Identification and molecular characterization of bacteriophage phiAxp-2 of Achromobacter xylosoxidans

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A novel Achromobacter xylosoxidans bacteriophage, phiAxp-2, was isolated from hospital sewage in China. The phage was morphologically and microbiologically characterized, and its one-step growth curve, host range, genomic sequence, and receptor were determined. Its morphology showed that phiAxp-2 belongs to the family Siphoviridae. Microbiological characterization demonstrated that pH 7 is most suitable for phage phiAxp-2; its titer decreased when the temperature exceeded 50 °C; phiAxp-2 is sensitive to ethanol and isopropanol; and the presence of calcium and magnesium ions is necessary to accelerate cell lysis and improve the formation of phiAxp-2 plaques. Genomic sequencing and a bioinformatic analysis showed that phage phiAxp-2 is a novel bacteriophage, consisting of a circular, double-stranded 62,220-bp DNA molecule with a GC content of 60.11% that encodes 86 putative open reading frames (ORFs). The lipopolysaccharide of A. xylosoxidans is involved in the adsorption of phiAxp-2.

Achromobacter xylosoxidans is a nonfermenting Gram-negative bacillus1. It is an uncommon opportunistic pathogen with low virulence, but can potentially cause invasive infections in immunocompromised patients, such as those with meningitis, empyema, pulmonary abscess, peritonitis, urinary tract infection, prosthetic valve endocarditis, chronic otitis media, keratitis, osteomyelitis, endophthalmitis, or septic arthritis1. A. xylosoxidans is frequently associated with antibiotic-resistant nosocomial infections. Bacteriophage therapy directed against A. xylosoxidans may be useful in combating these infections. Bacteriophages are potential therapeutic agents in the treatment of bacterial infections and useful diagnostic tools2, and since their discovery, attempts have been made to use bacteriophages to treat several infectious diseases3. Therefore, new phages are being isolated and characterized4. Because A. xylosoxidans infections are some of the most problematic nosocomial infections, the isolation and characterization of novel phages that infect this species is a priority. Many of these phages have been isolated in recent years, but only some have been fully sequenced and described in detail5. To develop an effective antimicrobial agent, we isolated a bacteriophage, designated phiAxp-2, from hospital sewage and described its morphology, host range, growth characteristics, whole genome sequence, and receptor usage. This phage may be an effective tool for the control of A. xylosoxidans infections in susceptible populations.

Results and Discussion

Phage morphology. Bacteriophage phiAxp-2 was isolated from A. xylosoxidans strain A22732 and observed with electron microscopy. A. xylosoxidans strain A22732 harbours a conjugative imipenem-encoding plasmid and is resistant to multiple β-lactam antibiotics, including imipenem and meropenem6. Cell lysis was observed after induction (phage yield: \(1 \times 10^9\) pfu/ml), by propagating the induced lysate on strain A22732. The plaques obtained had a clear pinpoint morphology, with well-defined boundaries (Fig. 1a). Electron micrographs

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of negatively stained phiAxp-2 virions showed an icosahedral head and a long noncontractile tail (Fig. 1b). The average particle had a capsid of approximately 56 nm in diameter and a tail length of approximately 230 nm, and the phage is therefore morphologically similar to phages in the order Caudovirales and family Siphoviridae. Host range tests suggested that among all the species tested (n = 14), phiAxp-2 was specifically virulent to only four strains of A. xylosoxidans (Table 1). Besides the reported multidrug-resistant strain A22732, the other three clinical A. xylosoxidans strains were shown to be resistant to aztreonam and tobramycin.

Latent period and burst size. There is a progressive relationship between burst size and the latent period, such that an optimal latent period leads to high phage fitness, and an increase in burst size may contribute to plaque size or larger plaques with higher burst sizes. A one-step growth curve of phage phiAxp-2 propagated on A. xylosoxidans A22732 was constructed. The latent period of phage phiAxp-2 was 180 min. The burst time was approximately 240 min and the burst size was 2,985 pfu/cell (burst size = the total number of phages liberated at the end of one cycle of growth/number of infected bacteria) (Fig. 1c).

