Deep-sea bacteriophages facilitate their host in utilizing polysaccharide

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Abstract

Viruses that infect bacteria (bacteriophages) are ubiquitous in the oceans and play key roles in marine ecosystems. They impact the microbial community composition and reprogram various metabolisms of host cells by the auxiliary metabolic genes (AMGs). However, whether bacteriophages are able to reprogram host polysaccharide metabolism through relevant AMGs remains unclear. Here, we found polysaccharide could effectively induce the production of different types of bacteriophages in two deep-sea *Lentisphaerae* strains, as well as other members belonging to the PVC (*Planctomycetes-Verrucomicrobia-Chlamydia*) superphylum. Through physiological assay, genomic sequencing and analysis, we found these bacteriophages were released via a chronic style and could assist their host in metabolizing polysaccharide through AMGs. Moreover, bacteriophages induced from *Lentisphaerae* strain WC36 by the supplement of polysaccharide could effectively assist a marine bacterium in metabolizing and utilizing polysaccharide to promote its growth. We also demonstrated for the first time that chronic phages released from *Lentisphaerae* strain WC36 facilitated the enrichment and cultivation of deep-sea difficult-to-culture microbes. The findings presented here, together with recent discoveries made on the reprogramming of host energy-generating metabolisms, shed light on the poorly explored marine virus-host interaction and bring us closer to understanding the potential role of viruses in marine ecosystems.

Introduction

Marine ecosystems always influence the operating conditions for life on earth via the microbial interaction networks[1,2], which are modulated by viruses through impacting their lifespan, gene flow and metabolic outputs[3]. Viruses are the most abundant biological entities in the oceans, which outnumbered their hosts by more than an order of magnitude[4,5]. Marine viruses infect 20-40% of surface ocean microbes, resulting in releasing carbon and other nutrients that impact the food web[6]. Majority of these viruses are bacteriophages whose hosts are bacteria, which widely exist in oceans[7]. Marine bacteriophages are known to play key roles in the ecology and biogeochemistry of marine ecosystems via affecting nutrient cycling, particle sinking rates, biodiversity and transfer of genetic information[8,9]. In addition to core viral genes encoding viral structural proteins[10], bacteriophages also encode various auxiliary metabolic genes (AMGs)[11] that provide the supplemental support to key steps of host metabolism, which is significant to phages and thereby developing a more successful phage-host interaction[12]. The role of AMGs has been well demonstrated with some marine bacteriophages, such as cyanobacterial viruses (cyanophages) both harbor[13] and express[14] core photosynthesis genes that allow host photosynthesis to continue and therefore provide the energy needed by the virus for its replication[15]. Recent studies have reported that the diurnal transcriptional rhythm of cyanophages was closely related to the photosynthetic activity of the host, which might impact cyanophage population-level dynamics in the oceans[16]. Additionally, AMGs of marine bacteriophages were also known to be involved in nitrogen-[9,17], sulfur-[18,19], phosphorus cycling[20], nucleotide metabolism[21-23], methane oxidation[24] and almost all central carbon metabolisms of host cells[25]. Large-scale metagenomic surveys of the ocean have led to the discovery of AMGs encompassing a wide diversity of metabolic functions[26,27], which might shed more light on bacteriophage-host synergies.

Bacteriophages are highly multifarious in shape (spherical, tailed, non-tailed, filamentous and fusiform), genetic material type (double-stranded or single-stranded

DNA or RNA) and gene content[5,28,29]. Currently, life cycles of bacteriophages were demonstrated to include lytic, lysogenic and chronic styles[30]. Among them, lytic cycle mainly performed by tailed phages is the most well studied so far[30]. Tailed phages contain dsDNA genome and use the tail for penetrating the bacterial cell surface and viral DNA injection, finally accomplish the lytic infection by holin-lysin-induced lysis of the host[31-33]. In contrast, the chronic life cycle with little studies is generally non-bactericidal phage infection[34], and chronic phages are released from host cells via extrusion or budding without killing the host[35-37]. This process occurred in phages lacking genes encoding tail proteins, which primarily identified to date belong to the family *Inoviridae* composed of filamentous ssDNA phage species[30,31,38]. Moreover, according to a latest study, the abundance of inoviruses in ecosystem may have been underestimated, and their diversity is prominent given that they have been associated with hosts across the domains Archaea and Bacteria ranging from aquatic to terrestrial[39]. In addition, tailless *Siphoviridae* phages enclosed in lipid membrane are also released from hosts by chronic life cycles[40]. These findings prompted that plenty of unexcavated phages in the biosphere may be produced through chronic life cycles, but whether these cycles are associated with specific environments or ecological conditions remains depth exploration[30].

Deep sea harbor abundant and undiscovered viruses, which potentially control the metabolism of microbial hosts and influence the biogeochemical cycling[41]. However, the vast majority of deep-sea bacteria could not be cultivated, thereby causing most bacteriophages are unknown. Thus, it is of great significance to further excavate unknown phages in the deep sea and explore their relationship with microbial hosts and even marine ecosystems. Here, we reported for the first time that polysaccharide could induce *Lentisphaerae* bacteria to release chronic bacteriophages. We confirmed these induced bacteriophages could reprogram the host metabolism in degrading polysaccharide and thereby promoting host growth. We also cultivated several deep-sea difficult-to-culture bacteria by the assistance of these induced bacteriophages in the presence of polysaccharide.

Results and Discussion

Polysaccharide promotes the growth of deep-sea *Lentisphaerae* strain WC36 and stimulates the expression of phage-associated genes. As a primary carbon source, polysaccharide is a ubiquitous energy source for microorganisms in both terrestrial and marine ecosystems[42]. In our previous work, we successfully isolated a novel *Bacteroidetes* species through a polysaccharide degradation-driven strategy from the deep-sea cold seep[42]. Of note, using the same approach and deep-sea sample replicates, we also cultured a bacterial strain WC36 that was identified as a member of the phylum *Lentisphaerae* by the 16S rRNA gene sequence-based phylogenetic analysis (Fig. 1a). As expected, the growth assays showed that two kinds of polysaccharides including laminarin and starch could significantly promote the growth of strain WC36 (Fig. 1b). In particular, the supplement of 10 g/L laminarin and 10 g/L starch in the 2216E medium respectively resulted in ~7-fold and ~4-fold growth promotion compared to that cultivated in the 2216E medium (Fig. 1b).

To explore the reasons of significant growth promotion by the supplement of polysaccharide, we performed the transcriptomic analysis of strain WC36 that cultured in the 2216E medium supplemented without or with 10 g/L laminarin for respective 5 days and 10 days. In addition to the up-regulation of genes related to glycan transport and degradation, the most up-regulated genes were found to be phage-associated (e. g. phage integrase, phage portal protein) when 10 g/L laminarin was added in the 2216E medium (Fig. 1c). Similarly, the expressions of these phage-associated genes were also evidently up-regulated (ranging from 4 to 40 folds) when prolonged the incubation time of strain WC36 in the medium supplemented with 10 g/L laminarin from 5 days to 10 days (Fig. 1d). Consistently, RT-qPCR results confirmed that the expression of some genes encoding phage-associated proteins shown in Figs. 1c and 1d was indeed significantly up-regulated (Fig. S1). We thus speculate that the metabolism of polysaccharide in strain WC36 might be closely connected with phage production.

Polysaccharide induces the production of chronic bacteriophages in

***Lentisphaerae* strain WC36.** To test our hypothesis, we cultured strain WC36 in the 2216E medium supplemented without/with laminarin or starch and performed the isolation of bacteriophages from the supernatant of cells suspension of strain WC36 according to the reported method[43]. Based on the growth curve of strain WC36, we found that the strictly anaerobic bacterium WC36 grew much slower than normal aerobic bacteria. Therefore, different with the typical sampling time (24 h) for bacteriophages isolation from other bacteria[44,45], we selected a longer sampling time (10 days) to extract bacteriophages. TEM observation showed that many different shapes of phages-like structures indeed existed in the supernatant of cells suspension of strain WC36 that cultured in the 2216E medium supplemented with laminarin (Fig. 2a, panels II-IV) or starch (Fig. 2a, panels V-VIII). In contrast, we did not observe any phages-like structures in the supernatant of cells suspension of strain WC36 that cultured in the 2216E medium (Fig. 2a, panel I) as well as in different media without culturing bacterial cells (Figs. S2a, 2b). These results suggested that polysaccharide indeed stimulated the production of various bacteriophages from strain WC36. Correspondingly, in the presence of laminarin, different forms of bacteriophages could be observed by TEM, while the filamentous ones were dominant (Fig. 2a). The length of the filamentous phage was about 1 to 8 μm (Fig. 2a, panel II), and the size of the hexagonal phage was less than 100 nm (Fig. 2a, panel IV). In the presence of starch, in addition to filamentous and hexagonal phages (Fig. 2a, panels V, VI and VIII), we also observed a kind of spherical cystovirus-like phage with a diameter of 25-30 nm (Fig. 2a, panel VII), indicating that different polysaccharides could induce the production of different types of phages.

Given that we found the presence of bacteriophages in the cultures of strain WC36, we next sought to explore whether bacteriophages adhering to bacterial cells could be observed. To this end, we checked the morphology of strain WC36 in the absent or present of polysaccharide. As expected, under TEM observation, we could observe many filamentous phage-like structures around the cells of strain WC36 that cultivated in the 2216E medium supplemented with laminarin (Fig. 2b, panel II) and starch (Fig. 2b, panels III-IV). In contrast, we could not find any phage-like structures

around the cells of strain WC36 cultivated in the 2216E medium (Fig. 2b, panel I) as well as in the 2216E medium supplemented with 10 g/L laminarin (Fig. S3a) or 10 g/L starch without culturing bacterial cells (Fig. S3b). Moreover, based on TEM observation of the ultrathin sections of strain WC36 cultured in the medium supplemented with polysaccharide, we even observed filamentous phages that being released from or entering into host cells via a kind of extrusion or budding structure around bacterial cells (Fig. 2c, panels II-IV), suggesting these bacteriophages might be released via a chronic style as those reported previously[30,40].

To further verify the release style of bacteriophages from strain WC36 in the presence of polysaccharide, we prolonged the incubation time of this strain up to 30 days in the medium supplemented with 5 g/L, 10 g/L laminarin. Strain WC36 showed a stable growth status after it entered the stationary phase (Fig. 3a). The bacterial cells could maintain intact cell shapes regardless of the presence of laminarin, indicating their healthy growing state after a longtime incubation (Fig. S4a, panels I-III). Apparently, the replication and release of bacteriophages in strain WC36 did not kill the host cell, consistent well with the key feature of chronic bacteriophages[34]. With the increase of culture time (5, 10, 30 days) and laminarin concentration (5, 10 g/L), the number of released bacteriophages showed an increasing trend (Fig. 3b, panels IV-IX; Fig. S4a, panels IV-VI). Specifically, the average number of filamentous phages extracted from the supernatant of strain WC36 that cultured in 2216E medium supplemented with 10 g/L laminarin for 5, 10, 30 days (around 9.7, 29, 65.3) were evidently higher than those cultured in 2216E medium supplemented with 5 g/L laminarin for 5, 10, 30 days (around 4.3, 13.7, 35.3), respectively (Fig. 3c). In addition, in the presence of laminarin we observed many extrusions or buddings around bacterial cells (Fig. S4b, panels II-III), which are typical cellular structures of host cells used for chronic bacteriophages release[36,46]. In contrast, phage-like structures with extrusions or buddings were not found in cells of strain WC36 cultivated in the 2216E medium (Fig. S4a, panels I-III; Fig. S4b, panel I).

