Quorum Sensing Promotes Phage Infection in *Pseudomonas aeruginosa* PAO1

Guanhua Xuan, Hong Lin, Lin Tan, Gang Zhao, Jingxue Wang

Food Safety Laboratory, College of Food Science and Engineering, Ocean University of China, Qingdao, China

**ABSTRACT** Quorum sensing (QS) is used to coordinate social behaviors, such as virulence and biofilm formation, across bacterial populations. However, the role of QS in regulating phage-bacterium interactions remains unclear. Preventing phage recognition and adsorption are the first steps of bacterial defense against phages; however, both phage recognition and adsorption are a prerequisite for the successful application of phage therapy. In the present study, we report that QS upregulated the expression of phage receptors, thus increasing phage adsorption and infection rates in *Pseudomonas aeruginosa*. In *P. aeruginosa* PAO1, we found that *las* QS, instead of *rhl* QS, upregulated the expression of *galU* for lipopolysaccharide synthesis. Lipopolysaccharides act as the receptor of the phage vB_Pae_QDWS. This *las* QS-mediated phage susceptibility is a dynamic process, depending on host cell density. Our data suggest that inhibiting QS may reduce the therapeutic efficacy of phages.

**IMPORTANCE** Phage resistance is a major limitation of phage therapy, and understanding the mechanisms by which bacteria block phage infection is critical for the successful application of phage therapy. In the present study, we found that *Pseudomonas aeruginosa* PAO1 uses *las* QS to promote phage infection by upregulating the expression of *galU*, which is necessary for the synthesis of phage receptor lipopolysaccharides. In contrast to the results of previous reports, we showed that QS increases the efficacy of phage-mediated bacterial killing. Since QS upregulates the expression of virulence factors and promotes biofilm development, which are positively correlated with lipopolysaccharide production in *P. aeruginosa*, increased phage susceptibility is a novel QS-mediated trade-off. QS inhibition may increase the efficacy of antibiotic treatment, but it will reduce the effectiveness of phage therapy.

**KEYWORDS** *P. aeruginosa*, *las* quorum sensing, phage, adsorption, *galU*, lipopolysaccharide

Bacteriophage (phage) therapy has been suggested as an alternative to conventional antibiotic treatment in clinical practice (1). However, a successful phage therapy requires that we first overcome the wide variety of antiphage defense strategies that are present in bacterial hosts, including the CRISPR-Cas system, abortive infection systems, and prevention of phage adsorption (2, 3). Although the mechanisms of phage resistance have been widely studied, little is known about phage-host dynamics in the context of the microbial community. Quorum sensing (QS) is widely used by bacteria to coordinate group behavior, and it depends on the production and release of signal molecules termed “autoinducers” (AIs) (4, 5).

Phage adsorption is the first step by which phages recognize and bind to the bacterial cell surface (6). Recently, QS has been found to be involved in the antiphage process by reducing the number of phage receptors. *Vibrio anguillarum* exhibits downregulation of phage receptor OmpK expression in response to *N*-acyl-L-homoserine lactones (AHL), a class of QS-signaling molecules used by many Gram-negative bacteria (7). *Vibrio cholerae* modulates its sensitivity to phage infection via a mechanism that
downregulates the phage receptor (lipopolysaccharide [LPS] O-antigen) and upregu-
lates the expression of the hemagglutinin protease HAP when supplemented with the
autoinducers CAI-1 or AI-2 (8). Both the aforementioned studies were based on the
assumption that QS negatively regulates the expression of receptors required for
phage infection. Phages can also communicate via the QS-like “arbitrium” system to al-
ter infection outcomes (9, 10). V. cholerae carrying the QS receptor VqmA expresses
the autoinducer 3,5-dimethylpyrazin-2-ol (DPO), which acts as a cue for prophage in-
duction when host cell densities are high (10). However, all QS-regulated phage resistance
models have been developed only in Vibrio spp., and even though QS has been
observed in several bacterial species, it is unknown whether QS plays a role in phage
infection by modulating phage adsorption in other bacterial species.

