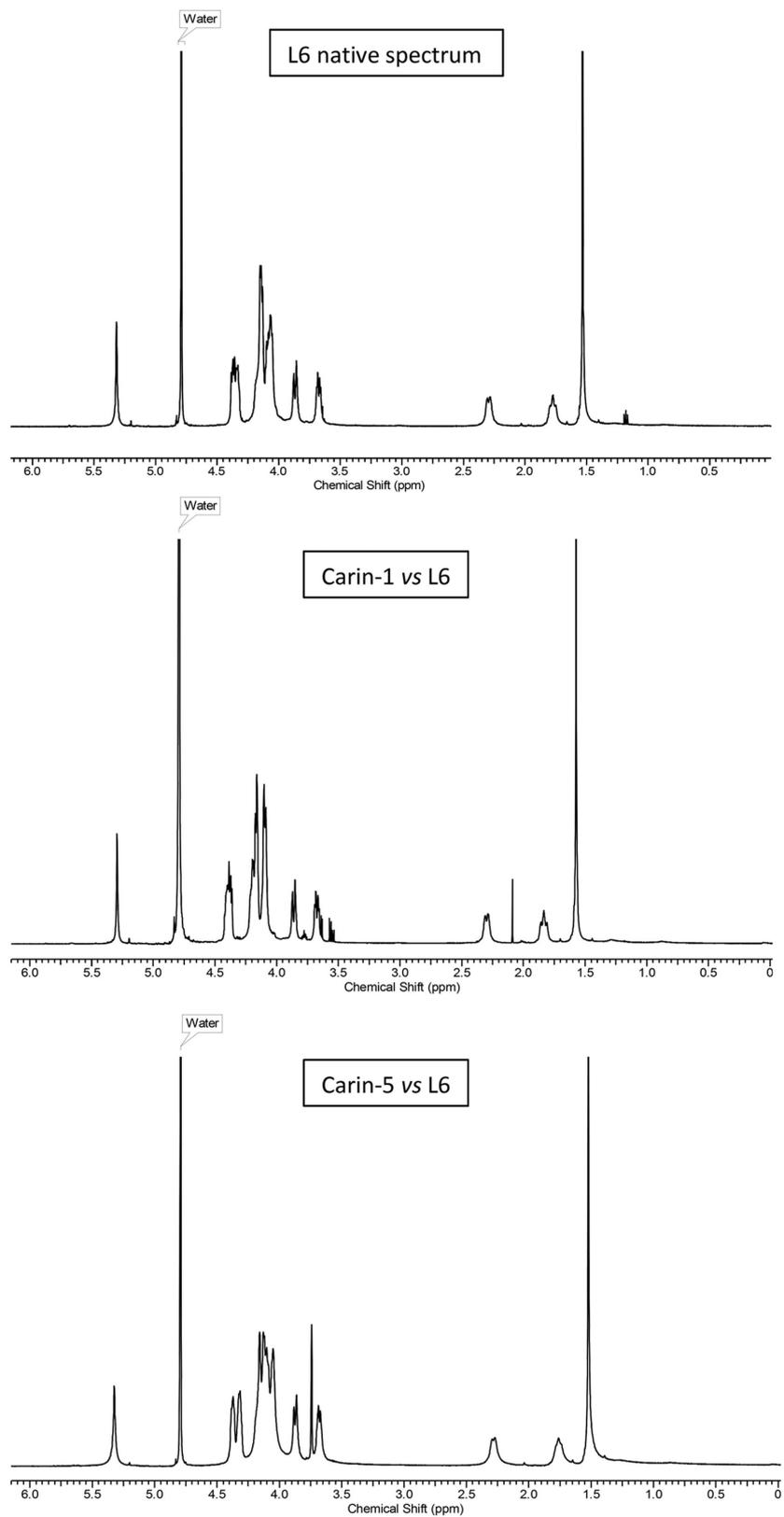


Figure 3. $^1\text{H-NMR}$ spectra of L_6 degradation products after Carin-1 and Carin-5 mediated depolymerization.

