

Bacteriophages from Arsenic-Resistant Bacteria Transduced Resistance Genes, which Changed Arsenic Speciation and Increased Soil Toxicity

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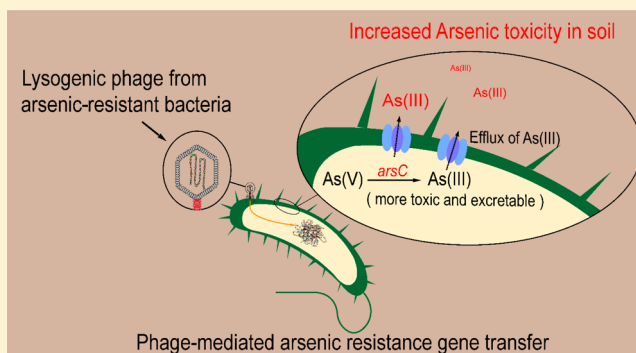
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Supporting Information

ABSTRACT: Lysogenic phages are known to serve as transfer vectors for bacterial genes involved in biotransformation of various environmental pollutants. However, their role in arsenic-contaminated environments is largely undocumented. Here, lysogenic phages were chemically induced (with mitomycin C) in soil samples from two contaminated sites, and arsenic resistance genes *arsC* (coding for As(V) reduction to excretable (via efflux pumps) but more toxic As(III)) and *arsM* (coding for As(III) methylation) were detected in these phage genomes. The relative abundance of these genes (per phage particle) was positively correlated with that in the corresponding indigenous soil bacterial communities (resistance gene per 16S rRNA), with $R^2 = 0.974$ for *arsC* and 0.761 for *arsM*. Microcosm studies with 100 mg/kg of arsenic soil showed that phages (amended at 5.0×10^7 phages per gram soil) enhanced the propagation of *arsC* by 122-fold and *arsM* by 575-fold, relative to unamended soil. This increased the As(III) concentration by 4.3 mg/kg (214%) after 15 days but also enabled arsenic methylation (to 0.8 mg/kg). Earthworm avoidance tests corroborated the increase in soil arsenic ecotoxicity after phage addition. Overall, this study demonstrates that arsenic resistance genes transduction by lysogenic phages can result in an overlooked but important phenomenon: a change in arsenic speciation and a significant increase in soil ecotoxicity.



INTRODUCTION

Phages (bacterial virus) shape the structure and function of bacterial communities through horizontal gene transfer (HGT) and bacterial predation in almost all identified ecosystems,^{1–3} which can in turn have profound impacts on biogeochemical cycling.⁴ Unlike lytic phages that rapidly kill their hosts,⁵ lysogenic phages can integrate their genome stably into the host genome to form prophages,^{6,7} which has the potential to introduce beneficial phage-harbored bacterial functional genes to the host.^{8,9}

Establishment of lysogeny often occurs when environmental factors negatively impact phage decay rates or productive infection (e.g., intense irradiation or nutrient limitation), leading phages to take refuge inside hosts.^{10,11} This linkage between phage and host survival may select for lysogenic phages to transduce genes that enhance host fitness.¹² For instance, some lysogenic phages encode genes that mitigate host photoinhibition under intense sunlight to protect themselves and their hosts against intense irradiation.¹⁰ However, very few studies have investigated the impact of lysogenic phages on contaminated soils, where microbial

communities have the potential to influence contaminant biotransformation and mobility.

Arsenic contamination of soil and groundwater poses a serious threat to public health due to its widespread occurrence, toxicity, and persistence.¹³ Resistant bacteria often harbor arsenic intracellular detoxification genes (AIDGs) that encode defense mechanisms such as arsenic oxidation (*aiiA*), arsenic respiratory reduction (*arrA*),¹⁴ As(V) reduction to As(III) (*arsC*) followed by As(III) excretion,¹⁵ or As(III) methylation (*arsM*).^{16,17} Lysogenic phages may infect arsenic-resistant hosts and acquire AIDGs for subsequent transduction and expression during lysogeny to avoid inactivation by high arsenic concentrations. However, the occurrence of prophages in arsenic-resistant bacteria and their interactions with their hosts remain largely unknown. Considering that phage-like elements have been identified in