Microbiological characterization. Figure 2a shows the pH sensitivity of phage phiAxp-2. The phage maintained its infectivity when incubated at 37°C in a pH range of 4–11. At pH 1 and pH 14, approximately 100% reductions in the phage particle counts were observed. The loss of viability when phage phiAxp-2 was subjected to various temperatures is shown in Fig. 2b. Phage phiAxp-2 displayed heat-sensitivity at 50°C, 60°C, 70°C, and 80°C. Treatment at 80°C for 75 min completely inactivated the phage. As shown in Fig. 2c,d, the activity of phage phiAxp-2 was affected by the presence of ethanol and isopropanol. The most effective concentrations of ethanol (95%, v/v) and isopropanol (95%, v/v) reduced the phage titer by 76% and 84%, respectively, after 90 min. Because many phages require divalent ions (such as Ca²⁺ or Mg²⁺) for optimal adsorption, the ion-dependence of phage phiAxp-2 was determined. The most efficient infection was achieved with concentrations of 15 mM Mg²⁺ and 10 mM Ca²⁺ (Fig. 2e).
| Species                        | ID       | Infection |
|-------------------------------|----------|-----------|
| Achromobacter xylosoxidans    | A22732   | +         |
| A. xylosoxidans               | 5271     | +         |
| A. xylosoxidans               | 844      | +         |
| A. xylosoxidans               | 6065     | +         |
| Escherichia coli              | ATCC 25922 | −        |
| Klebsiella pneumoniae         | ATCC BAA-1706 | −        |
| Serratia marcescens           | wk2050   | −         |
| Enterobacter aerogenes        | 3-SP     | −         |
| Enterobacter cloacae          | T5282    | −         |
| Leclercia adecarboxylata      | P10164   | −         |
| Raoultella ornithinolycica    | YNP001   | −         |
| Serratia marcescens           | 9665     | −         |
| Citrobacter freundii          | P10159   | −         |
| Vibrio parahaemolyticus       | J5421    | −         |
| Pseudomonas aeruginosa        | PA01     | −         |
| Acinetobacter baumannii       | N1       | −         |
| Shigella sonnei               | #1083    | −         |

Table 1. Host range analysis of the phage phiAxp-2 −, absent; +, present.

Figure 2. Resistance of phage phiAxp-2 to physical and chemical agents. (a) Effect of pH on the phage phiAxp-2 titer after incubation for 60 min in LB broth at 37 °C. (b) Inactivation kinetics of phage phiAxp-2 at 4 °C, 25 °C, 37 °C, 50 °C, 60 °C, 70 °C, and 80 °C. (c) Inactivation kinetics of phage phiAxp-2 in the presence of 10%, 50%, 75%, and 95% ethanol. (d) Inactivation kinetics of phage phiAxp-2 in the presence of 10%, 50%, and 95% isopropanol. (e) Viability of phage phiAxp-2 in LB broth with different Ca2+ and Mg2+ concentrations. On all graphs, the values are the means of three determinations.
Genome characterization. For the future application of phage phiAxp-2 to protect humans from *A. xylosoxidans* infections, the phage must be characterized in detail, including its genomic sequence. The genomic DNA of phiAxp-2 was extracted and purified, and its genome was completely sequenced and analyzed. Analysis of the sequence found that the restriction endonuclease *HindIII* had nine cutting sites in the genomic DNA. Thus, it was expected that when *HindIII* was used to digest the DNA, ten fragments would be generated if the DNA comprised a linear genome, but if the genome was circular, nine fragments would be generated. The *HindIII* digestion experiment generated nine fragments in the agarose gel (Fig. 3), revealing that the phiAxp-2 genome is a circular molecule. The complete circular double-stranded DNA genome of phage phiAxp-2 is 62,220 bp in length with a G+C content of 60.11%. This percentage is lower than those of the complete *Achromobacter* genomes sequenced so far (65–66% G+C content), but is higher than those of the sequenced *A. xylosoxidans*-specific phages, JWAlpha (KF787095) and JWDelta (KF787094), which are 54.4% and 54.2%, respectively. Analysis of the phage phiAxp-2 genome revealed 86 putative open reading frames (ORFs). The National Center for Biotechnology Information (NCBI) database was scanned for homologues of the proteins encoded by the predicted ORFs using BlastP. Because the genome of phiAxp-2 diverges from other available phage genomes, only a limited number (33%) of protein functions could be predicted with similarity searches, highlighting the novelty of this phage. Therefore, a more detailed investigation is required to fully understand the nature of this novel phage. Twenty-eight ORFs showed some similarity in the BlastP analysis. Putative functions were assigned to 22 ORFs based on these similarities. Despite the low number of annotated ORFs, different modules can be recognized in the phiAxp-2 genome, which encodes proteins for (i) DNA replication, regulation, and modification, (ii) DNA packaging, and (iii) head and tail morphogenesis (Fig. 4). All of these functional clusters are located in ORF1–ORF34, which constitutes approximately 60% of the whole genome length. The remaining ORFs showed less or no similarity to other proteins in the NCBI database.