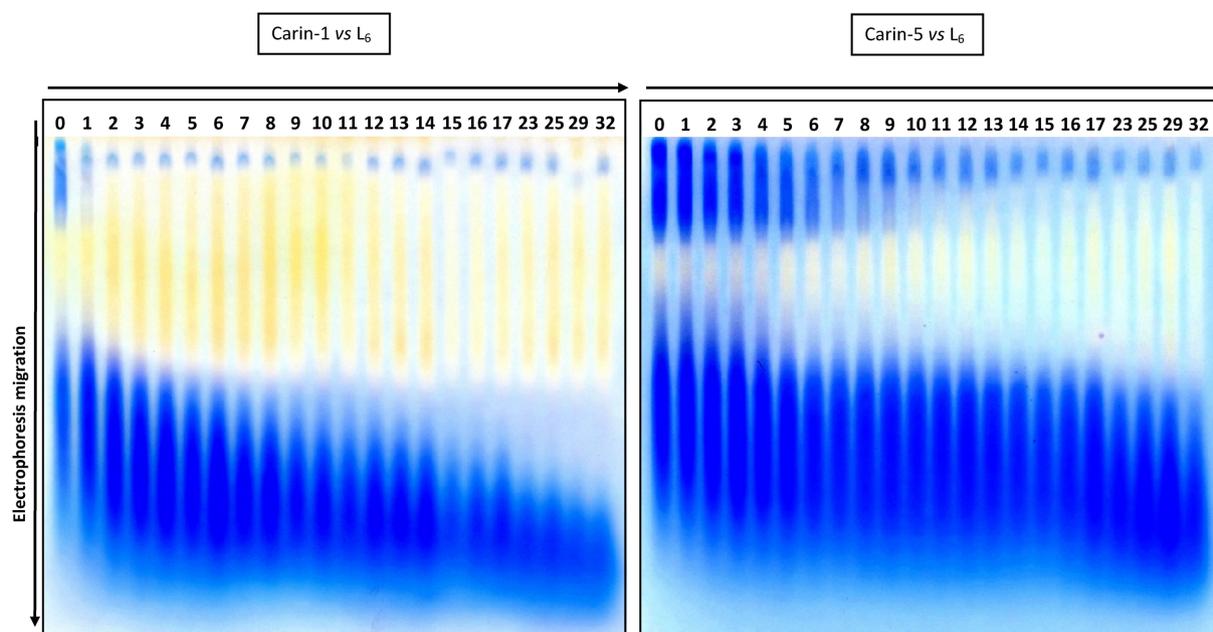


Figure 4. Degradation kinetics of the L_6 EPS (0.4 g.L^{-1}) by Carin-5 and Carin-1 (10^9 PFU) as assessed by agarose gel electrophoresis (Stains all coloration, blue smear: polysaccharides, yellow smear: proteins). The migration profiles suggest that both phages induced the formation of intermediate molecular weight species during the course of L_6 depolymerization, yet, at different velocity.

Table 1. Adsorption coefficient of Carin-1, 2,3 and 5 under control and EPS induced host growing conditions.

Bacteriophage	Adsorption coefficient Cd (ml.min^{-1})		
	EPS synthesis non-induced	EPS synthesis induced	Ratio $\text{Cd}_{\text{non-ind}}/\text{Cd}_{\text{ind}}$
Carin-1	$3,51.10^{-10}$	$7,78.10^{-11}$	4,51
Carin-2	$5,08.10^{-8}$	$3,32.10^{-9}$	15,30
Carin-3	$1,75.10^{-8}$	$3,32.10^{-11}$	527
Carin-4	nd	nd	nd
Carin-5	$1,56.10^{-9}$	$2,07.10^{-10}$	7,53

also the number of viral receptors. Nonetheless, the stimulation of host EPS synthesis decreased Cd values (Table 1) regardless of the viral isolate, suggesting that intensive EPS production acts as a physical barrier for phage adsorption. Interestingly, the amplitude of the changes in Cd varied depending on the ability of Carin phages to degrade *C. marina* EPS. While the Cd value of Carin-1, -5 and -2, which encode EPS polysaccharidase, were increased upon induction of EPS synthesis 5, 7, and 15-fold respectively, that of the phage Carin-3 was drastically augmented with a calculated 527-fold increase. The resulting Cd values show that phages that display polysaccharidase activity generally adsorb faster on their host under conditions mimicking intense EPS production. In nature, the amount and the chemical composition of EPS excreted by bacteria can be influenced by environmental parameters such as temperature, nutrient, pH, but also by the bacterial lifestyle (planktonic versus biofilm) (Sutherland 1972; Kumar, Mody and Jha 2007). Under such conditions, the presence of polysaccharidases may provide a competitive advantage to efficiently propagate on their hosts. Likewise, the isolation of both EPS-polysaccharidase-encoding and EPS-polysaccharidase-lacking phages from the same geographical area could also reflect the dynamics or the variability of EPS production by *Cobetia marina* under natural settings.