Polysaccharide promotes the growth of deep-sea *Lentisphaerae* strain zth2 and

induces the production of bacteriophages. To explore whether the production of bacteriophages induced by polysaccharide is an individual case, we further checked the growth promotion and bacteriophages induction by polysaccharide in another cultured deep-sea *Lentisphaerae* strain zth2, which was isolated by the same polysaccharide degradation-driven strategy as that used for strain WC36. Consistently, laminarin indeed promoted the growth of strain zth2 (Fig. 4a). Similar as the transcriptomic results of strain WC36 cultured in the medium supplemented without or with laminarin, many genes encoding critical factors associated with the phage life cycle were markedly up-regulated (ranging from 4 to 200 folds) (Figs. 4b, c), suggesting phages might also involve into the utilization of laminarin in strain zth2 as that in strain WC36.

To further confirm the existence of bacteriophages in the supernatant of cells suspension of strain zth2 that cultured in the medium supplemented with polysaccharide, we performed the bacteriophage isolation from the supernatant of strain zth2 cultures and observed through TEM. Indeed, many filamentous-, hexagonal- and spherical phages were clearly observed in the culture of strain zth2 grown in the presence of laminarin (Fig. 4d, panels I-III) and starch (Fig. 4d, panels IV-V), but not in the 2216E medium short of polysaccharide (Fig. 4d, panel VI), suggesting bacteriophages were induced by the supplement of polysaccharide in strain zth2. In addition, transcriptome and RT-qPCR results showed that the genes encoding bacteria type II/III secretion system-related proteins were evidently up-regulated in strains WC36 (Figs. S5a, 5b) and zth2 (Figs. S5c, 5d) that cultured in the medium supplemented with laminarin. It has been reported that filamentous phages were shown to share the host cell secretion systems for their own travel out of the bacterial cell[47,48]. Therefore, the filamentous phages induced from strains WC36 and zth2 might also get in and out of host cells by using bacterial type II/III secretion system. Taken together, we concluded that laminarin could effectively promote the growth of deep-sea *Lentisphaerae* strain WC36 and induce the production of chronic bacteriophages which kept the host cells viable[30]. Different with previous reports that bacteriophages were often induced under traditional conditions such as low

temperatures[49], mitomycin C[50], ultraviolet irradiation[51], this is the first report about bacteriophages induced from host cells by polysaccharide.

Given that polysaccharide is a kind of critical energy source for most microorganisms, we sought to ask whether polysaccharide also induces the production of bacteriophages in other deep-sea bacteria. To this end, we cultured deep-sea representatives from other six other phyla (including *Verrucomicrobia*, *Planctomycetes*, *Chloroflexi*, *Tenericutes*, *Proteobacteria*, *Actinobacteria*) in the medium supplemented with laminarin, and checked the supernatant of cells suspension through TEM as described above. The results showed that massive spherical cystovirus-like phages (100-150 nm in diameter) could be observed in supernatant concentrates of *Verrucomicrobia* and *Planctomycetes* strains (Figs. S6a, 6b), but none obvious phage-like structures were observed in other bacteria (Fig. S6c). Of note, the phyla *Lentisphaerae*, *Verrucomicrobia* and *Planctomycetes* belonged to the PVC (*Planctomycetes*-*Verrucomicrobia*-*Chlamydia*) superphylum[52], and many members of this superphylum were reported to be capable of degrading and consuming polysaccharide[53-55]. Combining our results presented in this study, we speculate polysaccharide could induce the production of phages in many PVC bacteria and phages in turn facilitate the metabolism of polysaccharide, which thereby promoting the host growth. Cell lysis was not observed among the isolated PVC strains in this study, suggesting that the identified phages may utilize a chronic infection cycle. We further speculate that chronic phage production induced by polysaccharide may be a common theme among other PVC lineages, however, further broad-scale examination of these lineages is required to be conclusive. As the oceans are estimated to capture half of human-caused carbon emissions[56], viruses are proposed to be as central players of the ocean “biological pump”[57]. Given that polysaccharide is a type of critical carbon source in the oceans, we thus propose that bacteriophages associated polysaccharide metabolism may be prevalent in PVC bacteria that widely distribute in the oceans[58-60]. In this sense, ecologists should reconsider the key functions of bacteriophages in the carbon cycling of the whole marine ecosystem.

Bacteriophages reprogram the polysaccharide metabolism of *Lentisphaerae* strains WC36 and zth2. In the ecosystem, viral infections indirectly influence microbial metabolic fluxes, metabolic reprogramming, and energy homeostasis of host cells[61]. For example, cyanoviruses possess some AMGs encoding the core photosynthetic reaction centers[62], which are highly expressed during the infection, thus stimulating photosynthesis and enhancing viral abundance[63]. In this study, we clearly showed that polysaccharide effectively promoted bacterial growth and simultaneously induced the production of bacteriophages, suggesting the close relationship between bacteriophages and host polysaccharide metabolism. Therefore, we sought to ask whether bacteriophages could reprogram the polysaccharide metabolism of deep-sea bacteria. For this purpose, the genomes of bacteriophages induced by polysaccharide in *Lentisphaerae* strains WC36 and zth2 were isolated and sequenced. Two assembled incomplete genomes (Phage-WC36-1, 8.0 kb; Phage-WC36-2, 28.3 kb) were obtained from the bacteriophages of strain WC36 (Figs. 5a, b). Meanwhile, one assembled incomplete genome (Phage-zth2-1, 8.0 kb) and one assembled complete genome (Phage-zth2-2, 40.4 kb) were obtained from the phages of strain zth2 (Figs. 5a, c). Subsequently, we carefully compared the genomes of different bacteriophages with those of corresponding hosts (strains WC36 and zth2), and found that the bacteriophage genomes were completely outside of the host chromosome, indicating these bacteriophages are extrachromosomal as that reported previously[30]. Through the genome sequence comparison, IMG database analysis and phylogenetic analysis (Fig. S7), we confirmed that Phage-WC36-1 was exactly same to Phage-zth2-1 (inoviruses, bacteriophages from the *Inoviridae* family), indicating inoviruses might be ubiquitous in the *Lentisphaerae* phylum. Inoviruses members were the dominant population in the active group of viruses from deep-sea sediments, which could encode some proteins contributing to host phenotypes and ultimately alter the fitness and other behaviors of their hosts[64,65]. The maximum likelihood tree of phage genomes placed Phage-WC36-2 and Phage-zth2-2 as a member of *Myoviridae* and *Podoviridae* family, respectively (Fig. S8). In addition to genes encoding phage-associated proteins within the genomes of assembled bacteriophages,

there are many genes encoding polysaccharides transport and degradation associated proteins present in these phage genomes. These polysaccharide metabolizing associated proteins included glycoside hydrolase family 18 protein (GH18, HMM e-value 1.5e-14) (Fig. 5a), TonB-system energizer ExbB[66], O-polysaccharide acetyltransferase[67], glucosaminidase, glycoside hydrolase family 127 protein (best BLASTP hit e-value 3e-25) (Fig. 5b) and transglycosylase (Fig. 5c). These CAZymes (GH18 and GH127) were specifically responsible for degrading polysaccharides[68]. The energizer ExbB was an important part of the TonB-system, which was often present in *Bacteroides* and responsible for transport oligosaccharides from the outer membrane into the periplasm[69,70], but they were absent in the genomes of strains WC36 and zth2. Therefore, these AMGs encoding TonB-system energizer ExbB and glycoside hydrolases could reprogram the polysaccharide metabolism of host cells (strains WC36 and zth2) by providing the supplemental support to key metabolisms. In general, bacteriophages induced by polysaccharide contained specific AMGs associated with polysaccharide degradation, which playing a key role in the degradation and utilization of polysaccharide by host cells.

Bacteriophages released from *Lentisphaerae* strain WC36 promote the growth and polysaccharide metabolism of a marine bacterium *Pseudomonas stutzeri* 273. Given that bacteriophages released from *Lentisphaerae* strain WC36 (named Phage-WC36 in this study) could reprogram bacterial polysaccharide metabolism and thereby promoting host growth, we next sought to determine the capability of these bacteriophages toward reprogramming of polysaccharide metabolism and growth promotion in other marine bacteria. To this end, we chose a marine bacterium *Pseudomonas stutzeri* 273[71] and tested the potential growth promotion driven by Phage-WC36. The growth assays showed that Phage-WC36 could significantly promote the growth of strain 273 by assisting its metabolism and utilization of starch (Fig. 6a). In particular, the supplement of Phage-WC36 and 5 g/L starch in basal medium resulted in 6~10-fold growth promotion compared to that cultivated in basal medium absent of Phage-WC36 or starch (Fig. 6a). In addition, we could observe

many filamentous phage-like structures in the supernatant of strain 273 that cultivated in basal medium supplemented with 5 g/L starch and Phage-WC36 for 24 h (Fig. 6b, panel IV). In contrast, we could not find any phage-like structures in the other three media (Fig. 6b, panels I, II and III). Specifically, the average number of filamentous phages extracted from the supernatant of strain 273 that cultured in basal medium supplemented with 5 g/L starch and Phage-WC36 (~60.3) was evidently higher than that in other three media (close to zero) (Fig. 6c). Consistently, the expressions of genes encoding TonB-dependent receptor and CAZymes of strain 273 that cultured in basal medium supplemented with 5 g/L starch and 20 μl/mL Phage-WC36 were significantly up-regulated in comparison to that cultured in basal medium amended with 5 g/L starch (Fig. 6d) or 20 μl/mL Phage-WC36 (Fig. 6e). These results suggested that chronic Phage-WC36 could proliferate in strain 273 and promote its growth by assisting its metabolism and utilization of starch. Consequently, we propose that chronic Phage-WC36 might infect other hosts and assist them in degradation and utilization of polysaccharides, which provides even stronger evidence that the relationship between Phage-WC36 and hosts is not the fight to the death between enemies, but the mutualistic relationship between partners[72]. Most of deep-sea microbes are anaerobic, which limits the investigation of bacteriophages in the original host. *P. stutzeri* 273 is a typical aerobic bacterium, thus, our study provides an effective way to replicate deep-sea bacteriophages and perform functional analysis in aerobic heterologous hosts.

Bacteriophages released from *Lentisphaerae* strain WC36 facilitate the enrichment and cultivation of deep-sea difficult-to-culture microorganisms. Based on our above results, it is clear that bacteriophages released from *Lentisphaerae* strain WC36 (Phage-WC36) evidently promoted the growth and polysaccharide metabolism of a marine bacterium *P. stutzeri* 273. We next sought to ask whether Phage-WC36 could assist other uncultured microorganisms in degradation and utilization of polysaccharides, which is helpful to overcome the cultivation bottleneck of huge amount of difficult-to-culture microbes[73]. We thus

designed a unique enrichment medium (inorganic medium supplemented with polysaccharide and Phage-WC36) to enrich and cultivate microbes from the deep-sea surface sediments. To understand the bacterial taxa within the enrichment samples (in the presence or absence of Phage-WC36), we performed the amplicon sequencing analysis. The amplicon results showed that Phage-WC36 could effectively enrich difficult-to-culture microorganisms (including *Cyanobacteria*, *Chloroflexi*, *Planctomycetes*, *Caldatribacteriota* and *Acidobacteria* bacteria) with the deep-sea surface sediments collected in three different years (2018, 2020 and 2021) (Fig. 7a). We continuously cultured the enrichment in the inorganic medium supplemented with polysaccharide and Phage-WC36, and eventually obtained seven novel pure isolates (including two *Planctomycetes* bacteria: strains ZRK32 and ZRK34; one *Verrucomicrobia* bacterium: strain ZRK36; one *Fusobacteria* bacterium: strain ZRK30 (Figs. 7b and 7c); one *Tenericutes* bacterium: strain zrk7; two *Firmicutes* bacteria: strains zrk8 and zrk18) and two enriched bacteria (one *Chloroflexi* bacterium: strain ZRK35; one *Cyanobacteria* bacterium: strain ZRK38) (Fig. 7b). Notably, several strains belonging to PVC superphylum were isolated by this strategy, confirming PVC bacteria were prone to coordinate with bacteriophages for polysaccharide utilization as shown in Figure S6. It is known that members belonging to *Cyanobacteria*, *Planctomycetes* and *Chloroflexi* are dominant in diverse ocean environments[74], and play crucial roles in carbon and nutrient cycling[75]. However, very few deep-sea members within these phyla have been cultured. Accordingly, they are recognized as typical difficult-to-culture bacteria. Therefore, the unique enrichment strategy associated with chronic phages performed in the present study is a very novel and effective method to enrich deep-sea uncultured microorganisms, which might be developed into a common technique for isolating difficult-to-culture microbes existing in diverse ocean environments.