_Pseudomonas aeruginosa_ is a Gram-negative opportunistic pathogen which is
responsible for the morbidity and mortality of patients with cystic fibrosis (11). Several
QS systems have been described in _P. aeruginosa_, including the las and rhl systems,
which recognize AHL signals (12, 13). In the las system, LasI synthesizes the signaling
molecule N-(3-oxododecanoyl)-L-homoserine lactone (3O-C_{12}-HSL). LasR binds to 3O-
C_{12}-HSL and functions as a transcriptional activator. In the rhl system, RhlI synthesizes
C4-homoserine lactone (C4-HSL), which, in conjunction with RhlR, activates the expres-
sion of a second set of QS-related genes. The las system positively regulates the rhl sys-
tem. Approximately 6% of _P. aeruginosa_ genes are regulated by AHL-based QS systems
(14, 15). Therefore, we speculated that certain receptors for _P. aeruginosa_ phages may
also be regulated by QS.

In the present study, we isolated and characterized _Pseudomonas_ phage vB_Pae_QDWS,
which could recognize and absorb LPS of _P. aeruginosa_. We identified a QS-regulated
phage infection mechanism in _P. aeruginosa_ PAO1, which is a model organism for the ge-
nus _Pseudomonas_. Our data showed that the expression of _galU_, which is a key gene for
LPS synthesis, was upregulated by las QS, resulting in an increase in phage adsorption
rate and subsequently increasing phage infection. In contrast to the results presented by
previous studies, our study suggests that inhibiting QS may reduce the therapeutic ef-
ciency of phage systems; this finding may help in filling several gaps in the field.

RESULTS

Phage genome analysis. The genome of phage vB_Pae_QDWS is a 43,170-bp, dou-
ble-stranded DNA molecule with 62.3% G+C content and contains 53 coding DNA
sequences (CDSs), which are transcribed in the same direction (Fig. 1). Bioinformatics anal-
ysis revealed 21 gene products with known functions, and the remaining 32 genes were
presumed to encode hypothetical proteins. An overview of the functional prediction of
phage-encoded gene products is provided in Table 1. No genes related to phage lysog-
eny were identified, confirming the lytic characteristics of phage vB_Pae_QDWS.
Compared to other phage genome sequences obtained from the NCBI GenBank reposi-
tory, the genome sequence of phage vB_Pae_QDWS most closely resembled that of the
_P. aeruginosa_ phage phiKMV (16, 17), with a similarity of 94% and a coverage of 92%. The
complete genome sequence of phage vB_Pae_QDWS has been deposited in GenBank
under the accession number MZ687409.

One-step growth curve analysis revealed that phage vB_Pae_QDWS had a latency
period of approximately 10 min (Fig. S1 in the supplemental material). The final titers
of phage exceeded 10^{11} PFU/mL, indicating that they were highly infective toward _P.
aeruginosa_ PAO1. Phylogenetic analysis based on the amino acid sequence of the large
subunit of the terminase protein from each phage showed that _P. aeruginosa_ phage vB_Pae_QDWS
was most closely related to _Phikmvirus_, which belongs to subfamily
Klyovirinae and family Autographiviridae (Fig. 2).

las QS influences phage resistance. We investigated the effects of the las and rhl
QS systems on phage vB_Pae_QDWS resistance. Deletion of lasI increased the resist-
ance of _P. aeruginosa_ PAO1 to phage infection. However, the deletion of rhlI did not
affect the transparency of the plaques, suggesting that this deletion did not affect the
resistance of the bacteria to this phage (Fig. 3). When exogenous 3O-C_{12}-HSL was
added, both PaΔlasI and PaΔlasIΔrhlI restored sensitivity toward phage vB_Pae_QDWS (Fig. 3). The plaques of the complemented strain ΔlasI::lasI were more transparent than that of the strain PaΔlasI (Fig. S2). These results suggest that the las QS system, but not the rhl QS system, positively regulates phage sensitivity of P. aeruginosa PAO1.

Phage vB_Pae_QDWS reduced cell density in the cultures of wild-type PAO1 and QS mutants PaΔlasI, PaΔrhlI, and PaΔlasIΔrhlI compared to that in control cultures without the phage. However, PaΔlasI and PaΔlasIΔrhlI exhibited a slower reduction in cell density within 2 h and a more rapid regrowth of cells during the remainder of the incubation period than PAO1 and PaΔrhlI (Fig. 4).