Received: September 26, 2019

Revised: October 23, 2019

Accepted: October 23, 2019

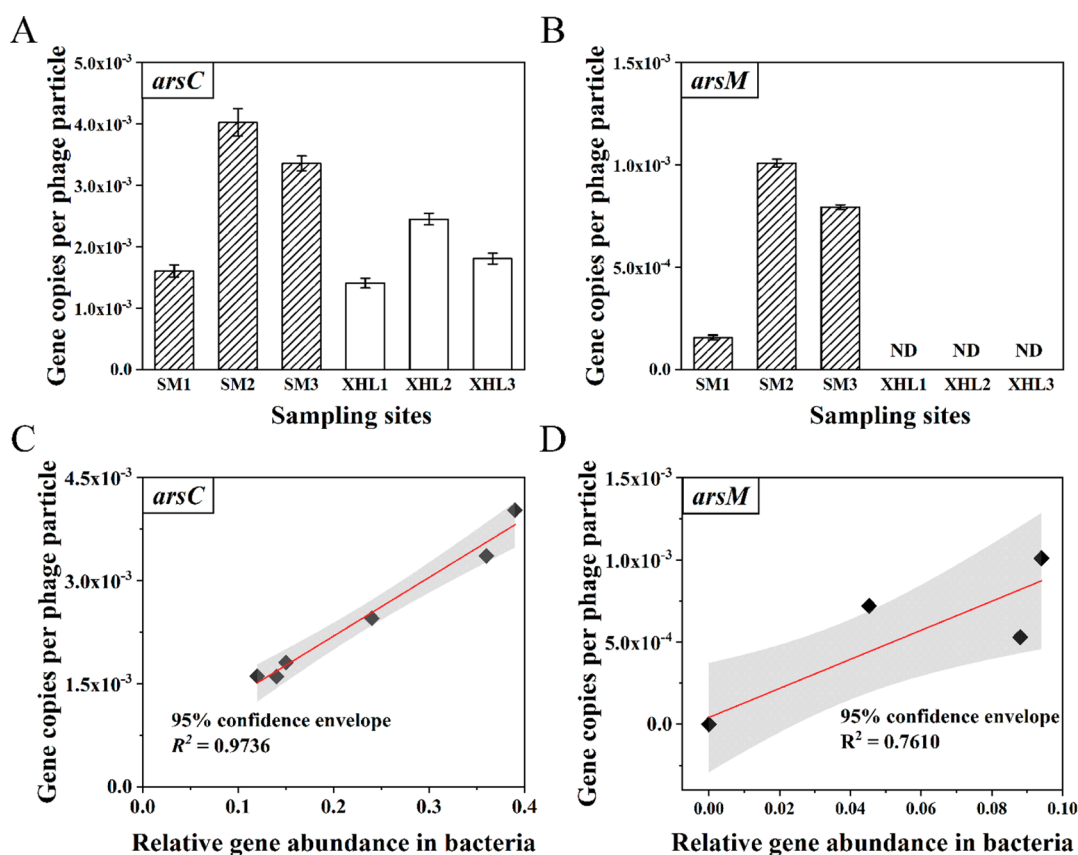


Figure 1. Relative abundance of lysogenic phage-encoded AIDGs in soil samples from three different locations at two contaminated sites. The lysogenic phages were chemically induced from the soil bacteria. The copy numbers of *arsC* (A) and *arsM* (B) genes were normalized to the total number of phage particles, counted as viral-like particles. Error bars indicate the standard deviation of triplicates. Gene *arsM* was not detected (ND) in the XHL site. Correlations are also shown for relative AIDG abundance in phages versus bacteria: (C) *arsC* and (D) *arsM*.

nearly all sequenced bacterial species,¹⁸ it is important to investigate the occurrence of AIDGs in prophages of arsenic-resistant bacteria and their implications on arsenic speciation, bioavailability, and toxicity.

This work discerns the role of lysogenic phages on bacterial arsenic resistance in contaminated environments. Lysogenic phages induced from arsenic-resistant bacteria were analyzed for presence of AIDGs in their genomes. Microcosm studies were conducted to investigate the effects of the lysogenic phages on AIDG propagation, arsenic species biotransformation, and overall arsenic toxicity in the impacted soil.