In summary, we demonstrate for the first time that polysaccharide could induce the production of bacteriophages (chronic type) in *Lentisphaerae* strain WC36, and these bacteriophages contain some AMGs encoding TonB-system energizer ExbB and CAZymes that reprogram the host metabolism of degradation and utilization of

polysaccharide, thereby promoting host growth (Figs. 1, 2, 3 and 5). And we find that polysaccharide could also induce the production of bacteriophages in some other PVC bacteria (*Lentisphaerae* strain zth2, *Verrucomicrobia* strain ZRK36 and *Planctomycetes* strain ZRK32) (Fig. 4 and Fig. S6). Therefore, the auxiliary role of bacteriophages in the degradation of polysaccharides by PVC bacteria is worthy of further study. We also demonstrate that chronic Phage-WC36 could assist other marine bacteria to utilize polysaccharides (Fig. 6) and facilitate the cultivation of difficult-to-culture microbes (Fig. 7), further expanding our understanding of the importance of deep-sea chronic phages.

Methods

Samples, media and cultivation conditions. Deep-sea samples used for bacteria isolation in this study were collected from a typical cold seep (E119º17'07.322", N22º06'58.598") at a depth of 1,146 m by *RV KEXUE* in July of 2018. The inorganic medium (including 1.0 g/L NH_4Cl, 1.0 g/L $NaHCO_3$, 1.0 g/L CH_3COONa, 0.5 g/L KH_2PO_4, 0.2 g/L $MgSO_4 \cdot 7H_2O$, 0.7 g/L cysteine hydrochloride, 500 µl/L 0.1 % (w/v) resazurin, 1 L filtered seawater, pH 7.0) supplemented with 1.0 g/L different polysaccharides (laminarin, fucoidan, starch and alginate) under a 100% N_2 atmosphere was used to enrich microbes at 28 °C for one month[42]. The detailed isolation and purification steps were performed as described previously[75]. After pure cultures of deep-sea bacteria were obtained, we cultured them in the anaerobic liquid 2216E medium (containing 1.0 g/L peptone, 5.0 g/L yeast extract, 0.7 g/L cysteine hydrochloride, 500 µl/L 0.1 % (w/v) resazurin, 1 L filtered seawater, pH 7.0) for better growth.

Phylogenetic analysis. The 16S rRNA gene tree was constructed based on full-length 16S rRNA gene sequences by the maximum likelihood method. The full-length 16S rRNA gene sequences of *Lentisphaerae* strains WC36 and zth2 were obtained from their genomes, and the 16S rRNA gene sequences of other related taxa used for phylogenetic analysis were obtained from NCBI (www.ncbi.nlm.nih.gov/). The

maximum likelihood phylogenetic trees of Phage-WC36-1 were based on the Zona occludens toxin (Zot) and single-stranded DNA-binding protein (ssDNA). The maximum likelihood phylogenetic tree of Phage-WC36-2 and Phage-zth2-2 was based on the terminase large subunit protein (*terL*). These proteins used to construct the phylogenetic trees were all obtained from the NCBI databases. The maximum likelihood phylogenetic tree of seven novel pure cultures (strains ZRK32, ZRK34, ZRK36, ZRK30, zrk7, zrk8 and zrk18) and two enriched cultures (strains ZRK35 and ZRK38) was based on almost complete 16S rRNA gene sequences. The 16S rRNA gene sequences of other phyla bacteria were all obtained from the NCBI databases. All the sequences were aligned by MAFFT version 7[76] and manually corrected. The phylogenetic trees were constructed using the W-IQ-TREE web server (http://iqtree.cibiv.univie.ac.at)[77]. Finally, we used the online tool Interactive Tree of Life (iTOL v5)[78] to edit the tree.

Growth assay and transcriptomic analysis of *Lentisphaerae* strains WC36 and zth2 cultured in the medium supplemented with laminarin. To assess the effect of polysaccharide on the growth of strain WC36, 100 µl freshly incubated cells were inoculated in a 15 mL Hungate tube containing 10 mL 2216E medium supplemented without/with 5 g/L or 10 g/L laminarin (or starch). To assess the effect of laminarin on the growth of strain zth2, 100 µL freshly incubated cells were inoculated in a 15 mL Hungate tube containing 10 mL 2216E medium supplemented without or with 3 g/L laminarin. Each condition was performed with three replicates. All the Hungate tubes were incubated at 28 °C. The bacterial growth status was monitored by measuring the OD_{600} value via a microplate reader (Infinite M1000 Pro; Tecan, Mannedorf, Switzerland) every day until the cell growth reached the stationary phase.

For transcriptomic analysis, cells suspension of strain WC36 cultured in 1.5 L 2216E medium supplemented without or with 10 g/L laminarin for 5 days and 10 days were harvested. Cells suspension of strain zth2 cultured in the 2216E medium supplemented without or with 3 g/L laminarin for 4 days was harvested. Thereafter, these collected samples were used for transcriptomic analysis by Novogene (Tianjin,

China). The detailed procedures of transcriptomic assays including library preparation, clustering and sequencing, and data analyses were described in the Supplementary information.

Bacteriophages isolation. To induce the production of bacteriophages, strain WC36 was inoculated in the 2216E medium supplemented with/without 10 g/L laminarin or 10 g/L starch, and strain zth2 was cultured in the 2216E medium supplemented with/without 3 g/L laminarin or 3 g/L starch. After 10 days incubation, different cultures were respectively collected and cells were removed by centrifugation at 8,000 *g*, 4 °C for 20 min. The supernatant was filtered through a 0.22 μm millipore filter (Pall Supor, New York, America) and further treated by 1 μg/mL DNase I and RNase A for 8 h, and subsequently 1 M NaCl was added to separate phage particles from bacterial fragments. Then the supernatant was collected by centrifugation at 8,000 *g*, 4 °C for 20 min, and phage particles were immediately precipitated with 100 g/L polyethylene glycol (PEG8000) at 4 °C for 6 h, and collected by centrifugation at 10,000 *g*, 4 °C for 20 min. The resulting phage particles were suspended in 2 mL SM buffer (0.01% gelatin, 50 mM Tris-HCl, 100 mM NaCl and 10 mM $MgSO_4$), then the suspension was extracted three times with an equal volume of chloroform[43] and collected by centrifugation at 4,000 *g*, 4 °C for 20 min. Finally, the clean phage particles were obtained.

Transmission electron microscopy (TEM). To observe the morphology of bacteriophages, phage virions were allowed to adsorb to the copper grid for 20 min, and then stained with the phosphotungstic acid for 30 s. Next, micrographs were taken with TEM (HT7700, Hitachi, Japan) with a JEOL JEM 12000 EX (equipped with a field emission gun) at 100 kV.

To observe the morphology of strain WC36, its cell suspension incubated in the 2216E medium supplemented with or without polysaccharide was centrifuged at 5,000 *g* for 10 min to obtain cell pellets. Subsequently, one part of the cell collection was adsorbed to the copper grids for 20 min, then washed with 10 mM phosphate

buffer solution (PBS, pH 7.4) for 10 min and dried at room temperature for TEM observation. Another part was first preserved in 2.5% glutaraldehyde for 24 h at 4 °C, and then washed three times with PBS and dehydrated in ethanol solutions of 30%, 50%, 70%, 90% and 100% for 10 min each time. Samples were further fixed in 1% osmium tetroxide for 2 h and embedded in plastic resin[79,80]. Thereafter, an ultramicrotome (Leica EM UC7, Germany) was used to prepare ultrathin sections (50 nm) of cells, and the obtained sections were stained with uranyl acetate and lead citrate[81,82]. Finally, all samples were observed with the JEOL JEM-1200 electron microscope as described above. In parallel, the filamentous phage' number in each condition were respectively calculated.

Observation of bacteriophages present in other deep-sea bacteria. To investigate whether polysaccharides could induce phage production in other deep-sea bacteria, we selected cultured representatives from six phyla (*Verrucomicrobia*, *Planctomycetes*, *Chloroflexi*, *Tenericutes*, *Proteobacteria*, *Actinobacteria*) in our laboratory. These six bacterial strains were all isolated from deep-sea cold seeps, and their 16S rRNA gene sequences had been deposited at GenBank under the accession numbers: MZ646149, MW376756, MN817941, MW132883, MT032312 and OL757559, respectively. All strains were cultivated in the anaerobic liquid 2216E medium supplemented without or with 10 g/L laminarin (or starch) at 28 °C for 15 days. Subsequently, we performed the phage isolation and TEM observation as described above.

Genome sequencing of bacteriophages. To sequence the genomes of bacteriophages, the phage genomic DNA was extracted from different purified phage particles. Briefly, 1 μg/mL DNase I and RNase A were added to the concentrated phage solution for nucleic acid digestion overnight at 37 °C. The digestion treatment was inactivated at 80 °C for 15 min, followed by extraction with a Viral DNA Kit (Omega Bio-tek, USA) according to the manufacturer's instructions. Then, the genome sequencing was performed by Biozeron Biological Technology Co.Ltd (Shanghai, China). The detailed process of library construction, sequencing, genome assembly and annotation

is described in the Supplementary information.

Growth assay of *Pseudomonas stutzeri* 273 cultured in basal medium supplemented with starch and Phage-WC36. In order to verify the function of bacteriophages induced from strain WC36 to assist host metabolism and utilization of polysaccharides, we selected *Pseudomonas stutzeri* 273 isolated from deep-sea sediment[71] as the research object. Specifically, 50 μL freshly incubated cells (*P. stutzeri* 273) were inoculated in 5 mL basal medium (10 g/L NaCl, 1 L sterilized distilled water), basal medium supplemented with 20 μl/L Phage-WC36, basal medium supplemented with 5 g/L starch, basal medium supplemented with 5 g/L starch and 20 μl/L Phage-WC36 for 24 h at 28 °C, respectively. Each condition was performed with three replicates. The bacterial growth status was monitored by measuring the OD_{600} value via a microplate reader (Infinite M1000 Pro; Tecan, Mannedorf, Switzerland) every 4 h until the cell growth reached the stationary phase. In addition, phages were extracted from the supernatants of the above four media and their morphology was observed by TEM. In parallel, the filamentous phages' number in each condition was respectively calculated.

For transcriptomic analysis, cells suspension of *P. stutzeri* 273 cultured in basal medium supplemented with 20 μl/L Phage-WC36, basal medium supplemented with 5 g/L starch, basal medium supplemented with 5 g/L starch and 20 μl/L Phage-WC36 for 24 h at 28 °C were harvested, respectively. Then, these collected samples were sent to Novogene (Tianjin, China) for transcriptome sequencing analysis. The detailed procedures were described in the Supplementary information.

Operational taxonomic units (OTUs) analysis of enrichment cultures supplemented with Phage-WC36. Since Phage-WC36 may have the ability to assist bacterial hosts in metabolizing polysaccharide, we designed unique enrichment media (inorganic medium supplemented with 3.0 g/L laminarin and 3.0 g/L starch, as the control group; inorganic medium supplemented with 3.0 g/L laminarin, 3.0 g/L starch and 20 μl/mL Phage-WC36, as the experimental group) to enrich and culture deep-sea

difficult-to-culture microorganisms. Specifically, we selected the surface sediments of deep-sea cold seep from three different years (2018, 2020 and 2021), which were cultured at 28 °C for one month in the above two unique enrichment media. Each medium had three biologically independent replicates. In order to understand the bacterial community structure of these enrichment cultures, 2 mL of each enrichment medium was harvested and sent to Novogene (Tianjin, China) for OTUs sequencing. The detailed procedures were described in the Supplementary information.