**FIG 1** Genome organization of phage vB_Pae_QDWS. The first circles represent the 53 open reading frames (ORFs) on the sense strand of the phage. The second circle shows G+C content. The red outward and blue inward arrows indicate that the G+C content of that region is higher or lower than the average G+C content of the whole genome, respectively. The third circle shows the G+C skew.
**las QS affects phage adsorption.** To investigate the mechanisms associated with the altered susceptibility of *P. aeruginosa* PAO1 strains to phage infections, the adsorption rate of phage vB_Pae_QDWS by different *P. aeruginosa* PAO1 strains was examined. The single Δ*lasI* and double Δ*lasIΔrhlI* mutants exhibited pronounced reduction in phage adsorption rates compared to that by the wild-type strain. The single Δ*rhlI* mutant, however, exhibited no differences in adsorption rate compared to that of the wild-type strain (Fig. 5). Thus, *las* QS positively regulated phage susceptibility by increasing the phage adsorption rate.

Na4IO4 was used to treat *P. aeruginosa* cells and damage LPS. Adsorption assay results showed that Na4IO4 treatment led to a dramatic reduction in the adsorption rates. In contrast, sodium acetate (CH3COONa) treatment resulted in a modest reduction in the adsorption rate, which may be due to the toxic effects of the solvent (Fig. 6A). The extracted LPS was used for adsorption assays. There was a significant

**TABLE 1** Functional genes of bacteriophage vB_Pae_QDWS

| ORF no. | Function                              | Sequence length (aa) |
|---------|---------------------------------------|----------------------|
| 14      | DNA-binding protein                   | 269                  |
| 15      | DNA primase                          | 180                  |
| 18      | DNA_B helicase                       | 397                  |
| 20      | DNA ligase                           | 315                  |
| 23      | DNA polymerase                       | 807                  |
| 27      | Endonuclease VII                     | 146                  |
| 31      | RNA polymerase                       | 815                  |
| 35      | Head-tail connector protein          | 510                  |
| 36      | Capsid and scaffold protein          | 322                  |
| 37      | Capsid protein                       | 335                  |
| 38      | Tail tubular protein A               | 184                  |
| 39      | Tail tubular protein B               | 835                  |
| 41      | Internal virion protein              | 898                  |
| 42      | Internal virion protein              | 1,337                |
| 43      | Particle protein                     | 251                  |
| 45      | Structural protein                   | 288                  |
| 46      | Tail fiber protein                   | 201                  |
| 48      | Terminase large subunit              | 601                  |
| 49      | Holin                                | 66                   |
| 50      | Endolysin                            | 160                  |
| 52      | Minor structural protein             | 104                  |
| Others  | Hypothetical protein                 |                      |

---

**FIG 2** Phylogenetic tree based on amino acid sequences of terminase large subunit protein of phage vB_Pae_QDWS and related phages.
increase in the adsorption rate when LPS was added to the reaction system (Fig. 6B). Thus, LPS was recognized as a receptor for *Pseudomonas* phage vB_Pae_QDWS.

**GalU expression is activated by las QS.** *GalU* is involved in *P. aeruginosa* LPS core synthesis (18, 19). The expression of *galU* is dependent on the growth phase, with its expression at high cell densities being higher than that at low cell densities (Fig. 7A). High cell density should lead to higher phage susceptibility due to the synthesis of more LPS receptors. As expected, the adsorption rate of stationary-phase cells was significantly higher than that of logarithmic-phase cells, and the optical density of stationary-phase cells decreased faster than that of the logarithmic-phase cells (Fig. S3). We also investigated *galU* expression in different *P. aeruginosa* PAO1 strains. When *lasI* was deleted, the expression level of *galU* was significantly decreased. However, *galU* expression did not change in strain PaΔ*rhlI* compared to that in the wild-type strain (Fig. 7B). Hence, we concluded that *galU* expression is regulated by *las* QS.