Besides influencing phage adsorption kinetics, the presence and the diversity of polysaccharidase may influence host specificities as reported by Leiman et al. (2007). This previous study showed that the acquisition of several polysaccharidases by a given phage broaden its host range. In the light of this finding, we determined the host specificities of Carin phages using three *C. marina* strains and six strains of the genetically related genus *Halomonas* (Table S1, Supporting Information). This assay indicates that the five Carin phages have a narrow host range. In addition to *C. marina* DSMZ 4741, the phages Carin-1, 2, 3 and 4 could infect *C. marina* LMG 6798 whereas Carin-5 could not replicate on any alternate host. Hence, we could not relate the divergent EPS degrading abilities of Carin phages with their specificity pattern. The host spectrum assay was however conducted on a limited number of strains.

CONCLUDING REMARKS

Viruses are undoubtedly the most abundant biological entities in the ocean (Suttle 2007). So far, their role has mainly been attributed to the mortality and the subsequent cell lysis that they impose on their host. Viral lysis mediates the release of up to 150 Gt C per year, and as such, viruses are responsible for one of the main fluxes of DOM in the ocean (Suttle 2005). Although the ecological and biogeochemical implications

of marine viruses are acknowledged internationally, the regulation of viral activities are still poorly understood. Our study provides evidence that viruses of marine bacteria can display structural polysaccharidases that likely initiate the infection cycle. In the model system *C. marina*-specific viruses, these enzymes were detected in four out of five virus isolates, they appeared to be constitutive, endo-acting and functionally diverse. The detailed biochemical characterization of these molecules, that is the spectrum of substrates they can degrade, their regulation by environmental factors (i.e. temperature, pH, pressure, salinity), their life-time, or their structure, should provide novel insights into the functional mechanisms and the ecology of marine viruses.

More importantly, polysaccharidases are widely distributed in the ocean and they play an important role in biogeochemical cycles, digesting much of the marine DOM (Arnosti 2011). To date, it is broadly assumed that these enzymes mostly derive from bacteria. The finding that tailed marine phages, that are widespread and abundant in the ocean, can display constitutive polysaccharidases active on bacterial EPS lead us to question whether viruses could also contribute to the degradation of marine DOM, and, therefore, the marine carbon cycling in the ocean. Most marine bacteria indeed produce EPS, which potentially could fuel the pool of DOM in the ocean (Carlson and Hansell 2014). Yet, the fate of these compounds remains largely unknown (Zhang et al. 2015). Complex bacterial EPS are thought to be rather recalcitrant to microbial degradation suggesting that they have long resident time in the ocean (Jiao et al. 2010). The depolymerization of bacterial EPS by viral polysaccharidases could provide an unexpected process affecting size distribution and the recycling of these compounds even at cold temperature. Thus, viral polysaccharidases, according to their mode of action and polysaccharide specificities, could be unexpected additional players in the size-reactivity continuum model in DOM biogeochemistry (Benner and Amon 2015). Bacterial EPS, usually negatively charged, are also involved in the complexation of trace metals such as iron, which limits primary production in more than 40% of the ocean (Martin, Fitzwater and Gordon 1990; Falkowski et al. 1998; Boyd et al. 2000; Boyd and Ellwood 2010; Moore et al. 2013). Besides the possible direct impact on DOM recycling, virally mediated degradation could also modify the bioavailability of EPS-bound trace metals. Considering the numerical dominance of viruses in the ocean, future studies should focus on the extent of virally mediated EPS degradation in the sea, and the bioavailability of the degradation products. Over the past decades, the bacteria *C. marina* has been used as a model for studies on EPS production and biofilm remediation (Maréchal et al. 2004; Ivanova et al. 2005; Mieszkin et al. 2012; Salaün et al. 2012). Given the structural singularity of its EPS (Lelchat et al. 2015b) and the recent finding that members of the genus *Cobetia* may actively contribute to the carbon export in the global ocean (Guidi et al. 2016), this bacterium and its associated phages emerge as a model of considerable interest for such biogeochemical studies.