Data availability. The complete genome sequences of strains WC36, zth2, ZRK32, ZRK34, ZRK36 and ZRK30 presented in this study have been deposited in the GenBank database with accession numbers CP085689, CP071032, CP066225, CP050848, CP080649 and CP102404-CP102408, respectively. The whole 16S rRNA gene sequences of strains WC36, zth2, ZRK32, ZRK34, ZRK36, ZRK30, zrk7, zrk8, zrk18, ZRK35 and ZRK38 have been deposited in the GenBank database with accession numbers OK614042, MW729759, MW376756, MT076070, MZ646149, OM892919, OP108612, MW581533, MW647584, MW581537 and OP108622, respectively. The genome sequences of Phage-WC36-1 (Phage-zth2-1), Phage-WC36-2 and Phage-zth2-2 have been deposited in the GenBank database with accession numbers ON698679, OL791266 and OL791268, respectively. The raw sequencing reads from the transcriptomics analysis have been deposited to the NCBI Short Read Archive (accession numbers: PRJNA785378, PRJNA756144 and PRJNA865570). The raw amplicon sequencing data have also been deposited to NCBI Short Read Archive (accession number: PRJNA865809).

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Author contributions. CW, RZ and CS conceived and designed the study; CW conducted most of the experiments; RZ helped to analyze the viral metagenomes; TZ helped to isolate the *Lentisphaerae* strain zth2; CW and CS led the writing of the manuscript; all authors contributed to and reviewed the manuscript.

Conflict of interest. The authors declare no competing interests.

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Figure Legends

Fig. 1. Polysaccharide promotes the growth of deep-sea *Lentisphaerae* strain WC36 and stimulates the expression of phage-associated genes. a, Maximum likelihood phylogenetic tree of 16S rRNA gene sequences from strain WC36 and some *Planctomycetes-Verrucomicrobia-Chlamydia* (PVC) group bacteria. The *Bacillus cereus* ATCC 14579 was used as the outgroup. Bootstrap values (%) > 80 are indicated at the base of each node with the gray dots (expressed as percentages of 1,000 replications). **b**, Growth assays of strain WC36 cultivated in the 2216E medium supplemented either with or without 5 g/L, 10 g/L laminarin or starch. **c**, Transcriptomics based heat map showing all up-regulated genes encoding phage-associated proteins in strain WC36 that cultured in the 2216E medium supplemented with laminarin. "2216E" indicates strain WC36 was cultivated in the 2216E medium; "Lam" indicates strain WC36 was cultivated in the 2216E medium supplemented with 10 g/L laminarin. **d**, Transcriptomics based heat map showing all up-regulated genes encoding phage-associated proteins in strain WC36 that cultured in the 226E medium supplemented with laminarin for 10 days compared to those for 5 days. "Lam5D" and "Lam10D" indicate strain WC36 cultivated in the 2216E medium supplemented with 10 g/L laminarin for 5 days and 10 days, respectively. The heat map is generated by the Heml 1.0.3.3. The numbers in panels **c** and **d** represent the multiple of difference in gene expression (by taking $\log_2$ value).

Fig. 2. Polysaccharide induces the production of bacteriophages in *Lentisphaerae* strain WC36. a, TEM observation of phages extracted from the supernatant of cells suspension of strain WC36 that cultured in the 2216E medium supplemented without or with polysaccharide. Panel I, absence of phages in the cells supernatant of strain WC36 cultivated in the 2216E medium. Panels II-IV, observation of the morphology of phages present in the cells supernatant of strain WC36 cultivated in the 2216E medium supplemented with 10 g/L laminarin. Yellow-hollow arrows indicated the typical granular structure of filamentous phage, and green arrows indicated phage-like particles with other shapes. Panels V-VIII, observation of the morphology of phages present in the cells supernatant of strain WC36 cultivated in the 2216E medium supplemented with 10 g/L starch. Typical filamentous phages were indicated with

yellow-hollow arrows, and other two kinds of phage-like particles with different shapes were indicated by orange and green arrows, respectively. **b**, TEM observation of strain WC36 cultured in the 2216E medium supplemented without or with 10 g/L laminarin or 10 g/L starch. Panel I, representative morphology of strain WC36 cultivated in the 2216E medium. Panel II, morphology of strain WC36 cultivated in the 2216E medium supplemented with 10 g/L laminarin. Panels III-IV, morphology of strain WC36 cultivated in the 2216E medium supplemented with 10 g/L starch. Red arrows indicated the filamentous phages associated with bacterial cells. **c**, TEM observation of the ultrathin section of strain WC36 cultured in the 2216E medium supplemented without or with 10 g/L laminarin or 10 g/L starch. Panel I, observation of the ultrathin section of strain WC36 cultivated in the 2216E medium; Panels II-III, observation of the ultrathin section of strain WC36 cultivated in 2216E medium supplemented with 10 g/L laminarin; Panel IV, observation of the ultrathin section of strain WC36 cultivated in 2216E medium supplemented with 10 g/L starch. Red arrows indicated the filamentous phages being released from or entering into bacterial cells.

Fig. 3. Bacteriophages induced from *Lentisphaerae* strain WC36 by polysaccharides are released via a chronic style. a, Growth curve of strain WC36 cultivated in the 2216E medium supplemented without or with 5 g/L, 10 g/L laminarin for 30 days. **b**, TEM observation of filamentous phages extracted from the supernatant of cells suspension of strain WC36 that cultured in the 2216E medium supplemented without or with 5 g/L, 10 g/L laminarin for different days (5, 10 and 30 days). Panels I-III, absence of phages in the cells supernatant of strain WC36 cultivated in the 2216E medium for 5, 10 and 30 days, respectively. Panels IV-VI, observation the morphology of filamentous phages present in the cells supernatant of strain WC36 cultivated in the 2216E medium supplemented with 5 g/L laminarin for 5, 10 and 30 days, respectively. Panels VII-IX, observation of the morphology of filamentous phages present in the cells supernatant of strain WC36 cultivated in the 2216E medium supplemented with 10 g/L laminarin for 5, 10 and 30 days,

respectively. **c**, The number of filamentous phages extracted from the supernatant of cells suspension of strain WC36 that cultured in the 2216E medium supplemented without or with 5 g/L, 10 g/L laminarin for different days (5, 10 and 30 days). The average number of filamentous phages is shown at the top of the bar chart (calculated based on 6 photographs taken randomly for each condition).

Fig. 4. Polysaccharide promotes the growth of deep-sea *Lentisphaerae* strain zth2 and induces the production of bacteriophages. a, Growth assays of strain zth2 cultivated in the 2216E medium supplemented either with or without 3 g/L laminarin for 4 days. **b**, Transcriptomics based heat map showing all up-regulated genes encoding phage-associated proteins in strain zth2 that cultured in the 2216E medium supplemented with 3 g/L laminarin. **c**, RT-qPCR detection of the expressions of some genes encoding phage-associated proteins shown in panel b. **d**, TEM observation of phages extracted from the supernatant of cells suspension of strain zth2 that cultured in the 2216E medium supplemented without or with polysaccharides. Panels I-III, observation of the morphology of phages presented in the cells supernatant of strain zth2 that cultivated in the 2216E medium supplemented with 3 g/L laminarin. Typical filamentous phage was indicated with yellow-hollow arrows, and other two kinds of phage-like particles with different shapes were indicated by orange and green arrows, respectively. Panels IV-V, observation of the morphology of phages present in the cells supernatant of strain zth2 cultivated in the 2216E medium supplemented with 3 g/L starch. Typical filamentous phage was indicated with yellow-hollow arrows, and other phage-like particles with different shapes were indicated by green arrows. Panel VI, none phages were observed in the cells supernatant of strain zth2 that cultivated in the 2216E medium. “Control” indicates strain zth2 was cultivated in the 2216E medium; “Lam” indicates strain zth2 was cultivated in the 2216E medium supplemented with 3 g/L laminarin.

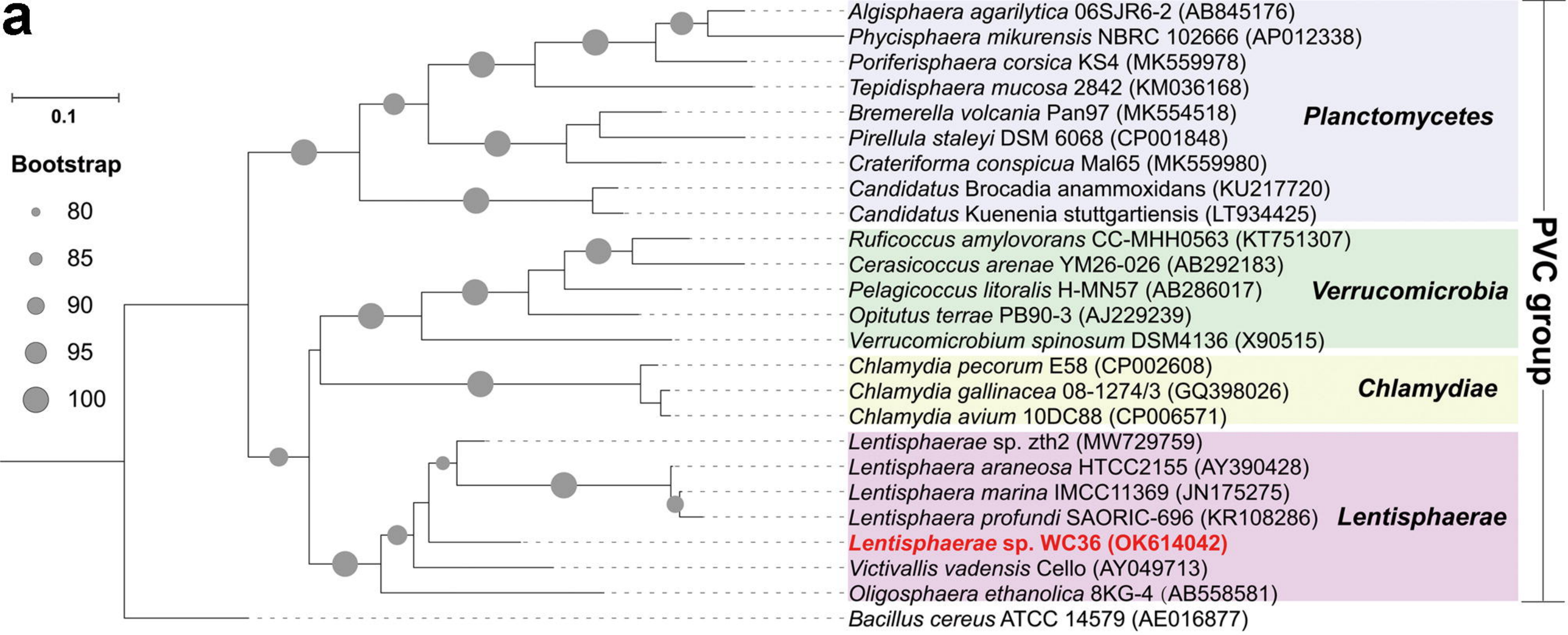
Fig. 5. Genomic organization of bacteriophages released from *Lentisphaerae* strains WC36 and zth2 that cultured in the 2216E medium supplemented with polysaccharide. **a**, A diagram showing the genomic composition of Phage-WC36-1