**DISCUSSION**

Taken together, our findings indicate that *las* QS regulates *galU* expression, which is essential for LPS receptor synthesis and subsequently affects the susceptibility of *P. aeruginosa* PAO1 to phage vB_Pae_QDWS infection. A schematic of the proposed mechanism is shown in Fig. 8. Disruption of *las* QS led to an increase in bacterial resistance to phage infection; however, this resistance decreased after the addition of synthetic 3O-C<sub>12</sub>-HSL (Fig. 3). These results were further supported by the results of growth and adsorption assays of *P. aeruginosa* PAO1 and its QS mutants (Fig. 4 and 5). Cells at high density express more receptors and are more susceptible to phage infection than cells at low cell density. Thus, our results suggest that QS positively regulates phage susceptibility in PAO1 cells.

The expression of CRISPR-Cas is regulated by QS. *Pseudomonas aeruginosa* strain PA14 and *Serratia* use QS to activate *cas* gene expression, which protects the bacteria against phage infection (20, 21). In contrast, in the present study, we showed that PAO1 QS could improve the efficacy of phage therapy. Broniewski reported that inhibiting QS may reduce the therapeutic efficacy of phages (15), which is consistent with our results. It is likely that QS plays a dual role by decreasing phage adsorption rates and favoring the evolution of CRISPR immunity in *P. aeruginosa*. Since PAO1 does not possess the CRISPR-Cas system, whereas PA14 does (22), QS may have different effects upon phage infection in both strains. Cells of strain PAO1 are easily lysed by phages...
that use LPS as a receptor, under high cell density. We showed that *P. aeruginosa* PAO1 QS increased phage adsorption, which is different from previous observations in *V. anguillarum*, *V. cholerae*, and *Escherichia coli* (10, 23, 24). However, type IV pili are recognized by many *Pseudomonas* phages and are positively regulated by QS, which is in agreement with our results (15, 25, 26). The regulation of phage resistance by QS is clearly diverse and complex.

The genes *wzy*, *wbpD*, *galU*, and *wzz* are closely related to LPS synthesis (18, 19, 27, 28). In the present study, transcriptional analysis revealed that *galU* expression was closely related to *las* QS (Fig. 7), but the expression of *wzy*, *wbpD*, and *wzz* was not related to *las* QS (Fig. S4). Furthermore, the expression of *galU* was elevated at high cell density and regulated by *las* QS. However, *rhl* QS had no effect on *galU* expression or the susceptibility of the bacterial strain to phage vB_Pae_QDWS (Fig. 3). Transcriptional data also showed that *galU* was not controlled by *rhl* QS (Fig. 7B). The *las* system exerts positive control over the *rhl* system in *P. aeruginosa* (29). In some cases, the two systems have opposing effects on the same target. There are many genes that are specifically regulated by either the *las* or the *rhl* system (30, 31). The *las* and *rhl* QS systems regulate 315 genes, while the *rhl* system regulates approximately 112 genes (32, 33). It is, therefore, expected that phage vB_Pae_QDWS infection efficiency is controlled by *las* QS rather than by *rhl* QS.
QS-mediated phage infection is a dynamic process. las QS is usually affected by different growth conditions (31, 34) and bacterial community composition (35–37), which may, in turn, affect phage resistance. In the present study, we found that phage resistance also depends on the growth phase of the host. Stationary-phase PAO1 cells were more susceptible to infection than logarithmic-phase cells (Fig. S3). This discovery will be significant for guiding the preparation of high-titer phage vB_Pae_QDWS because there is no optimal, universal method for phage amplification (38).

Our findings represent an example of evolutionary trade-offs. P. aeruginosa relies on QS to regulate several functions, including the expression of virulence factors and biofilm development (11, 39). The virulence factor LPS acts as a phage receptor that is conducive to phage infection, and its synthesis pathway is positively regulated by QS (Fig. 7) (40). QS has the potential to mediate trade-offs between LPS-based bacterial virulence and phage sensitivity. LPS is one of the factors involved in biofilm formation (41), which is positively regulated by QS (42), increases the resistance of microorganisms toward biocides, and reduces antibiotic treatment efficacy. Although the coevolutionary mechanisms involved in antibiotic resistance and phage sensitivity have been widely studied (43, 44), our discovery adds another example of pleiotropy involving antibiotic resistance and phage sensitivity driven by QS.