SUPPLEMENTARY DATA

Supplementary data are available at [FEMSEC](https://www.femsec.org/) online.

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REFERENCES

- Ackermann HW. 5500 Phages examined in the electron microscope. *Arch Virol* 2007;**152**:227–43.
- Ackermann HW. Basic phage electron microscopy. In: *Bacteriophages*. New York City: Humana Press, 2009, 113–26.
- Arahal DR, Castillo AM, Ludwig W et al. Proposal of *Cobetia marina* gen. nov., comb. nov., within the family Halomonadaceae, to include the species *Halomonas marina*. *Syst Appl Microbiol* 2002;**25**:207–11.
- Arnosti C. Microbial extracellular enzymes and the marine carbon cycle. *Ann Rev Mar Sci* 2011;**3**:401–25.
- Arntzen MØ, Várnai A, Mackie RI et al. Outer membrane vesicles from *Fibrobacter succinogenes* S85 contain an array of carbohydrate-active enzymes with versatile polysaccharide-degrading capacity. *Environ Microbiol* 2017;**19**:2701–14.
- Bachrach U, Friedmann A. Practical procedures for the purification of bacterial viruses. *Appl Environ Microbiol*. 1971;**22**:706–15.
- Baumann L, Bowditch RD, Baumann P. Description of *Deleya* gen. nov. created to accommodate the marine species *Alcaligenes aestus*, *A. pacificus*, *A. cupidus*, *A. venustus*, and *Pseudomonas marina*. *Int J Syst Evol Microbiol* 1983;**33**:793–802.
- Bayer ME, Thurow H, Bayer MH et al. Penetration of the polysaccharide capsule of *Escherichia coli* (Bi161/42) by bacteriophage K29. *Virology* 1979;**94**:95–118.
- Benner R, Amon RMW. The Size-Reactivity continuum of major bioelements in the ocean. *Ann Rev Mar Sci* 2015;**7**:185–205.
- Bessler W, Fehmel F, Freund-Mölbert E et al. *Escherichia coli* capsule bacteriophages. IV. Free capsule depolymerase 29. *J Virol* 1975;**15**:976–84.
- Biersmith A, Benner R. Carbohydrates in phytoplankton and freshly produced dissolved organic matter. *Mar Chem* 1998;**63**:131–44.
- Boyd PW, Ellwood MJ. The biogeochemical cycle of iron in the ocean. *Nat Geosci* 2010;**3**:675–82.
- Boyd PW, Watson A, Law C et al. A mesoscale phytoplankton bloom in the polar Southern Ocean stimulated by iron fertilization. *Nature* 2000;**407**:6805:695–702.
- Brussaard CPD. Optimization of Procedures for Counting Viruses by Flow Cytometry. *Appl Environ Microbiol* 2004;**70**:1506–13.
- Carlson CA, Hansell DA. DOM sources, sinks, reactivity, and budgets. In: *Biogeochemistry of Marine Dissolved Organic Matter: Second Edition*. London: Elsevier Inc., 2014.
- Casillo A, Lanzetta R, Parrilli M et al. Exopolysaccharides from marine and marine extremophilic bacteria: structures, properties, ecological roles and applications. *Marine drugs* 2018;**16**:69.