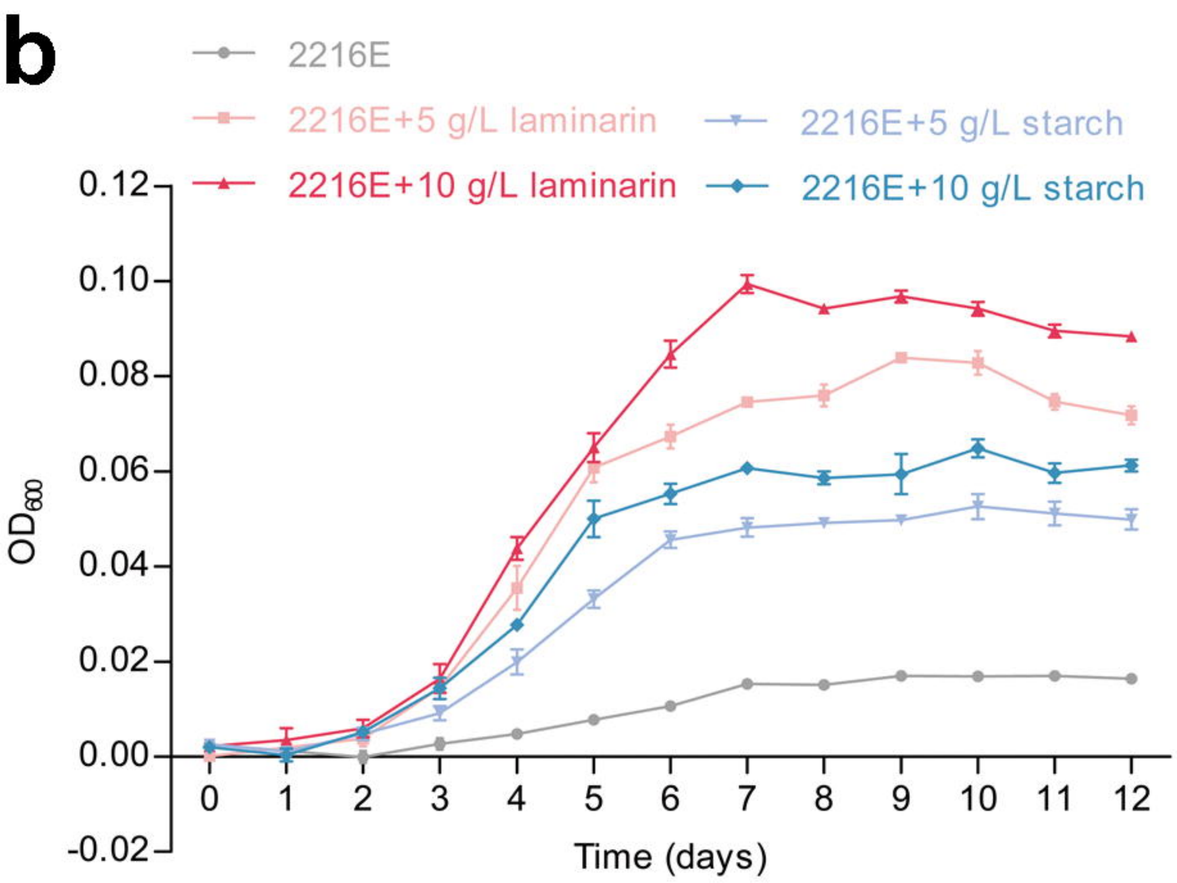
and Phage-zth2-1. **b**, A diagram showing the genomic composition of Phage-WC36-2. **c**, A diagram showing the genomic composition of Phage-zth2-2. Arrows represent different ORFs and the direction of transcription. The main putative gene products of phages are shown, and the numbers in brackets indicate the numbers of amino acides within each ORF. The hypothetical proteins are indicated with gray arrows, the structural modules are indicated by green arrows, the replication module is indicated by blue-grey arrows, the regulatory module is indicated by golden arrows, the assembly and secretion modules are indicated by blue arrows and auxiliary metabolic genes (AMGs) encoding proteins associated with polysaccharide transport and degradation are indicated by red arrows. The size of phage genomes is shown behind each gene cluster.

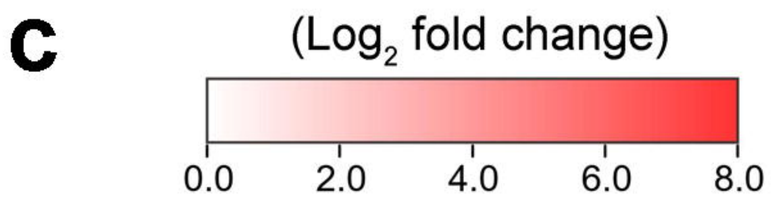
Fig. 6. Bacteriophages released from *Lentisphaerae* strain WC36 promote the growth and polysaccharide metabolism of a marine bacterium *Pseudomonas stutzeri* 273. **a**, Growth curve and status of *P. stutzeri* 273 that cultivated in basal medium, basal medium supplemented with 20 μl/mL Phage-WC36, basal medium supplemented with 5 g/L starch, basal medium supplemented with 5 g/L starch and 20 μl/mL Phage-WC36. **b**, TEM observation of phages extracted from the supernatant of cells suspension of *P. stutzeri* 273 that cultured in four different media (Panel I, basal medium; Panel II, basal medium supplemented with 20 μl/mL Phage-WC36; Panel III, basal medium supplemented with 5 g/L starch; Panel IV, basal medium supplemented with 5 g/L starch and 20 μl/mL Phage-WC36). **c**, Counting of the number of filamentous phages extracted from the supernatant of cells suspension of *P. stutzeri* 273 that cultured in four different media shown in panel b. The average number of filamentous phages is shown at the top of the bar chart (calculated based on 9 photographs taken randomly for each condition). **d** and **e**, Transcriptomics based heat map showing all up-regulated genes encoding TonB-dependent receptor (**d**) and CAZymes (**e**) of *P. stutzeri* 273 that cultured in the basal medium supplemented with 5 g/L starch and 20 μl/mL Phage-WC36 (B+S+P) in comparison to the basal medium supplemented with 5 g/L starch (B+S) or the basal medium supplemented with 20

μl/mL Phage-WC36 (B+P).

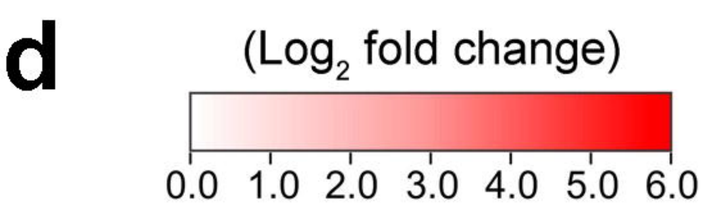
Fig. 7. Bacteriophages released from *Lentisphaerae* strain WC36 facilitate the enrichment and cultivation of deep-sea difficult-to-culture bacteria. a, Analysis of bacterial community structure of deep-sea sediments (collected in the year of 2018, 2020 or 2021) in the medium supplemented with or without Phage-WC36 for 30 days. Data shown are mean ± standard deviation (n = 3 biologically independent replicates). "Control"indicates samples were inoculated in the inorganic medium supplemented with 3.0 g/L laminarin and 3.0 g/L starch;"Phage"indicates samples were inoculated in the inorganic medium supplemented with 3.0 g/L laminarin, 3.0 g/L starch and 20 μl/mL Phage-WC36. **b**, Maximum-likelihood phylogenetic tree of seven novel pure cultures (red) and two enriched cultures (black) based on almost complete 16S rRNA gene sequences. Bar, 0.1 substitutions per nucleotide position. **c**, TEM observation and circular genome diagram of four deep-sea difficult-to-culture bacteria: *Planctomycetes* strain ZRK32 (I), *Planctomycetes* strain ZRK34 (II), *Verrucomicrobia* strain ZRK36 (III) and *Fusobacteria* strain ZRK30 (IV), respectively.







2216E	Lam	Locus tag	Product
0.0	6.5	LJT99_00015	Phage portal protein
0.0	4.9	LJT99_00040	Phage tail assembly chaperone
0.0	5.9	LJT99_00070	Phage small terminase subunit
0.0	6.6	LJT99_00075	Phage major capsid protein, P2 family
0.0	5.1	LJT99_01680	Phage integrase
0.0	3.7	LJT99_02055	Phage integrase
0.0	8.5	LJT99_05395	Phage integrase
0.0	8.1	LJT99_06855	Phage integrase
0.0	5.2	LJT99_07095	Phage terminase large subunit (GpA)
0.0	5.2	LJT99_07540	Phage head-tail connector protein
0.0	3.2	LJT99_07930	Phage integrase
0.0	9.3	LJT99_09120	Phage integrase
0.0	7.5	LJT99_09900	Phage integrase
0.0	6.4	LJT99_09950	Phage tail tape measure protein
0.0	4.1	LJT99_09990	Phage portal protein
0.0	7.2	LJT99_10035	Phage terminase large subunit (GpA)
0.0	4.7	LJT99_10050	Phage integrase
0.0	4.1	LJT99_15275	Phage integrase
0.0	8.0	LJT99_15310	Phage major capsid protein
0.0	4.9	LJT99_15320	Phage tail protein
0.0	4.4	LJT99_15330	Phage baseplate assembly protein V
0.0	5.2	LJT99_15350	Phage tail protein I
0.0	6.7	LJT99_15355	Phage tail-collar fibre protein
0.0	7.7	LJT99_15365	Phage tail sheath protein
0.0	5.8	LJT99_15370	Phage tail tube protein FII
0.0	6.1	LJT99_15375	Phage tail assembly protein
0.0	3.2	LJT99_15385	Phage tail protein
0.0	3.7	LJT99_15395	Phage late control gene D protein (GPD)