In summary, we discovered that las QS plays a significant role in regulating phage vB_Pae_QDWS susceptibility in PAO1. GalU, which contributes to LPS synthesis, is positively regulated by las QS. Since LPS is a common receptor for Pseudomonas phages, las QS-regulated phage killing is probably a conserved mechanism.

**FIG 5** Adsorption rate of phage vB_Pae_QDWS by its host strain Pseudomonas aeruginosa PAO1 wild-type (WT) and quorum-sensing (QS) mutants. Data are averages of six samples with standard deviations (error bars). ***, P < 0.01 (Student’s paired t test).

**FIG 6** Identification of lipopolysaccharide (LPS) as an important receptor for Pseudomonas phage vB_Pae_QDWS infection. (A) NaJIO₃ treatment significantly reduced the adsorption of Pseudomonas phage vB_Pae_QDWS. (B) Extracted LPS was used for adsorption assays. The adsorption rate was increased in the LPS-treated group compared to that in the control group. Data are averages of three samples with standard deviations (error bars).
MATERIALS AND METHODS

Strains, plasmids, and growth conditions. Detailed information of the strains and plasmids used in the present study is presented in Table S1 in the supplemental material. All PCR primers used in the study are listed in Table S2. *P. aeruginosa* was cultured in Luria-Bertani (LB) medium at 37°C. Gentamicin (30 μg/mL) and tetracycline (30 μg/mL) were added as required.

Isolation and purification of phages. Phages specific for *P. aeruginosa* PAO1 were isolated from sewage samples collected in Qingdao, China. The sewage samples were centrifuged at 2,348 × g for 10 min and then filtered through a 0.22-μm-pore-size filter (Millipore, Burlington, MA, USA). The filtrate was mixed with 50 mL of log-phase *P. aeruginosa* PAO1 cells and incubated at 37°C with 200-rpm rotary agitation for 12 h. The resulting culture suspension was centrifuged and filtered, as described above. Phages were isolated using the double-layer agar plate method (45). Single plaques were separated by stinging with a pipette tip into the plaque followed by resuspending the phages in SM buffer (100 mM NaCl, 8 mM MgSO4, 50 mM Tris-HCl, pH 7.5). After multiple rounds of purification, the phage was verified by electron microscopy.

Gene sequencing and bioinformatic analysis. Genomic DNA of phage vB_Pae_QDWS was extracted using a bacterial DNA kit (Omega) according to the manufacturer’s instructions. DNA sequencing was performed by Shanghai Biozeron Biotechnology Co., Ltd. (Shanghai, China). Phage DNA library construction and genome sequencing were performed using the Illumina MiSeq sequencing platform to obtain paired-end reads. The genome sequence was assembled using ABySS (http://www.bcgsc.ca/platform/bioinfo/software/abyss). GapCloser software (https://sourceforge.net/projects/soapdenovo2/files/GapCloser/) was subsequently used to fill the remaining local internal gaps and correct single nucleotide polymorphisms (SNPs) for final assembly. Genome annotation was performed using the ab initio prediction method. Gene

**FIG 7** las QS activates *galU* expression. (A) Relative *galU* expression measured by RT-qPCR in *Pseudomonas aeruginosa* PAO1 cells at low and high cell densities (OD600, 0.8 and 2.5, respectively). The reference gene was *rplS*. (B) Relative *galU* expression at high cell density in wild-type (WT) PAO1 and the designated QS mutants. Data are averages of three samples with standard deviations (error bars). ***, P < 0.01 (paired t test).

**FIG 8** Schematic representation of the mechanism by which las QS regulates the resistance of *Pseudomonas aeruginosa* PAO1 to phage vB_Pae_QDWS. The las QS positively regulates the expression of *galU*, which is involved in LPS biosynthesis, thereby promoting phage adsorption.
models were identified using GeneMark server (http://topaz.gatech.edu/GeneMark/generemarks.cgi). All gene models were evaluated by performing BLAST searches using the nonredundant (nr) NCBI GenBank database, Swiss-Prot, KEGG, and COG to perform functional annotation.