- Chertkov OV, Chuprov-Netochin RN, Legotskiy SV et al. Properties of the peptidoglycan-degrading enzyme of the *Pseudomonas aeruginosa* ϕ PMG1 bacteriophage. *Russ J Bioorg Chem* 2011;**37**:732–8.
- Cornelissen A, Ceyssens P-J, Krylov VN et al. Identification of EPS-degrading activity within the tail spikes of the novel *Pseudomonas putida* phage AF. *Virology* 2012;**434**:251–6.
- Cornelissen A, Ceyssens P-J, T'Syen J et al. The T7-Related *Pseudomonas putida* phage ϕ 15 displays Virion-Associated biofilm degradation properties. *PLoS One* 2011;**6**:e18597
- Davies G, Henrissat B. Structures and mechanisms of glycosyl hydrolases. *Structure* 1995 **3**:853–9.
- Elsässer-Beile U, Stirm S. Substrate specificity of the glycanase activity associated with particles of *Klebsiella* bacteriophage no. 6. *Carbohydr Res* 1981;**88**:315–22.
- Falkowski PG, Barber RT, Smetacek V. Biogeochemical controls and feedbacks on ocean primary production. *Science* 1998;**281**:200–6.
- Fischetti VA. Bacteriophage lysins as effective antibacterials. *Curr Opin Microbiol* 2008;**11**:393–400.
- Fuhrman JA. Marine viruses and their biogeochemical and ecological effects. *Nature* 1999;**399**:541–8.
- Fujimura R, Kaesberg P. The adsorption of bacteriophage ϕ X174 to its host. *Biophys J* 1962;**2**:433–49.
- Gallet R, Kannoly S, Wang I-N. Effects of bacteriophage traits on plaque formation. *BMC Microbiol* 2011;**11**:181.
- Glonti T, Chanishvili N, Taylor PW. Bacteriophage-derived enzyme that depolymerizes the alginic acid capsule associated with cystic fibrosis isolates of *Pseudomonas aeruginosa*. *J Appl Microbiol* 2010;**108**:695–702.
- Guidi L, Chaffron S, Bittner L et al. Plankton networks driving carbon export in the oligotrophic ocean. *Nature* 2016;**532**:465–70.
- Gutiérrez D, Martínez B, Rodríguez A et al. Genomic characterization of two *Staphylococcus epidermidis* bacteriophages with anti-biofilm potential. *BMC Genomics* 2012;**13**:228.
- Gutiérrez D, Martínez B, Rodríguez A et al. Isolation and characterization of bacteriophages infecting *Staphylococcus epidermidis*. *Curr Microbiol* 2010;**61**:601–8.
- Heissenberger A, Leppard GG, Herndl GJ. Relationship between the intracellular integrity and the morphology of the capsular envelope in attached and free-living marine bacteria. *Appl Environ Microbiol* 1996;**62**:4521–8.
- Henrissat B. A classification of glycosyl hydrolases based on amino acid sequence similarities. *Biochem J* 1991;**280**:309–16.
- Hyman P, Abedon ST. Practical methods for determining phage growth parameters. In: *Bacteriophages*. New York City: Humana Press, 2009, 175–202.
- Ivanova EP, Christen R, Sawabe T et al. Presence of ecophysiologically diverse populations within *Cobetia marina* strains isolated from marine invertebrate, algae and the environments. *Microbes Environ* 2005;**20**:200–7.
- Jakobsson E, Jokilampi A, Aalto J et al. Identification of amino acid residues at the active site of endosialidase that dissociate the polysialic acid binding and cleaving activities in *Escherichia coli* K1 bacteriophages. *Biochem J* 2007;**405**:465–72.
- Jiao N, Herndl GJ, Hansell DA et al. Microbial production of recalcitrant dissolved organic matter: long-term carbon storage in the global ocean. *Nat Rev Micro* 2010;**8**:593–9.
- Krause-Jensen D, Duarte CM. Substantial role of macroalgae in marine carbon sequestration. *Nature Geosci* 2016;**9**:737–42.