MATERIALS AND METHODS

Arsenic-Resistant Microbe Collection and Lysogenic Phages Induction. Arsenic-resistant microorganisms were enriched *in situ* for two months using microbial immobilization beads from two contaminated sites (SM and XHL) in Hunan Province, China (Figure S1A and Table S1). The beads (magnesium hydroxide aluminum colloids) with a high surface area (25.4 m²/g based on BET adsorption method) and bacterial affinity (Figure S2) were synthesized by hydrothermal reactions followed by calcination,¹⁹ then equilibrated in sodium arsenate solution with similar total arsenic levels as the contaminated soil, and then buried in the arsenic-contaminated sites for two months to allow for the attachment and enrichment of arsenic-resistant bacteria. The details of the bead preparation and microbe enrichment are available in the Supporting Information. Induction of lysogenic phages (to transform to a lytic lifecycle and be released from bacterial

hosts) can be achieved by chemical inducers (e.g., mitomycin C) that damage bacterial genomes and activate the SOS repair system.²⁰ The bacteria were dispersed in a PBS buffer and then exposed to 1 μg/mL of mitomycin C overnight for lysogenic phage induction and release,¹⁹ which was verified by epifluorescence microscopy (EFM) after SYBR gold staining and transmission electron microscopy (TEM) (Figure S3).^{21,22} The number of phages was expressed as viral-like particles (VLPs) based on fluorescent VLPs under EFM.^{23,24}

Bacterial and Viral Genome Analysis. Microbial DNA was extracted using a DNeasy PowerSoil Kit (QIAGEN, GERMANY) following the manufacturer's protocol. The induced phages were treated with DNase I (Thermo Scientific) to degrade extracellular DNA before being concentrated by centrifugation and ultrafiltration using an ultra-4 centrifugal tube (Amicon 100kD). Phage DNA was extracted using a TIANamp Virus DNA/RNA Kit following the manufacturer's protocol. PCR reactions were conducted to screen viral AIDGs, and the abundance of bacterial 16S rRNA and AIDGs that had been detected in the induced phage genome (i.e., *arsC* and *arsM*) was measured by qPCR.¹⁶ The details of the primer design, reagent mixture, and thermal programs are included in the Supporting Information, Table S2.

Phage Transduction Test in Arsenic-Contaminated soil. Microcosm studies were carried out in triplicate with 50 g of homogenized soil from Yuelu Mountain (located in Changsha city (CS)) at 20% soil moisture under four arsenic levels (indigenous arsenic level (11), 100, 200, and 300 mg/kg). Sodium arsenate solution (50 mg/mL) was used to