The left half of the phiAxp-2 genome (ORF1–ORF29) has a similar genomic structure and encodes proteins most similar to each of the *Burkholderia* phage AH2 (IN564907) proteins from ORF78–ORF50 (the right half of the AH2 genome; excluding nine proteins) in the reverse transcription direction (Fig. 4 and Table 2). However, the range of overall similarity was as low as 29–57% at the amino acid level, and genomic comparisons with AH2 showed that in many instances, only small parts or domains of the ORFs were conserved. The most similar of these protein was the portal protein (57% identity with AH2 gp65) and the least similar was the decorator protein (24% identity with AH2 gp65) (Table 2). Phage AH2 is most closely related to *Burkholderia* phage BcepNazgul.
(NC005091)\textsuperscript{10}, and a multiple genome alignment of the chromosomes of phiAxp-2, AH2, and BcepNazgul confirmed their relatedness (Fig. 5). Despite the similarities in their virion morphologies (the AH2 virion also has a noncontractile tail of approximately 220 nm and a capsid approximately 60 nm in diameter\textsuperscript{10}), a phylogenetic analysis of the DNA polymerase and the terminase large subunit predicted that phiAxp-2 is most closely related to AH2 (Fig. 6a,b). Because the proteins of phiAxp-2 have largely uncharacterized functions, its genome must encode several new viral proteins. We predict that the large-scale genomic rearrangement in phiAxp-2 was mediated by transposase genes. However, we did not identify any transposase gene. Such genomic rearrangements may have caused the genomic diversity observed in the phages, resulting in the biological differences that distinguish them.