- Kropinski AM, Mazzocco A, Waddell TE et al. Enumeration of bacteriophages by double agar overlay plaque assay. In: *Bacteriophages*. New York City: Humana Press, 2009, 69–76.
- Kumar AS, Mody K, Jha B. Bacterial exopolysaccharides – a perception. *J Basic Microbiol* 2007;**47**:103–17.
- Kwiatkowski B, Boschek B, Thiele H et al. Substrate specificity of two bacteriophage-associated endo-N-acetylneuraminidases. *J Virol* 1983;**45**:367–74.
- Lee HG, Cowman MK. An agarose gel electrophoretic method for analysis of hyaluronan molecular weight distribution. *Anal Biochem* 1994;**219**:278–87.
- Leiman PG, Battisti AJ, Bowman VD et al. The structures of bacteriophages K1E and K1-5 explain processive degradation of polysaccharide capsules and evolution of new host specificities. *J Mol Biol* 2007;**371**:836–49.
- Lelchat F, Cozien J, Costaeuec TL et al. Exopolysaccharide biosynthesis and biodegradation by a marine hydrothermal *Alteromonas* sp. strain. *Appl Microbiol Biotechnol* 2015a;**99**:2637–47.
- Lelchat F, Cérantola S, Brandily C et al. The marine bacteria *Cobetia marina* DSMZ 4741 synthesizes an unexpected K-antigen-like exopolysaccharide. *Carbohydr Polym* 2015b;**124**:347–56.
- Li J, Azam F, Zhang S. Outer membrane vesicles containing signalling molecules and active hydrolytic enzymes released by a coral pathogen *Vibrio shilonii* AK1. *Environ Microbiol* 2016;**18**:3850–66.
- Linnerborg M, Weintraub A, Albert MJ et al. Depolymerization of the capsular polysaccharide from *Vibrio cholerae* O139 by a lyase associated with the bacteriophage JA1. *Carbohydr Res* 2001;**333**:263–9.
- Lombard V, Golaconda Ramulu H, Drula E et al. The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Res* 2014;**42**:D490–5.
- Martin JH, Fitzwater SE, Gordon RM. Iron deficiency limits phytoplankton growth in Antarctic waters. *Global Biogeochem Cycles* 1990;**4**:5–12.
- Maréchal J-P, Culioli G, Hellio C et al. Seasonal variation in antifouling activity of crude extracts of the brown alga *Bifurcaria bifurcata* (Cystoseiraceae) against cyprids of *Balanus amphitrite* and the marine bacteria *Cobetia marina* and *Pseudoalteromonas haloplanktis*. *J Exp Mar Biol Ecol* 2004;**313**:47–62.
- McCarthy MD, Hedges JI, Benner R. The chemical composition of dissolved organic matter in seawater. *Chem Geol* 1993;**107**:503–7.
- Middelboe M, Jørgensen N, Kroer N. Effects of viruses on nutrient turnover and growth efficiency of noninfected marine bacterioplankton. *Appl Environ Microbiol* 1996;**62**:1991–7.
- Mieszkin S, Martin-Tanchereau P, Callow ME et al. Effect of bacterial biofilms formed on fouling-release coatings from natural seawater and *Cobetia marina*, on the adhesion of two marine algae. *Biofouling* 2012;**28**:953–68.
- Miyake K, Muraki T, Hattori K et al. Screening of bacteriophages producing endo-N-acetylneuraminidase. *J Ferment Bioeng* 1997;**84**:90–93.
- Moldovan R, Chapman-McQuiston E, Wu XL. On kinetics of phage adsorption. *Biophys J* 2007;**93**:303–15.
- Moore CM, Mills MM, Arrigo KR et al. Processes and patterns of oceanic nutrient limitation. *Nat Geosci* 2013;**6**:9701–10.
- Murray AG, Jackson GA. Viral dynamics: a model of the effects of size, shape, motion and abundance of single-celled planktonic organisms and other particles. *Mar Ecol Prog Ser* 1992;**89**:103–16.
- Møller EF. Sloppy feeding in marine copepods: prey-size-dependent production of dissolved organic carbon. *J Plankton Res* 2005;**27**:27–35.