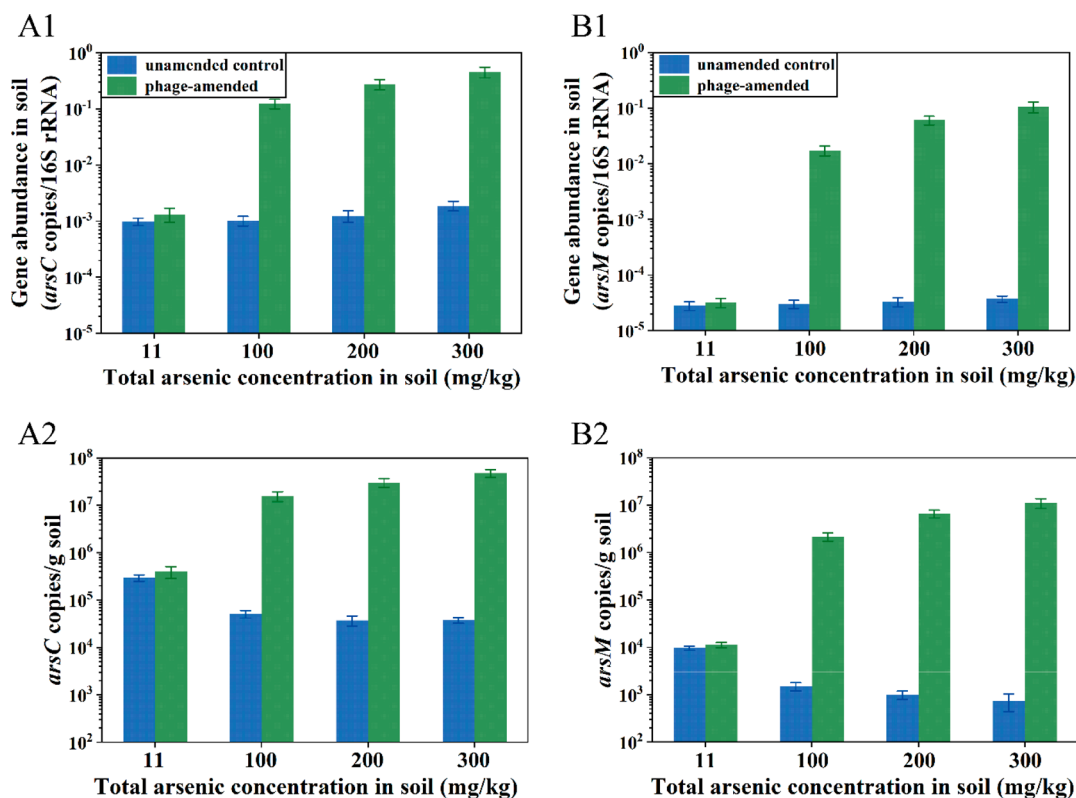


Figure 2. Lysogenic phage inoculation enhances microbial AIDG propagation in soil microcosms. Spiked with 5.0×10^7 phages per gram soil, the microcosms were incubated for 15 days in the dark. Relative abundances of *arsC* (A1) and *arsM* (B1) are compared between the phage-amended and unamended (control) treatments. Absolute abundances of *arsC* (A2) and *arsM* (B2) are also compared. Error bars indicate the standard deviation of triplicates.

achieve the desired total arsenic concentration. The microcosms were treated with free lysogenic phages (from SM site), heat-inactivated phages, or an SM buffer as detailed in the [Supporting Information](#). The phage dosage (5.0×10^7 VLPs g^{-1}) was representative of indigenous phage concentrations in soils (ranging from 10^7 to 10^8 g^{-1}).²⁵ After 15 days of incubation in darkness at room temperature, the abundance of *arsC* and *arsM* genes in the soil samples was measured by qPCR, and the arsenic species (i.e., As(V), As(III), and methyl As) were measured by liquid chromatography coupled with an atomic fluorescence instrument (Haiguang LC-AFS 6500, Beijing, China) as previously described.²⁶ Multiple samples ($n = 5$) were analyzed for each microcosm to reduce variability. The recovery rates and detection limits of arsenic species are also included in the [Supporting Information](#).

Soil Toxicity Tests. Earthworm avoidance tests were conducted using a stainless-steel avoidance wheel²⁷ to compare the ecotoxicity of phage-amended and unamended (control) soil. Briefly, 60 adult earthworms (10 for each compartment) (*Pheretima guillelmi*) were added in the wheel with alternating compartments filled with soils from the treated and control microcosms that had been spiked with the same initial total arsenic concentration. After 48 h, the number of earthworms in the control and treated soils were counted to calculate the avoidance rate (AR) following eq 1 with higher ecotoxicity reflected by larger AR.²⁸ Analysis of variance for multiple comparisons was performed to determine statistical significance at a 95% confidence level ($p = 0.05$).

$$AR = \frac{C - T}{2N} \times 100\% \quad (1)$$

where C and T are the average numbers of earthworms in the control and treated soils after the tests, while N is the number of earthworms initially added to each compartment.

RESULTS AND DISCUSSION

Lysogenic Phages from Arsenic-Resistant Bacteria Harbored AIDGs. Higher arsenic levels in the contaminated sites were correlated with more abundant and diverse AIDGs in indigenous phage genomes. Both *arsC* and *arsM* genes (3.0×10^{-3} and 6.5×10^{-4} copies per phage, respectively) were detected in lysogenic phages in arsenic-resistant microbes from the SM site (with higher arsenic levels), while only the *arsC* gene (2.0×10^{-3} copies per phage) was found in phages from the XHL site (with lower arsenic levels) (Figure 1A and B). Moreover, the relative viral AIDG levels were positively correlated with those in the indigenous soil bacterial communities ($R^2 = 0.974$ for *arsC* and 0.761 for *arsM*) (Figure 1C and D). The final product of arsenic methylation (i.e., TMA) can be volatilized, which eliminates the arsenic toxicity in the soil.²⁹ However, efflux of As(III) is usually a more common defense mechanism than the multistep methylation pathway under acute exposure to arsenic.^{30,31} The increased As(III) in the surrounding soil would provide a competitive advantage during interspecies competition against bacteria with low arsenic resistance.³² Accordingly, *arsC* genes were more abundant than *arsM* genes in lysogenic phage genomes.^{15,33}