Lam5D	Lam10D	Locus tag	Product
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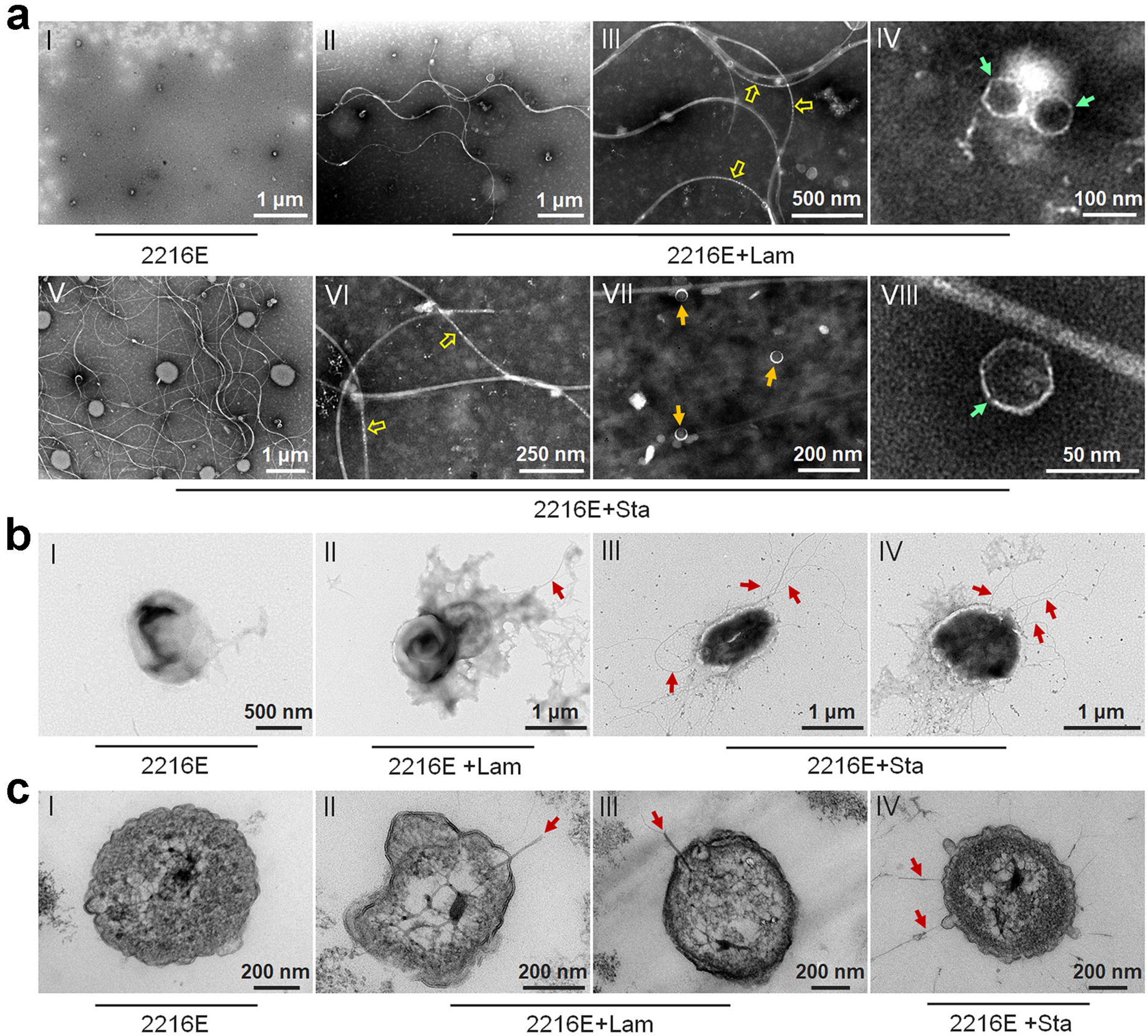
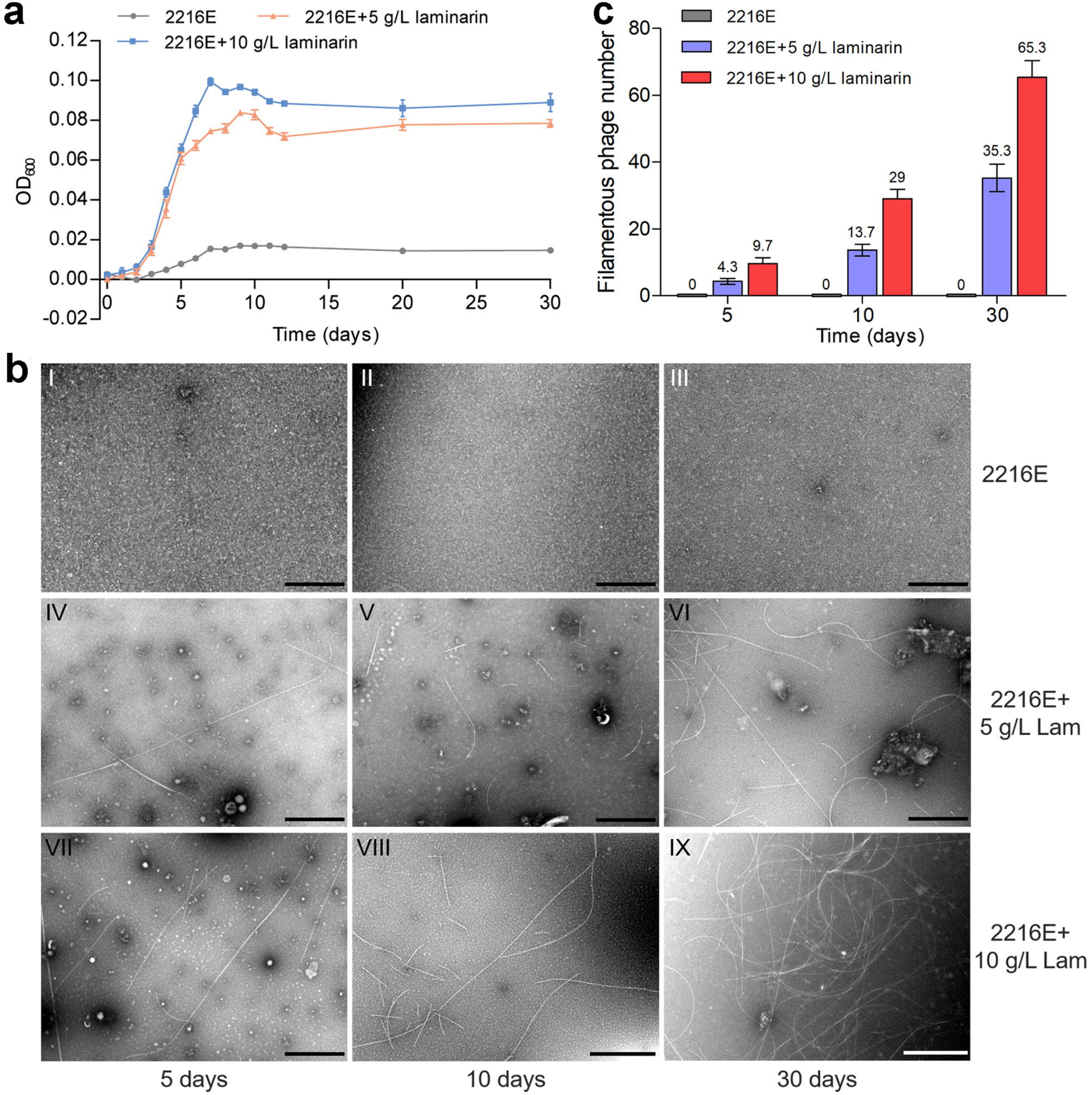
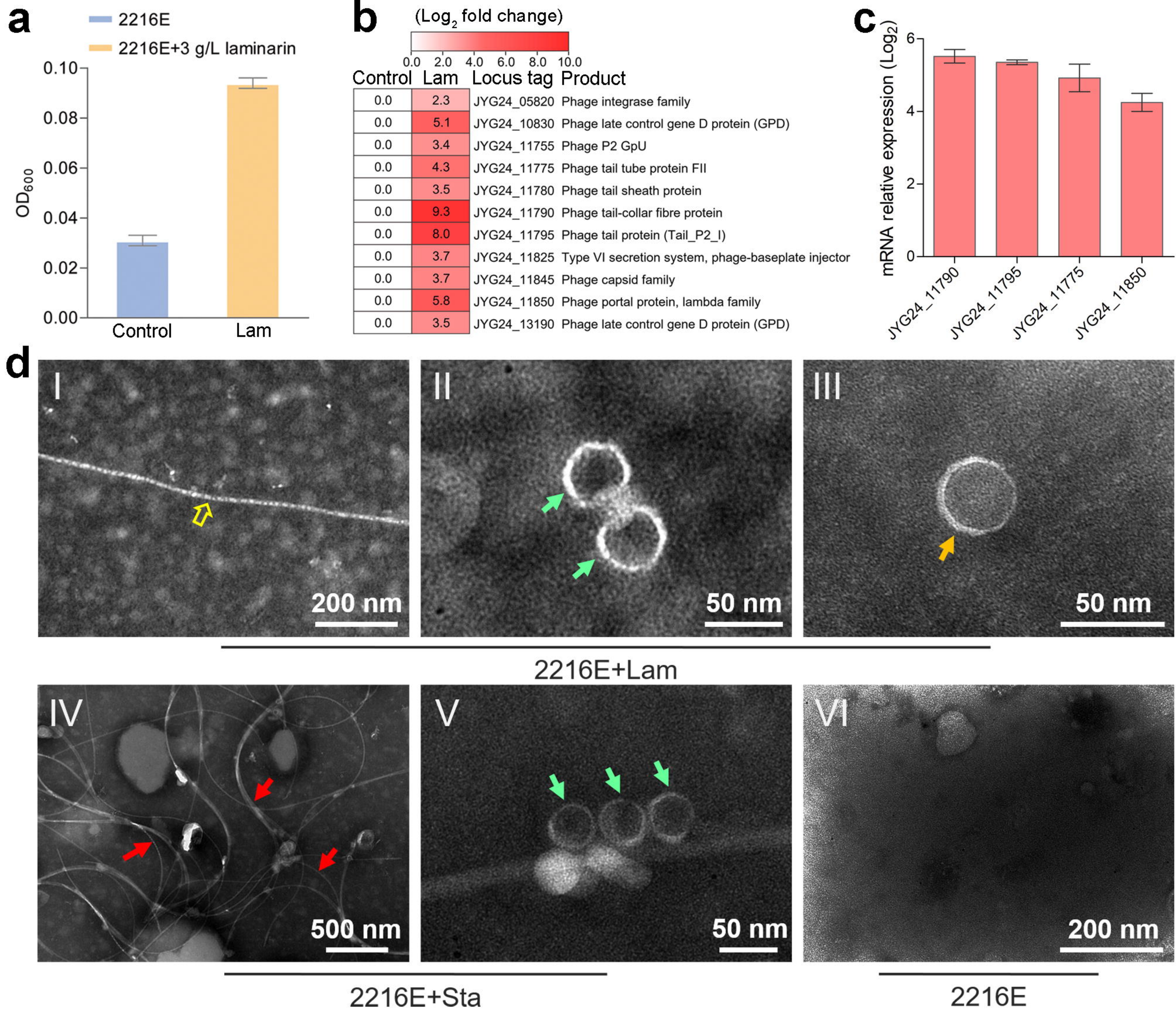
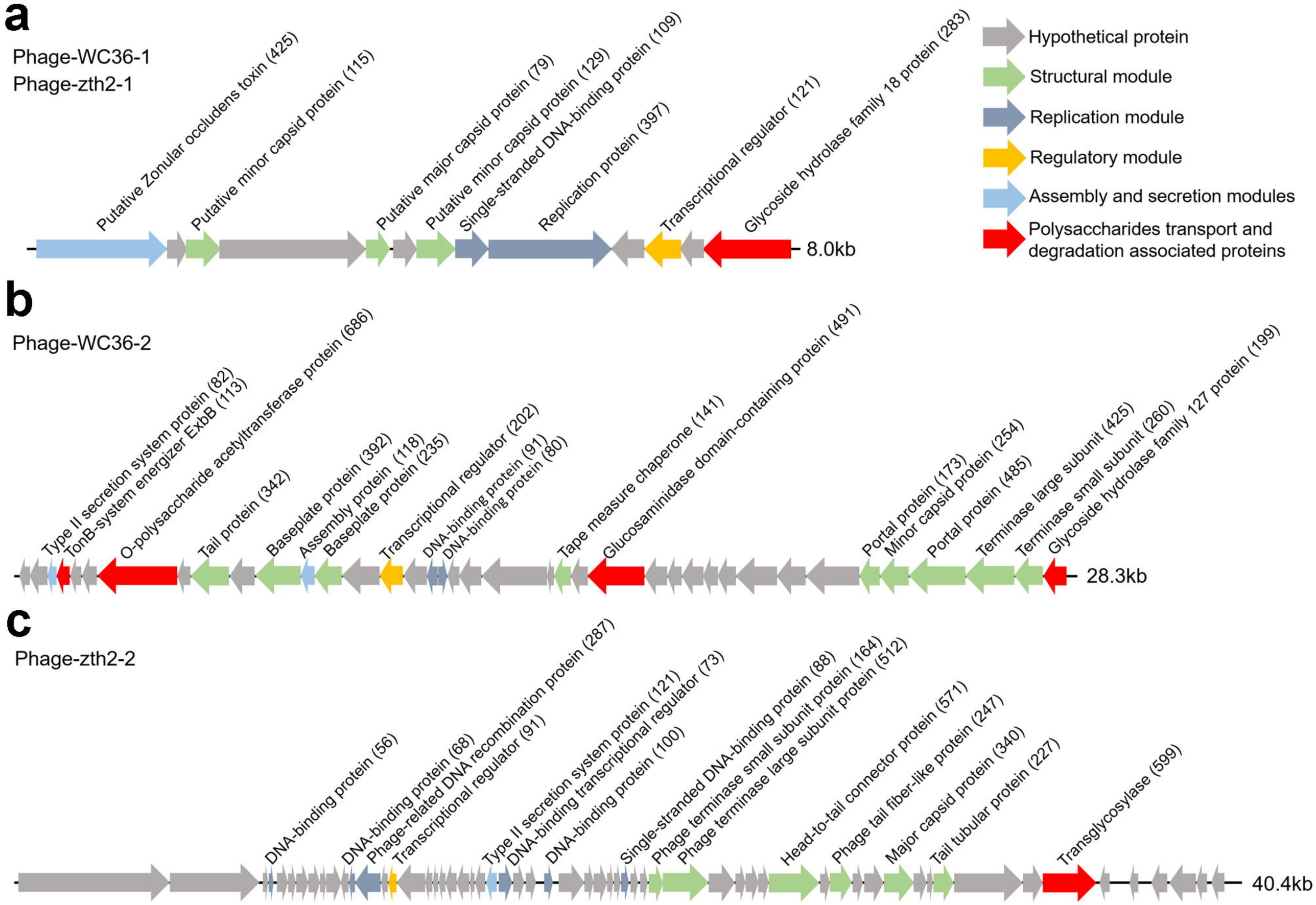
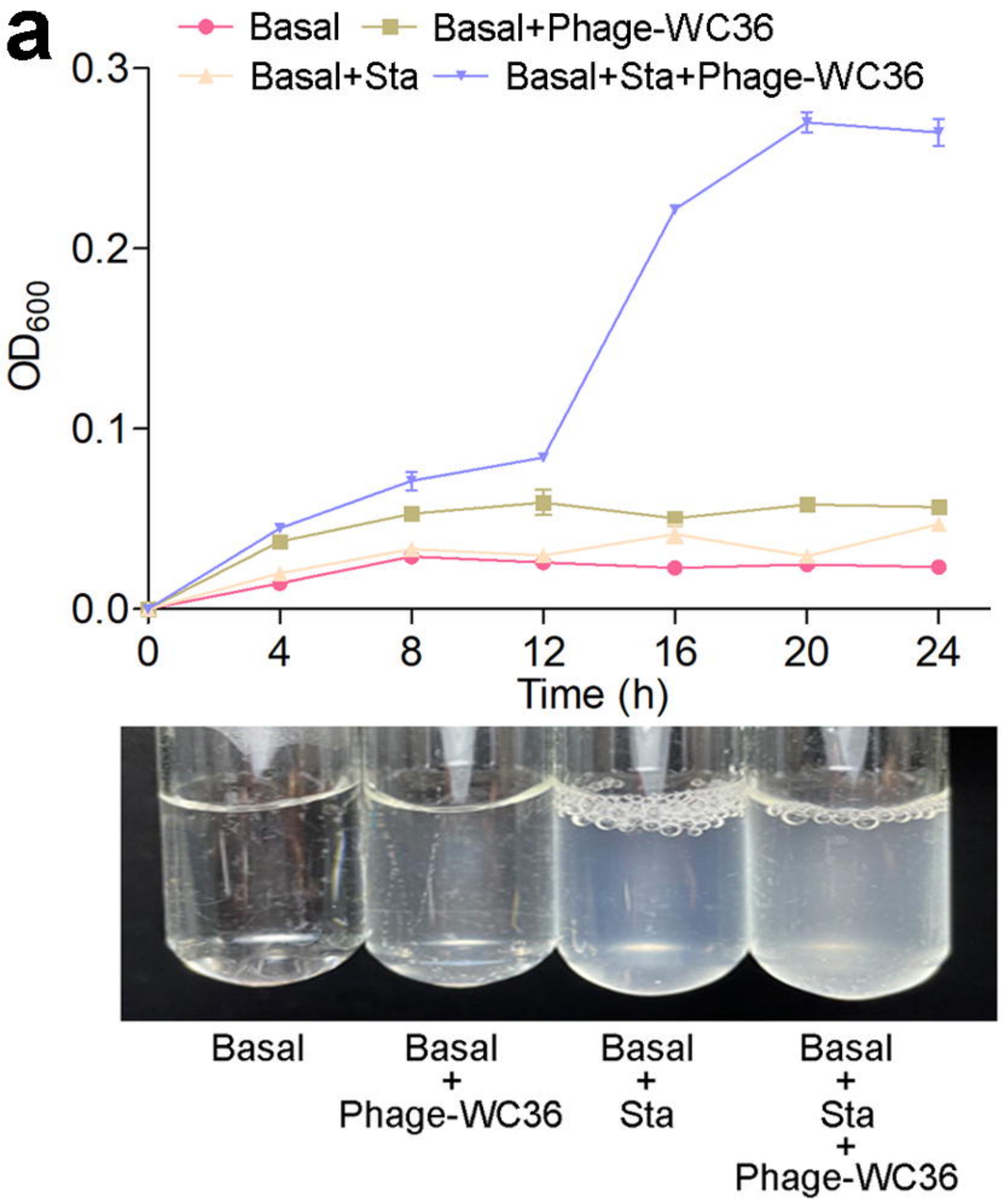
0.0	3.3	LJT99_00015	Phage portal protein
0.0	2.2	LJT99_00030	Phage tail tape measure protein
0.0	4.9	LJT99_00080	Phage capsid scaffolding protein
0.0	4.2	LJT99_05230	Phage integrase
0.0	4.2	LJT99_05290	Phage terminase large subunit
0.0	4.6	LJT99_09990	Phage portal protein
0.0	4.6	LJT99_15275	Phage integrase
0.0	2.0	LJT99_15350	Phage tail protein I