Module analysis. Genes for DNA replication and DNA metabolism occur at the beginning of the phiAxp-2 genome, followed by packaging genes and the structural genes. ORF1–9 encode proteins for DNA replication, regulation, and modification, and six of them encode proteins with homology to AH2 proteins (35–45% identity): DNA primase (ORF1), exonuclease (ORF5), single-stranded DNA binding protein (ORF6), DNA polymerase (ORF7), resolvase (ORF8), and helicase (ORF9). The DNA packaging genes (the terminase subunits, ORF11–ORF12) of phiAxp-2 both have counterparts in AH2. phiAxp-2 ORF11 codes a 199-aa protein with limited similarity (32%) to the known small terminase subunit of phage AH2. A DUF1441 superfamily member was detected using BlastP-based tools, and appears to be distantly related to other helix–turn–helix DNA-binding motif families, so may also be involved in the recognition of viral DNA and the subsequent initiation of viral packaging\textsuperscript{11}. The gene immediately downstream from ORF11 encodes a 695-aa protein with 54% identity to the large terminase subunit of AH2, which implies a similar function in DNA packaging. ORF12 is predicted to have a GpA (pfam05876) domain, which is actively involved in the late stages of packaging, including DNA translocation. ORF13–ORF17 make up the capsid morphogenesis module, containing genes encoding the head–tail joining protein (ORF13), portal protein (ORF14), prohead protease (ORF15), decorator protein (ORF16), and major capsid protein (ORF17). Each of these proteins is similar to an AH2 protein, with percentage identities of 29–57% (Table 2). Among these proteins, the portal protein is thought to form the hole through which DNA is packaged into the prohead, and is also a part of the packaging motor\textsuperscript{11}. All the genes encoded by ORF20–25 have counterparts in AH2 (31–55% identity), and three were annotated as tail proteins: ORF22, ORF24, and ORF25. Like most tailed phages, phiAxp-2 encodes two tail proteins proximal to the tail tape measure gene\textsuperscript{12}. The tape measure protein of phiAxp-2, encoded by ORF26, has no sequence similarity to that of AH2, although the two proteins have similar functions in the assembly of the phage tails and in tail length determination\textsuperscript{12,13}. phiAxp-2 ORF27 is distantly related to the Escherichia phage N4 tail sheath protein, which is known to interact with the N4 outer membrane receptor, NfrA\textsuperscript{14}. phiAxp-2 ORF28 encodes a capsid and scaffold protein that is absent in AH2. The scaffold protein assists in the assembly of the outer shell and dissociates from the capsid during subsequent DNA packaging\textsuperscript{11}. ORF29 encodes a tail assembly protein that has 31% amino acid identity with the AH2 tail assembly protein. ORF33 and ORF34 encode a tail assembly protein and a virion-associated protein, respectively. Neither is present in AH2. Following the structural components, there is a region encoding small and uncharacterized proteins, which spans about 24 kb. No genes similar to the genes for lysin or holin have yet been detected in the phiAxp-2 genome, which are responsible for host cell disruption during the burst steps of phages\textsuperscript{13}, although the clearing of the bacterial culture at a specific time point strongly suggest that they exist.