Lysogenic Phage Enhanced AIDGs Propagation in Indigenous Soil Microorganisms. The addition of phages from the SM site increased the abundance of *arsC* and *arsM*

genes in the CS soil compared with the unamended control (Figure 2). In the 100 mg/kg of arsenic microcosm spiked with active phages, the relative abundance of *arsC* and *arsM* genes increased by 122-fold and 575-fold, respectively, after 15 days compared with heat-inactivated phage and phage-free groups (Figure 2). The total abundance of *arsC* directly introduced by lysogenic phages (1.5×10^5 copies per gram soil) was much lower than the final *arsC* abundance after treatment (1.6×10^7 copies per gram soil), indicating that AIDGs propagation was caused by phage-mediated HGT and subsequent replication in transduced bacteria. Moreover, higher soil arsenic levels resulted in faster AIDG propagation (Figure 2). Specifically, the relative abundance of *arsC* increased significantly with the total arsenic concentration in soil (i.e., $1.2 \pm 0.2 \times 10^{-1}$ for 100 mg/kg, $2.7 \pm 0.5 \times 10^{-1}$ for 200 mg/kg, and $4.5 \pm 0.8 \times 10^{-1}$ for 300 mg/kg). This trend was also observed for *arsM* (i.e., $1.7 \pm 0.03 \times 10^{-2}$, $6.0 \pm 0.1 \times 10^{-2}$, and $1.1 \pm 0.2 \times 10^{-1}$, respectively) (Figure 2). Therefore, the proliferation of AIDGs due to transduction by lysogenic phages could be significant in microbial communities at arsenic-contaminated sites. Notably, AIDG propagation was not significant under the original arsenic concentration (11 mg/kg), which suggests that there may be a threshold arsenic level to drive significant phage-mediated transduction and subsequent replication of AIDGs in soil.

It has been theorized that the introduction of foreign genes by lysogenic phages could confer beneficial phenotypes and improve bacterial fitness in unfavorable conditions,¹⁸ which was supported by our results. The prophages may repress not only their own lytic genes but also unnecessary host metabolic genes, which further facilitate the host survival in unfavorable conditions.¹⁸ Lysogenic phages harboring AIDGs may confer intracellular detoxification to host bacteria, which is crucial for the host (and the prophage) survival under arsenic stress.³⁴ Therefore, higher soil arsenic levels resulted in faster AIDG propagation.

Lysogenic Phages Induced Changes in Arsenic Speciation and Increased Overall Soil Toxicity. The introduction of AIDG-encoding lysogenic phages enhanced microbial reduction of As(V) to As(III). Microcosm studies with 100 mg/kg of arsenic soil showed that As(V) concentrations decreased by 5.3 ± 0.4 mg/kg ($5.4 \pm 0.4\%$), while the As(III) concentrations increased by 4.3 ± 0.4 mg/kg ($214.0 \pm 50.4\%$) compared with the unamended soil (Figure 3A and Table S4). The transformation of arsenic species was verified to be caused by transduction since no significant difference in arsenic speciation was observed when inactivated phages were used (Figure S4). Phage treatment also stimulated the methylation of arsenic, resulting in the production of methyl arsenic (0.78 ± 0.10 mg/kg), and higher arsenic levels were associated with higher methylated arsenic levels (Figures 3A and Table S4). The earthworm avoidance tests showed that the total arsenic toxicity of the phage-amended soil was significantly higher than the unamended soil after 15 days (Figure 3B).³⁵ As with the case that higher arsenic levels facilitated AIDG propagation, higher arsenic levels also enhanced the reduction of As(V) to As(III) and increased soil toxicity to a greater extent. The earthworms were more likely to escape from the phage-amended soil to the control soil without added phages (Figure 3B).

Implications. There seems to be a beneficial bacteria–phage association in microbial communities under arsenic stress, as the phage transduces resistance genes to bacteria,