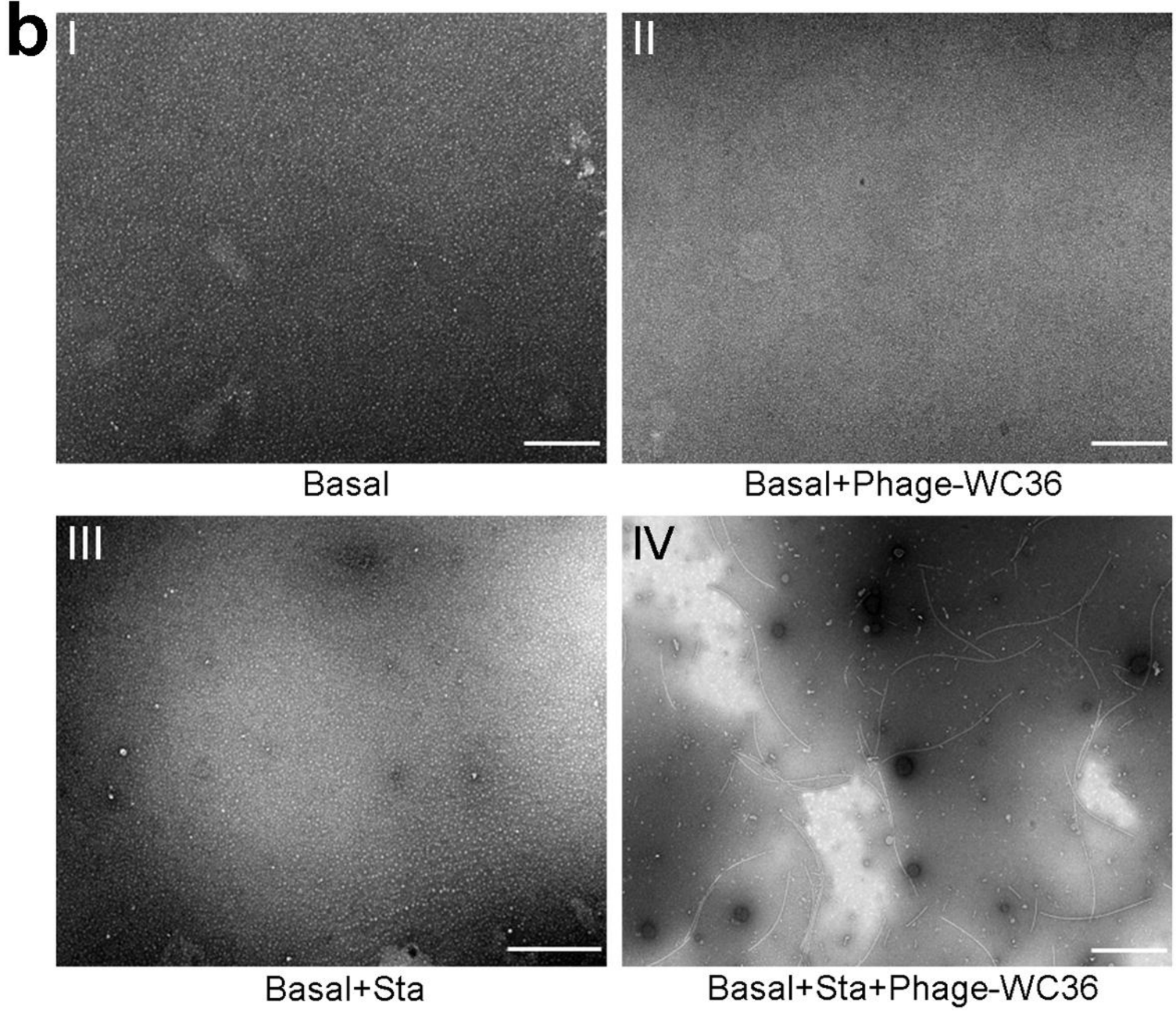

a
I
1 μm
II
1 μm
III
500 nm
IV
100 nm
2216E
2216E+Lam
V
1 μm
VI
250 nm
VII
200 nm
VIII
50 nm
2216E+Sta
b
I
500 nm
II
1 μm
III
1 μm
IV
1 μm
2216E
2216E +Lam
2216E+Sta
c
I
200 nm
II
200 nm
III
200 nm
IV
200 nm
2216E
2216E+Lam
2216E +Sta

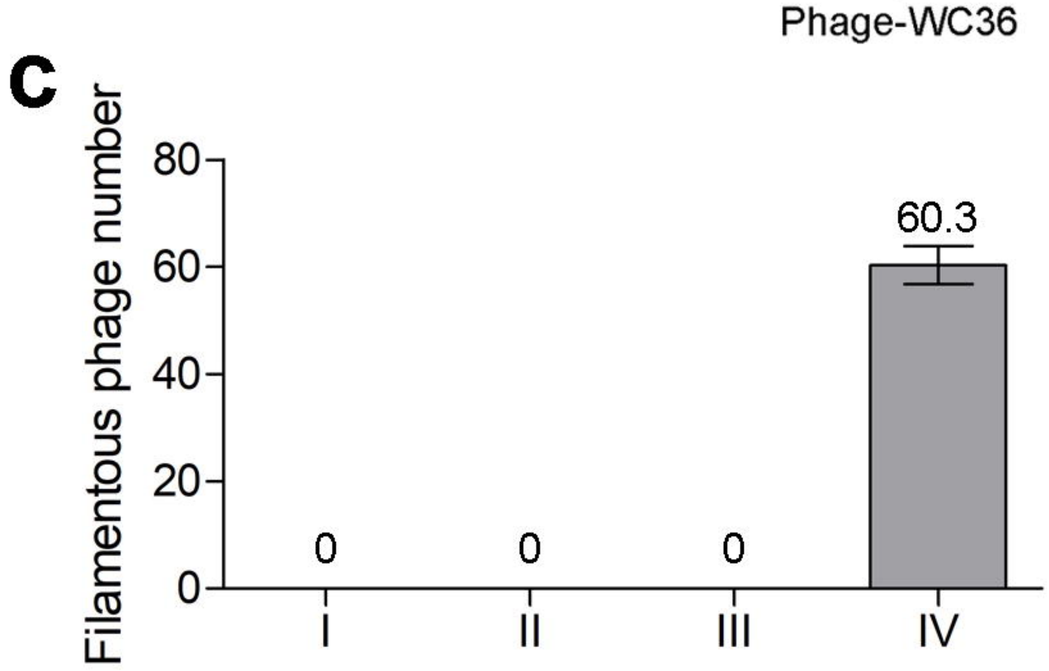

a
2216E
2216E+5 g/L laminarin
2216E+10 g/L laminarin
OD600
Time (days)
c
Filamentous phage number
Time (days)
0
4.3
9.7
13.7
29
35.3
65.3
b
I
II
III
IV
V
VI
VII
VIII
IX
2216E
2216E+
5 g/L Lam
2216E+
10 g/L Lam
5 days
10 days
30 days