- Niemann H, Birch-Andersen A, Kjems E et al. Streptococcal bacteriophage 12/12-Borne hyaluronidase and its characterization as a lyase (ec 4.2.99.1) by means of streptococcal hyaluronic acid and purified bacteriophage suspensions. *Acta Pathol Microbiol Scand B* 1976;**84B**:145–53.
- Niemann H, Kwiatkowski B, Westphal U et al. Klebsiella serotype 25 capsular polysaccharide: primary structure and depolymerization by a bacteriophage-borne glycanase. *J Bacteriol* 1977;**130**:366–74.
- Nimmich W, Krallmann-Wenzel U, Müller B et al. Isolation and characterization of bacteriophages specific for capsular antigens K3, K7, K12, and K13 of *Escherichia coli*. *Zentralblatt für Bakteriologie* 1992;**276**:213–20.
- Olkkonen VM, Bamford DH. Quantitation of the adsorption and penetration stages of bacteriophage ϕ 6 infection. *Virology* 1989;**171**:229–38.
- Panagiotopoulos C, Sempéré R. Analytical methods for the determination of sugars in marine samples: A historical perspective and future directions. *Limnol Oceanogr Methods* 2005;**3**:419–54.
- Pires DP, Oliveira H, Melo LDR et al. Bacteriophage-encoded depolymerases: their diversity and biotechnological applications. *Appl Microbiol Biotechnol* 2016;**100**:2141–51.
- Pleteneva EA, Bourkaltseva MV, Shaburova OV et al. TL, the new bacteriophage of *Pseudomonas aeruginosa* and its application for the search of halo-producing bacteriophages. *Russ J Genet* 2011;**47**:1–5.
- Rieger-Hug D, Stirn S. Comparative study of host capsule depolymerases associated with *Klebsiella* bacteriophages. *Virology* 1981;**113**:363–78.
- Roach DR, Sjaarda DR, Castle AJ et al. Host exopolysaccharide quantity and composition impact *erwinia amylovora* bacteriophage pathogenesis. *Appl Environ Microbiol* 2013;**79**:3249–56.
- Romanenko LA, Tanaka N, Svetashev VI et al. Description of *Cobetia amphilecti* sp. nov., *Cobetia litoralis* sp. nov. and *Cobetia pacifica* sp. nov., classification of *Halomonas halodurans* as a later heterotypic synonym of *Cobetia marina* and emended descriptions of the genus *Cobetia* and *Cobetia marina*. *Int J Syst Evol Microbiol* 2013;**63**:288–97.
- Salaün S, Barre SL, Santos-Goncalvez MD et al. Influence of exudates of the kelp *laminaria digitata* on biofilm formation of associated and exogenous bacterial epiphytes. *Microb Ecol* 2012;**64**:359–69.
- Scholl D, Adhya S, Merrill C. *Escherichia coli* K1's capsule is a barrier to bacteriophage T7. *Appl Environ Microbiol* 2005;**71**:4872–4.
- Shaburova OV, Krylov SV, Veiko VP et al. Search for destruction factors of bacterial biofilms: Comparison of phage properties in a group of *Pseudomonas putida* bacteriophages and specificity of their halo-formation products. *Russ J Genet* 2009;**45**:161–70.
- Smith ARW, Zamze SE, Hignett RC. Morphology and hydrolytic activity of A7, a typing phage of *Pseudomonas syringae* pv. *morsprunorum*. *Microbiology* 1994;**140**:905–13.
- Stoderegger K, Herndl GJ. Production and release of bacterial capsular material and its subsequent utilization by marine bacterioplankton. *Limnol Oceanogr* 1998;**43**:877–84.
- Stoderegger KE, Herndl GJ. Production of exopolymer particles by marine bacterioplankton under contrasting turbulence conditions. *Mar Ecol Prog Ser* 1999;**189**:9–16.
- Stoderegger KE, Herndl GJ. Visualization of the exopolysaccharide bacterial capsule and its distribution in oceanic environments. *Aquat Microb Ecol* 2001;**26**:195–9.
- Storms ZJ, Arsenault E, Sauvageau D et al. Bacteriophage adsorption efficiency and its effect on amplification. *Bioprocess Biosyst Eng* 2010;**33**:823–31.
- Sutherland IW, Hughes KA, Skillman LC et al. The interaction of phage and biofilms. *FEMS Microbiol Lett* 2004;**232**:1–6.
- Sutherland IW. Bacterial exopolysaccharides. *Adv Microb Physiol* 1972;**8**:143–213.
- Sutherland IW. Polysaccharases for microbial exopolysaccharides. *Carbohydr Polym* 1999;**38**:319–28.
- Sutherland IW. Polysaccharide lyases. *FEMS Microbiol Rev* 1995;**16**:323–47.
- Suttle CA. Marine viruses – major players in the global ecosystem. *Nat Rev Microbiol* 2007;**5**:801–12.
- Suttle CA. Viruses in the sea. *Nature* 2005;**437**:356–61.
- Swanstrom M, Adams MH. Agar layer method for production of high titer phage stocks. *Proc Soc Exp Biol Med* 1951;**78**:372–5.
- Volpi N, Maccari F. Detection of submicrogram quantities of glycosaminoglycans on agarose gels by sequential staining with toluidine blue and Stains-All. *Electrophoresis* 2002;**23**:4060–6.
- Yele AB, Thawal ND, Sahu PK et al. Novel lytic bacteriophage AB7-IBB1 of *Acinetobacter baumannii*: isolation, characterization and its effect on biofilm. *Arch Virol* 2012;**157**:1441–50.
- Yumoto I, Hirota K, Iwata H et al. Temperature and nutrient availability control growth rate and fatty acid composition of facultatively psychrophilic *Cobetia marina* strain L-2. *Arch Microbiol* 2004;**181**:345–51.
- Zhang Z, Chen Y, Wang R et al. The fate of marine bacterial exopolysaccharide in natural marine microbial communities. *PLoS One* 2015;**10**:e0142690.