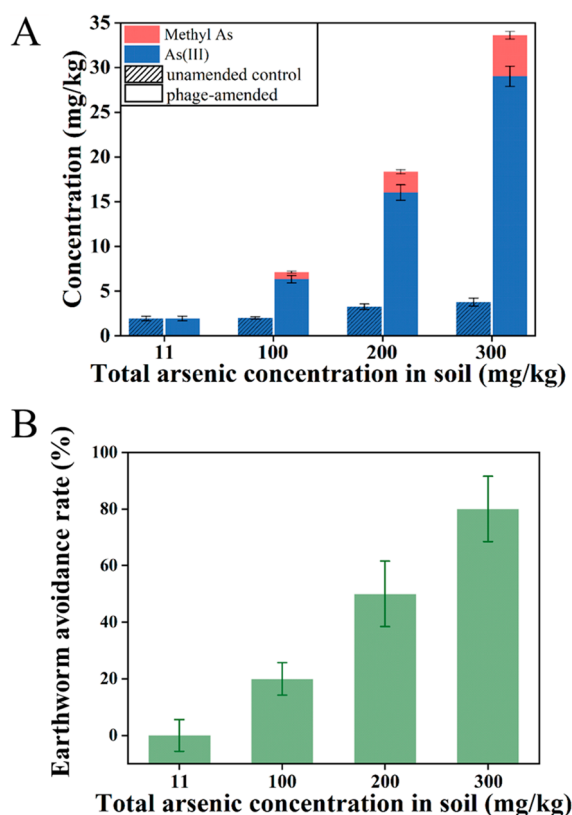


Figure 3. Lysogenic phage inoculation altered soil arsenic speciation and increased overall toxicity in microcosms. (A) Arsenic speciation profile in soil microcosm with different initial total arsenic concentrations (100, 200, 300 mg/kg soil). (B) Increase in overall arsenic toxicity due to phage inoculation based on earthworm avoidance tests. The initial total arsenic concentration in the CS soil was 11 mg/kg. Error bars indicate the standard deviation of triplicate measurements.

where they can take refuge and avoid exposure to toxic arsenic. Previous studies usually focused on bacterial community structure and function while overlooking the impact of coexisting lysogenic phages,³⁶ and our results suggest that more attention should be paid to their indirect biogeochemical effects. In this work, the lysogenic phages from arsenic-resistant bacteria harbored AIDGs, and the inoculation of such phages resulted in a surprising increase in soil arsenic toxicity. These results suggest the contribution of phages in the spread of heavy metal resistance, antibiotic resistance, and pathogenic genes may be underestimated in certain environments.

In summary, lysogenic phages from arsenic-resistant bacteria mediated the transfer of AIDGs between different microbial communities, and lysogenic phage introduction in the arsenic-contaminated soil promoted arsenic transformation from As(V) to As(III) in soil, which increased the total arsenic toxicity. This work is the first demonstration of phage-mediated HGT of AIDGs with a resulting significant alteration of arsenic speciation and activation in contaminated soil and highlights the overlooked potential role of transduction in increasing ecotoxicity in arsenic-contaminated environments.

■ ASSOCIATED CONTENT

● Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.estlett.9b00600](https://doi.org/10.1021/acs.estlett.9b00600).

(1) Methods of sampling collection and characterization, microbial immobilization bead synthesis and characterization, and quantitation and verification of selected biomarkers. (2) Figures of soil microbe enrichment, test of microbial adsorption capacity, lysogenic phage induction, effects of inactivated phage treatment, and earthworm respiration abilities. (3) Tables of physical chemical parameters of the soil samples, primers and thermal program for qPCR analysis, correlation coefficient between arsenic species and arsenic resistance genes, and arsenic species in soils after microcosm tests. (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (51679084, 51709100, 51579096, 51521006) and a National Science Foundation PIRE grant (OISE-1545756). We thank Professor Zhigang Qiu for his help in the synthesis of microbial immobilization beads.