a
2216E
2216E+3 g/L laminarin
OD600
0.10
0.08
0.06
0.04
0.02
0.00
Control
Lam
b
(Log2 fold change)
0.0 2.0 4.0 6.0 8.0 10.0
Control Lam Locus tag Product
0.0 2.3 JYG24_05820 Phage integrase family
0.0 5.1 JYG24_10830 Phage late control gene D protein (GPD)
0.0 3.4 JYG24_11755 Phage P2 GpU
0.0 4.3 JYG24_11775 Phage tail tube protein FII
0.0 3.5 JYG24_11780 Phage tail sheath protein
0.0 9.3 JYG24_11790 Phage tail-collar fibre protein
0.0 8.0 JYG24_11795 Phage tail protein (Tail_P2_I)
0.0 3.7 JYG24_11825 Type VI secretion system, phage-baseplate injector
0.0 3.7 JYG24_11845 Phage capsid family
0.0 5.8 JYG24_11850 Phage portal protein, lambda family
0.0 3.5 JYG24_13190 Phage late control gene D protein (GPD)
c
mRNA relative expression (Log2)
6
4
2
0
JYG24_11790
JYG24_11795
JYG24_11775
JYG24_11850
d
I
200 nm
II
50 nm
III
50 nm
2216E+Lam
IV
500 nm
V
50 nm
VI
200 nm
2216E+Sta
2216E


a
Phage-WC36-1
Phage-zth2-1
Putative Zonular occludens toxin (425)
Putative minor capsid protein (115)
Putative major capsid protein (79)
Putative minor capsid protein (129)
Single-stranded DNA-binding protein (109)
Replication protein (397)
Transcriptional regulator (121)
Glycoside hydrolase family 18 protein (283)
8.0kb
Hypothetical protein
Structural module
Replication module
Regulatory module
Assembly and secretion modules
Polysaccharides transport and degradation associated proteins
b
Phage-WC36-2
Type II secretion system protein (82)
TonB-system energizer ExbB (113)
O-polysaccharide acetyltransferase protein (686)
Tail protein (342)
Baseplate protein (392)
Assembly protein (118)
Baseplate protein (235)
Transcriptional regulator (202)
DNA-binding protein (91)
DNA-binding protein (80)
Tape measure chaperone (141)
Glucosaminidase domain-containing protein (491)
Portal protein (173)
Minor capsid protein (254)
Portal protein (485)
Terminase large subunit (425)
Terminase small subunit (260)
Glycoside hydrolase family 127 protein (199)
28.3kb
c
Phage-zth2-2
DNA-binding protein (56)
DNA-binding protein (68)
Phage-related DNA recombination protein (287)
Transcriptional regulator (91)
Type II secretion system protein (121)
DNA-binding transcriptional regulator (73)
DNA-binding protein (100)
Single-stranded DNA-binding protein (88)
Phage terminase small subunit protein (164)
Phage terminase large subunit protein (512)
Head-to-tail connector protein (571)
Phage tail fiber-like protein (247)
Major capsid protein (340)
Tail tubular protein (227)
Transglycosylase (599)
40.4kb







d

(Log$_2$ fold change)

0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0

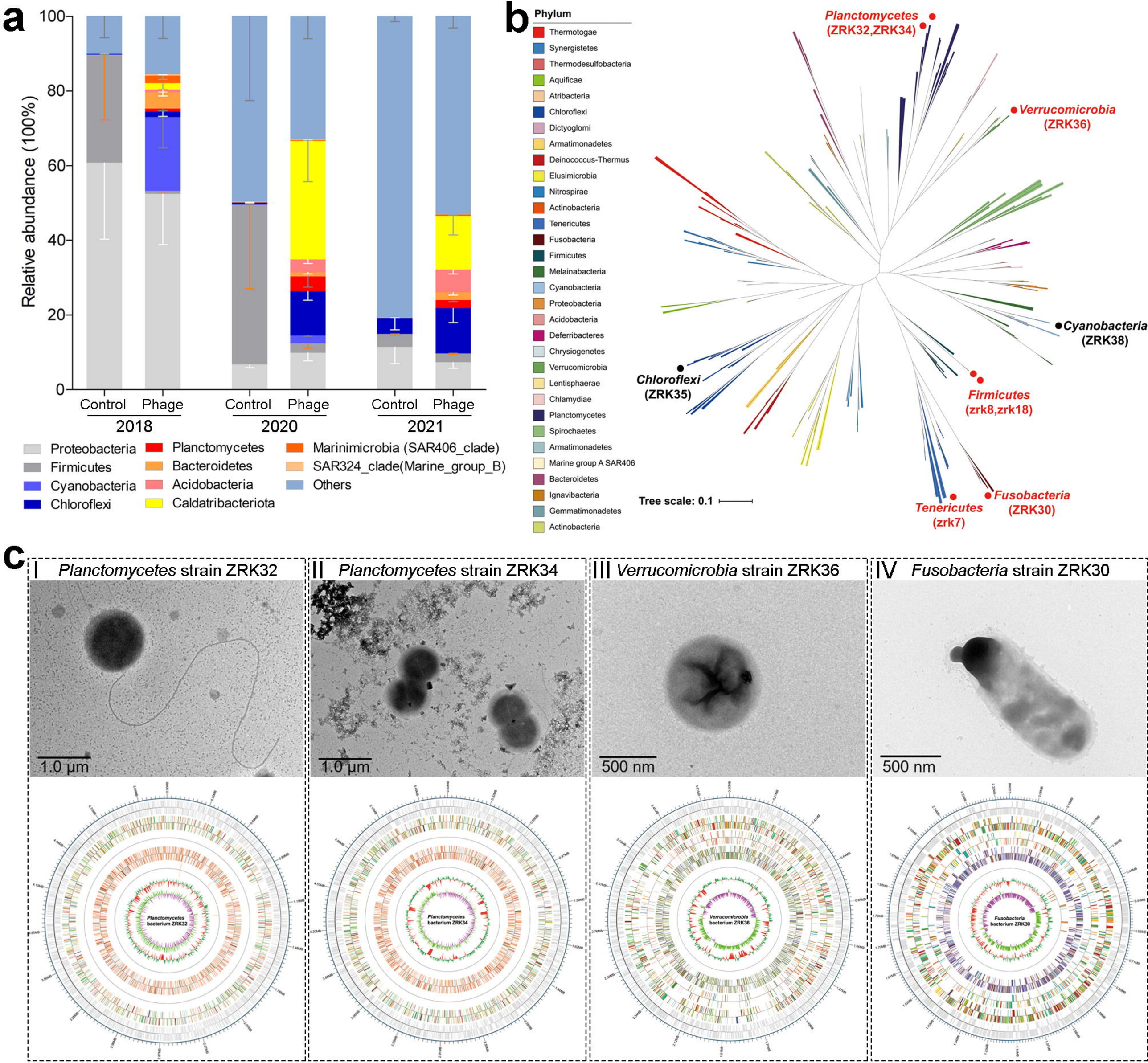
B+S	B+S+P	Locus tag	Product
0.0	2.9	PS273GM_RS07045	TonB dependent receptor
0.0	1.9	PS273GM_RS09170	TonB dependent receptor
0.0	3.2	PS273GM_RS16550	TonB-dependent receptor
0.0	2.1	PS273GM_RS16570	TonB dependent receptor
0.0	1.6	PS273GM_RS00755	Glycoside hydrolase family 15 protein
0.0	2.1	PS273GM_RS03090	Glycoside hydrolase family 5 protein
0.0	3.2	PS273GM_RS09250	Glycoside hydrolase family 65 protein
0.0	4.0	PS273GM_RS09850	Alpha-amylase family glycosyl hydrolase
0.0	2.9	PS273GM_RS03080	Glycosyltransferase family 4 protein
0.0	4.0	PS273GM_RS03105	Glycosyltransferase
0.0	2.0	PS273GM_RS04635	Glycosyltransferase
0.0	2.4	PS273GM_RS08005	Glycosyltransferase family 1 protein
0.0	2.0	PS273GM_RS08550	Glycosyltransferase family 2 protein
0.0	3.0	PS273GM_RS08605	Glycosyltransferase
0.0	3.4	PS273GM_RS14580	Glycosyltransferase
0.0	2.7	PS273GM_RS15905	Glycosyltransferase

e

(Log$_2$ fold change)

0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0

B+P	B+S+P	Locus tag	Product
0.0	2.0	PS273GM_RS07045	TonB-dependent receptor
0.0	2.3	PS273GM_RS16415	TonB-dependent receptor
0.0	2.2	PS273GM_RS16550	TonB-dependent receptor
0.0	1.7	PS273GM_RS00755	Glycoside hydrolase family 15 protein
0.0	2.2	PS273GM_RS03090	Glycoside hydrolase family 5 protein
0.0	1.6	PS273GM_RS09250	Glycoside hydrolase family 65 protein
0.0	2.7	PS273GM_RS09850	Alpha-amylase family glycosyl hydrolase
0.0	1.9	PS273GM_RS02235	Glycosyltransferase family 20 protein
0.0	3.4	PS273GM_RS03080	Glycosyltransferase family 4 protein
0.0	2.6	PS273GM_RS03105	Glycosyltransferase
0.0	1.7	PS273GM_RS04635	Glycosyltransferase
0.0	2.7	PS273GM_RS08005	Glycosyltransferase family 1 protein
0.0	2.4	PS273GM_RS08550	Glycosyltransferase family 2 protein
0.0	1.6	PS273GM_RS15145	Glycosyltransferase
0.0	3.5	PS273GM_RS15315	Glycosyltransferase family 4 protein
0.0	2.1	PS273GM_RS15335	Glycosyltransferase family 2 protein
0.0	2.0	PS273GM_RS15415	Glycosyltransferase family 1 protein
0.0	2.0	PS273GM_RS15905	Glycosyltransferase
0.0	3.3	PS273GM_RS19510	Glycosyltransferase family 1 protein
0.0	2.4	PS273GM_RS20900	Glycosyltransferase family 2 protein
0.0	1.5	PS273GM_RS23165	Glycosyltransferase family 2 protein


a
Relative abundance (100%)
100
80
60
40
20
0
Control
Phage
2018
2020
2021
Proteobacteria
Firmicutes
Cyanobacteria
Chloroflexi
Planctomycetes
Bacteroidetes
Acidobacteria
Caldatribacteriota
Marinimicrobia (SAR406_clade)
SAR324_clade(Marine_group_B)
Others
b
Phylum
Thermotogae
Synergistetes
Thermodesulfobacteria
Aquificae
Atribacteria
Chloroflexi
Dictyoglomi
Armatimonadetes
Deinococcus-Thermus
Elusimicrobia
Nitrospirae
Actinobacteria
Tenericutes
Fusobacteria
Firmicutes
Melainabacteria
Cyanobacteria
Proteobacteria
Acidobacteria
Deferribacteres
Chrysiogenetes
Verrucomicrobia
Lentisphaerae
Chlamydiae
Planctomycetes
Spirochaetes
Armatimonadetes
Marine group A SAR406
Bacteroidetes
Ignavibacteria
Gemmatimonadetes
Actinobacteria
Planctomycetes (ZRK32,ZRK34)
Verrucomicrobia (ZRK36)
Cyanobacteria (ZRK38)
Chloroflexi (ZRK35)
Firmicutes (zrk8,zrk18)
Tenericutes (zrk7)
Fusobacteria (ZRK30)
Tree scale: 0.1
c
I Planctomycetes strain ZRK32
II Planctomycetes strain ZRK34
III Verrucomicrobia strain ZRK36
IV Fusobacteria strain ZRK30
1.0 μm
1.0 μm
500 nm
500 nm
Planctomycetes bacterium ZRK32
Planctomycetes bacterium ZRK34
Verrucomicrobia bacterium ZRK36
Fusobacteria bacterium ZRK30