The terminal enzyme large subunit sequence of phage vB_Pae_QDWS was used as a query to identify homologues in sequenced bacterial genomes at NCBI (http://blast.ncbi.nlm.nih.gov/). Eleven terminase large subunit protein sequences of different phages with high identity were selected, combined with the seed protein from phage vB_Pae_QDWS for phylogenetic tree analysis. Multiple-sequence alignment was carried out using ClustalW (46), and the tree was constructed by MEGA version 7.0 (47) using neighbor joining with a pairwise deletion, p-distance distribution, and bootstrap analysis of 1,000 repeats as the parameters.

Gene knockout and complementation. All deletions in *P. aeruginosa* PAO1 were performed according to a previously published method (48). The primers used for inactivation of *P. aeruginosa* PAO1, *P. aeruginosa* PAO1 and *P. aeruginosa* PAO1 and *P. aeruginosa* PAO1 were listed in Table S2. The mutants *P. aeruginosa* PAO1, *P. aeruginosa* PAO1, and *P. aeruginosa* PAO1 were selected using colony PCR. The complemented strain was constructed by transforming pBBR::lasI with gentamicin resistance into *P. aeruginosa* PAO1 to generate *P. aeruginosa* PAO1.

**Phage sensitivity assay.** Overnight cultures of *P. aeruginosa* PAO1, *P. aeruginosa* PAO1, and *P. aeruginosa* PAO1 strains were inoculated in fresh LB medium for 5 h until the early stationary phase (optical density at 600 nm (OD600) 2) was reached. Then, 100 μL of the culture was mixed with 5 mL of melted 1% agar and LB medium to prepare double-layered agar plates. For N-(3-oxododecanoyl)-l-homoserine lactone (3O-C12-HSL) chemical complementation experiments, 3O-C12-HSL was stored in dimethyl sulfoxide (DMSO) and added to the melted 1% agar and LB medium to form a double layer of agar at a concentration of 10 μM. In control samples, an equivalent volume of DMSO was added as a solvent control. The phages were then subjected to 10-fold gradient dilution in SM buffer, and 3-μL aliquots were spotted onto a plate and incubated at 37°C for 12 h.

**Adsorption rate assay.** Overnight cultures (OD600 0.05) of *P. aeruginosa* PAO1, *P. aeruginosa* PAO1, and *P. aeruginosa* PAO1 were inoculated in fresh LB medium. The cells were cultured until the OD600 reached 2.5, followed by 10-fold dilution in LB medium. To facilitate phage adsorption, 0.5 mL of phage solution (10^6 PFU/mL) was mixed with the diluted cell suspension (0.5 mL) and incubated at 37°C for 5 min. LB broth mixed with phage without bacteria was used as the control. The cultures were then centrifuged at 4°C for 10 min, and then their titers were determined.

**Identification of phage receptor.** Overnight cultures of the *P. aeruginosa* strains were diluted (1:100) in LB medium and incubated at 37°C until the OD600 reached 2. The cells were then treated with 50 mM Na4IO4 at 37°C for 30 min. A control cell suspension containing only solvent CH3COONa was prepared. The phage adsorption rate was determined as described above.

**RT-qPCR.** Cells were harvested at the indicated OD600, RNA was purified using the TRIzol RNA purification kit (catalog no. 12183555; Invitrogen). Total cDNA was synthesized using the HiScript II reverse transcriptase kit (Vazyme). Real-time quantitative reverse transcription-PCR (RT-qPCR) was performed using the SYBR green real-time PCR master mix and StepOnePlus real-time PCR system (ABI). To calculate the relative expression levels of the tested genes, *P. aeruginosa* PAO1 was used as the reference gene.

**Statistical analysis.** Data were expressed as means ± standard deviation, and differences between groups were evaluated using Student’s *t* test for individual measurements (Fig. 5 and 7) or two-way analysis of variance (ANOVA) for data containing repeated measurements of the same cultures (Fig. 4). Analysis was carried out using GraphPad Prism v5.0 software.

**Data availability.** The complete genome sequence of phage vB_Pae_QDWS has been deposited in GenBank under the accession number MZ2687409.

**SUPPLEMENTAL MATERIAL**

Supplemental material is available online only.

**FIG S1**, TIF file, 0.8 MB.
**FIG S2**, TIF file, 1.5 MB.
**FIG S3**, TIF file, 0.8 MB.
**FIG S4**, TIF file, 0.9 MB.
**TABLE S1**, DOCX file, 0.03 MB.
**TABLE S2**, DOCX file, 0.02 MB.