■ REFERENCES

- (1) Wu, B.; Wang, R.; Fane, A. G. The Roles of Bacteriophages in Membrane-based Water and Wastewater Treatment Processes: A review. *Water Res.* **2017**, *110*, 120–132.
- (2) Wichels, A.; Biel, S. S.; Gelderblom, H. R.; Brinkhoff, T.; Muyzer, G.; Schütt, C. Bacteriophage Diversity in the North Sea. *Appl. Environ. Microbiol.* **1998**, *64* (11), 4128–4133.
- (3) Sun, C. L.; Thomas, B. C.; Barrangou, R.; Banfield, J. F. Metagenomic Reconstructions of Bacterial CRISPR Loci Constrain Population Histories. *ISME J.* **2016**, *10*, 858–870.
- (4) Weinbauer, M. G. Ecology of Prokaryotic Viruses. *FEMS Microbiol. Rev.* **2004**, *28* (2), 127–181.
- (5) Yu, P.; Mathieu, J.; Lu, G. W.; Gabiatti, N.; Alvarez, P. J. J. S. Control of Antibiotic Resistant Bacteria in Activated Sludge Using Polyvalent Phages in Conjunction with a Production Host. *Environ. Sci. Technol. Lett.* **2017**, *4* (4), 137–142.
- (6) Shropshire, J. D.; On, J.; Layton, E. M.; Zhou, H.; Bordenstein, S. R. One Prophage WO Gene Rescues Cytoplasmic Incompatibility in *Drosophila Melanogaster*. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115* (19), 4987–4991.
- (7) Nobrega, F. L.; Costa, A. R.; Kluskens, L. D.; Azeredo, J. Revisiting phage therapy: new applications for old resources. *Trends Microbiol.* **2015**, *23* (4), 185–191.
- (8) Boyd, E. F.; Davis, B. M.; Hochhut, B. Bacteriophage–bacteriophage Interactions in the Evolution of Pathogenic Bacteria. *Trends Microbiol.* **2001**, *9* (3), 137–144.
- (9) Waldor, M. K.; Mekalanos, J. J. Lysogenic Conversion by a Filamentous Phage Encoding Cholera Toxin. *Science* **1996**, *272* (5270), 1910–1914.
- (10) Mann, N.; Cook, A.; Millard, A.; Bailey, S.; Clokie, M. Bacterial Photosynthesis Genes in a Virus. *Nature* **2003**, *424* (6950), 741–741.
- (11) Yu, Z.-C.; Chen, X.-L.; Shen, Q.-T.; Zhao, D.-L.; Tang, B.-L.; Su, H.-N.; Wu, Z.-Y.; Qin, Q.-L.; Xie, B.-B.; Zhang, X.-Y.; Yu, Y.; Zhou, B.-C.; Chen, B.; Zhang, Y.-Z. Filamentous Phages Prevalent in *Pseudoalteromonas* spp. Confer Properties Advantageous to Host Survival in Arctic Sea Ice. *ISME J.* **2015**, *9*, 871–881.
- (12) Bettstetter, M.; Peng, X.; Garrett, R. A.; Prangishvili, D. AFV1, a Novel Virus Infecting Hyperthermophilic Archaea of the Genus *Acidianus*. *Virology* **2003**, *315* (1), 68–79.
- (13) Garbinski, L. D.; Rosen, B. P.; Chen, J. Pathways of arsenic uptake and efflux. *Environ. Int.* **2019**, *126*, 585–597.
- (14) Zhu, Y.-G.; Yoshinaga, M.; Zhao, F.-J.; Rosen, B. P. Earth Abides Arsenic Biotransformations. *Annu. Rev. Earth Planet. Sci.* **2014**, *42* (1), 443–467.
- (15) Zhang, S.-Y.; Su, J.-Q.; Sun, G.-X.; Yang, Y.; Zhao, Y.; Ding, J.; Chen, Y.-S.; Shen, Y.; Zhu, G.; Rensing, C.; Zhu, Y.-G. Land scale biogeography of arsenic biotransformation genes in estuarine wetland. *Environ. Microbiol.* **2017**, *19* (6), 2468–2482.
- (16) Zhang, S.-Y.; Zhao, F.-J.; Sun, G.-X.; Su, J.-Q.; Yang, X.-R.; Li, H.; Zhu, Y.-G. Diversity and Abundance of Arsenic Biotransformation Genes in Paddy Soils from Southern China. *Environ. Sci. Technol.* **2015**, *49* (7), 4138–4146.
- (17) Qin, J.; Rosen, B. P.; Zhang, Y.; Wang, G.; Franke, S.; Rensing, C. Arsenic Detoxification and Evolution of Trimethylarsine Gas by a Microbial Arsenite S-adenosylmethionine Methyltransferase. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103* (7), 2075–2080.
- (18) Paul, J. H. J. I. Prophages in marine bacteria: dangerous molecular time bombs or the key to survival in the seas? *ISME J.* **2008**, *2* (6), 579–589.
- (19) Ghosh, D.; Roy, K.; Williamson, K. E.; White, D. C.; Wommack, K. E.; Sublette, K. L.; Radosevich, M. Prevalence of Lysogeny among Soil Bacteria and Presence of 16S rRNA and trzN Genes in Viral-Community DNA. *Appl. Environ. Microbiol.* **2008**, *74* (2), 495–502.
- (20) Weinbauer, M.; Suttle, C. Lysogeny and prophage induction in coastal and offshore bacterial communities. *Aquat. Microb. Ecol.* **1999**, *18* (3), 217–225.
- (21) Williamson, K. E.; Wommack, K. E.; Radosevich, M. Sampling Natural Viral Communities from Soil for Culture-Independent Analyses. *Appl. Environ. Microbiol.* **2003**, *69* (11), 6628–6633.
- (22) Yu, P.; Mathieu, J.; Li, M.; Dai, Z.; Alvarez, P. J. J. Isolation of Polyvalent Bacteriophages by Sequential Multiple-Host Approaches. *Appl. Environ. Microbiol.* **2016**, *82* (3), 808–815.
- (23) Choi, J.; Kotay, S. M.; Goel, R. Various Physico-chemical Stress Factors Cause Prophage Induction in *Nitrosospira* Multiformis 25196-an Ammonia Oxidizing Bacteria. *Water Res.* **2010**, *44* (15), 4550–4558.
- (24) Motlagh, A. M.; Bhattacharjee, A. S.; Goel, R. Microbiological study of bacteriophage induction in the presence of chemical stress factors in enhanced biological phosphorus removal (EBPR). *Water Res.* **2015**, *81*, 1–14.
- (25) Ghosh, D.; Roy, K.; Williamson, K.; White, D.; Wommack, K.; Sublette, K.; Radosevich, M. J. A. E. M. Prevalence of lysogeny among soil bacteria and presence of 16S rRNA and trzN genes in viral-community DNA. *Appl. Environ. Microbiol.* **2008**, *74* (2), 495–502.
- (26) Wang, H.-T.; Zhu, D.; Li, G.; Zheng, F.; Ding, J.; O'Connor, P. J.; Zhu, Y.-G.; Xue, X.-M. Effects of Arsenic on Gut Microbiota and Its Biotransformation Genes in Earthworm *Metaphire sieboldi*. *Environ. Sci. Technol.* **2019**, *53* (7), 3841–3849.
- (27) Li, D.; Alvarez, P. J. J. Avoidance, weight loss, and cocoon production assessment for *Eisenia fetida* exposed to C60 in soil. *Environ. Toxicol. Chem.* **2011**, *30* (11), 2542–2545.