**ACKNOWLEDGMENTS**

This work was supported by the National Key Research and Development Program (2017YFC1600703 and 2016YFD0400105) and China Agriculture Research System (CARS-47).
G.X. acquired and analyzed most data, L.T. and G.Z. performed the adsorption rate assay, H.L. supervised the research, and J.W. designed the study and wrote the manuscript. We have no conflict of interests to declare.

REFERENCES

1. Kortright KE, Chan BK, Koff JL, Turner PE. 2019. Phage therapy: a renewed approach to combat antibiotic-resistant bacteria. Cell Host Microbe 25: 210–232. https://doi.org/10.1016/j.chom.2019.01.014.

2. Torres-Barcelo C. 2018. Phage therapy faces evolutionary challenges. Viruses 10:323. https://doi.org/10.3390/v10060323.

3. Labrie SJ, Samson JE, Moineau S. 2010. Bacteriophage resistance mechanisms. Nat Rev Microbiol 8:317–327. https://doi.org/10.1038/nrmicro2315.

4. Miller MB, Bassler BL. 2001. Quorum sensing in bacteria. Annu Rev Microbiol 55:165–199. https://doi.org/10.1146/annurev.micro.55.1.165.

5. Pearson JP, Pesci EC, Iglewski BH. 1997. Roles of Pseudomonas aeruginosa WbpM in regulating wbpA expression and virulence. Mol Microbiol 24:685–695. https://doi.org/10.1111/j.1365-2958.1997.tb00264.x.

6. Li P, Lin H, Mi ZQ, Xing SZ, Tong YG, Wang JX. 2019. Screening of polyvalent bacteriophages. Sci Rep 6:37956. https://doi.org/10.1038/srep37956.

7. G.X. acquired and analyzed most data, L.T. and G.Z. performed the adsorption rate assay, H.L. supervised the research, and J.W. designed the study and wrote the manuscript. We have no conflict of interests to declare.

8. Saraf MS, Gomila M, Benkeser A, Lalaucet J, Garcia-Valdes E. 2014. Genome analysis of environmental and clinical P. aeruginosa isolates from sequence type-1146. PLoS One 9:e107754. https://doi.org/10.1371/journal.pone.0107754.

9. Silpe JE, Bassler BL. 2019. Phage-encoded LuxR-type receptors responsive to host-produced bacterial quorum-sensing autoinducers. mBio 10:e00638-19.https://doi.org/10.1128/mBio.00638-19.
38. Skaradzińska A, Ochocka M, Śliwka P, Kuźmińska-Bajor M, Skaradziński G, Friese A, Roschanski N, Murugaiyan J, Roesler U. 2020. Bacteriophage amplification - a comparison of selected methods. J Virol Methods 282:113856. https://doi.org/10.1016/j.jviromet.2020.113856.

39. Xuan G, Lv C, Xu H, Li K, Liu H, Xia Y, Xun L. 2021. Sulfane sulfur regulates LasR-mediated quorum sensing and virulence in Pseudomonas aeruginosa PAO1. Antioxidants 10:1498. https://doi.org/10.3390/antiox10091498.

40. Bartell JA, Blazier AS, Yen P, Thogersen JC, Jelsbak L, Goldberg JB, Papin JA. 2017. Reconstruction of the metabolic network of Pseudomonas aeruginosa to interrogate virulence factor synthesis. Nat Commun 8:14631. https://doi.org/10.1038/ncomms14631.

41. Alshalchi SA, Anderson GG. 2015. Expression of the lipopolysaccharide biosynthesis gene lpxD affects biofilm formation of Pseudomonas aeruginosa. Arch Microbiol 197:135–145. https://doi.org/10.1007/s00203-014-1030-y.

42. Sakuragi Y, Kolter R. 2007. Quorum-sensing regulation of the biofilm matrix genes (pel) of Pseudomonas aeruginosa. J Bacteriol 189:5383–5386. https://doi.org/10.1128/JB.00137-07.

43. Burmeister AR, Turner PE. 2020. Trading-off and trading-up in the world of bacteria-phage evolution. Curr Biol 30:R1120–R1124. https://doi.org/10.1016/j.cub.2020.07.036.