(28) Garcia, M.; Rombke, J.; de Brito, M. T.; Scheffczyk, A. Effects of three pesticides on the avoidance behavior of earthworms in laboratory tests performed under temperate and tropical conditions. *Environ. Pollut.* **2008**, *153* (2), 450–456.

(29) Chen, J.; Sun, G.-X.; Wang, X.-X.; Lorenzo, V. d.; Rosen, B. P.; Zhu, Y.-G. Volatilization of Arsenic from Polluted Soil by *Pseudomonas putida* Engineered for Expression of the arsM Arsenic(III) S-Adenosine Methyltransferase Gene. *Environ. Sci. Technol.* **2014**, *48* (17), 10337–10344.

(30) Liu, S.; Zhang, F.; Chen, J.; Sun, G. Arsenic removal from contaminated soil via biovolatilization by genetically engineered bacteria under laboratory conditions. *J. Environ. Sci.* **2011**, *23* (9), 1544–1550.

(31) Slyemi, D.; Bonnefoy, V. How prokaryotes deal with arsenic. *Environ. Microbiol. Rep.* **2011**, *4* (6), 571–586.

(32) Bleeker, P. M.; Hakvoort, H. W. J.; Bliet, M.; Souer, E.; Schat, H. Enhanced Arsenate Reduction by a CDC25-like Tyrosine Phosphatase Explains Increased Phytochelatin Accumulation in Arsenate-tolerant *Holcus Lanatus*. *Plant J.* **2006**, *45* (6), 917–929.

(33) Ashelford, K. E.; Day, M. J.; Fry, J. C. %J. Applied; Microbiology, E., Elevated abundance of bacteriophage infecting bacteria in soil. *Appl. Environ. Microbiol.* **2003**, *69* (1), 285–289.

(34) Zhao, Y.; Su, J.-Q.; Ye, J.; Rensing, C.; Tardif, S.; Zhu, Y.-G.; Brandt, K. K. AsChip: A High-Throughput qPCR Chip for Comprehensive Profiling of Genes Linked to Microbial Cycling of Arsenic. *Environ. Sci. Technol.* **2019**, *53* (2), 798–807.

(35) Langdon, C. J.; Meharg, A. A.; Feldmann, J.; Balgar, T.; Charnock, J.; Farquhar, M.; Pearce, T. G.; Semple, K. T.; Cotter-Howells, J. Arsenic-speciation in Arsenate-resistant and Non-resistant Populations of the Earthworm, *Lumbricus rubellus*. *J. Environ. Monit.* **2002**, *4* (4), 603–608.

(36) Turpeinen, R.; Kairesalo, T.; Häggblom, M. M. Microbial community structure and activity in arsenic-, chromium- and copper-contaminated soils. *FEMS Microbiol. Ecol.* **2004**, *47* (1), 39–50.