Identification of the host receptor. The adsorption of the phage to the bacterial surface is the first and most important step in the phage infection process. Both the lipopolysaccharide (LPS) and outer membrane proteins located on the surfaces of Gram-negative bacteria can be used as phage receptors. In the present study, protease K and periodate were used to destroy the A. xylosoxidans outer membrane proteins and LPS, respectively.
| ORFs | Strand | Start | End   | Length (aa)* | aa identity (%) | Function                                      |
|------|--------|-------|-------|--------------|----------------|-----------------------------------------------|
| orf01 | −      | 444   | 2966  | 840          | 42             | DNA primase [Burkholderia phage AH2]          |
| orf02 | −      | 3002  | 3301  | 99           | −              | —                                             |
| orf03 | +      | 3451  | 3957  | 168          | 37             | hypothetical protein Nazgul2 [Burkholderia phage BcepNazgul] |
| orf04 | +      | 4042  | 4437  | 131          | −              | —                                             |
| orf05 | +      | 4453  | 5745  | 430          | 38             | exonuclease [Burkholderia phage AH2]         |
| orf06 | +      | 5792  | 6430  | 212          | 35             | single-stranded DNA binding protein [Burkholderia phage AH2] |
| orf07 | +      | 6507  | 8600  | 697          | 43             | DNA polymerase [Burkholderia phage AH2]      |
| orf08 | +      | 8597  | 8990  | 92           | 38             | resolvase [Burkholderia phage AH2]           |
| orf09 | +      | 8937  | 10481 | 514          | 45             | helicase [Burkholderia phage AH2]            |
| orf10 | +      | 10528 | 10737 | 69           | 57             | hypothetical protein Q051_01461 [Pseudomonas aeruginosa BWHPSA046] |
| orf11 | +      | 10727 | 11326 | 199          | 32             | terminase small subunit [Burkholderia phage AH2] |
| orf12 | +      | 11313 | 13400 | 1688         | 54             | terminase large subunit [Burkholderia phage AH2] |
| orf13 | +      | 13400 | 13651 | 251          | 39             | head-tail joining protein [Burkholderia phage AH2] |
| orf14 | +      | 13682 | 15292 | 6810         | 57             | portal protein [Burkholderia phage AH2]      |
| orf15 | +      | 15296 | 16675 | 459          | 42             | prohead protease [Burkholderia phage AH2]    |
| orf16 | +      | 16703 | 17080 | 125          | 29             | decorator protein [Burkholderia phage AH2]   |
| orf17 | +      | 17108 | 18163 | 351          | 47             | major capsid protein [Burkholderia phage AH2] |
| orf18 | +      | 18233 | 18523 | 691          | 38             | hypothetical protein [Pseudomonas aeruginosa] |
| orf19 | +      | 18550 | 18783 | 77           | −              | −                                             |
| orf20 | +      | 18783 | 19205 | 140          | 32             | hypothetical protein AH2_00060 [Burkholderia phage AH2] |
| orf21 | +      | 19208 | 19834 | 227          | 55             | hypothetical protein AH2_00059 [Burkholderia phage AH2] |
| orf22 | +      | 19827 | 20420 | 593          | 34             | minor tail protein [Burkholderia phage AH2]   |
| orf23 | +      | 20456 | 21253 | 797          | 50             | hypothetical protein AH2_00057 [Burkholderia phage AH2] |
| orf24 | +      | 21263 | 21754 | 531          | 31             | tail protein [Burkholderia phage AH2]        |
| orf25 | +      | 21757 | 21966 | 69           | 43             | tail protein [Burkholderia phage AH2]        |
| orf26 | +      | 21950 | 26752 | 1603         | 33             | tape measure protein [Bradyrhizobium sp. WSM3983] |
| orf27 | +      | 26749 | 30924 | 1387         | 42             | tail sheath [Escherichia phage N4]            |
| orf28 | +      | 30927 | 33446 | 315          | 51             | capsid and scaffold protein [Delftia phage RG-2014] |
| orf29 | +      | 33448 | 34290 | 280          | 31             | tail assembly protein [Burkholderia phage AH2] |
| orf30 | +      | 34301 | 34426 | 45           | −              | −                                             |
| orf31 | +      | 34414 | 34653 | 79           | −              | −                                             |
| orf32 | +      | 34686 | 34877 | 201          | −              | −                                             |
| orf33 | +      | 34874 | 37261 | 1397         | 44             | tail assembly protein [Bradyrhizobium sp. WSM3983] |
| orf34 | +      | 37258 | 38034 | 258          | 36             | virion associated protein [Xylella phage Salvo] |
| orf35 | +      | 38046 | 38780 | 244          | −              | −                                             |
| orf36 | +      | 38850 | 39266 | 136          | −              | −                                             |
| orf37 | +      | 39424 | 39699 | 275          | −              | −                                             |
| orf38 | +      | 39996 | 40481 | 485          | −              | −                                             |
| orf39 | +      | 40536 | 40745 | 129          | −              | −                                             |
| orf40 | +      | 40729 | 40887 | 58           | −              | −                                             |
| orf41 | −      | 40934 | 41860 | 927          | −              | −                                             |
| orf42 | −      | 41857 | 42147 | 290          | −              | −                                             |
| orf43 | −      | 42149 | 42520 | 171          | −              | −                                             |
| orf44 | −      | 42510 | 42731 | 71           | −              | −                                             |
| orf45 | −      | 42728 | 42982 | 84           | −              | −                                             |
| orf46 | −      | 42979 | 43158 | 79           | −              | −                                             |
| orf47 | −      | 43179 | 43490 | 121          | −              | −                                             |
| orf48 | −      | 43480 | 43917 | 437          | −              | −                                             |
| orf49 | −      | 44030 | 44233 | 203          | −              | −                                             |
| orf50 | −      | 44230 | 44595 | 365          | −              | −                                             |
| orf51 | −      | 44598 | 45155 | 557          | −              | −                                             |
| orf52 | −      | 45216 | 45710 | 504          | −              | −                                             |
| orf53 | −      | 45764 | 46351 | 587          | −              | −                                             |
| orf54 | −      | 46353 | 46538 | 185          | −              | −                                             |
| orf55 | −      | 46816 | 47238 | 422          | −              | −                                             |
| orf56 | −      | 47302 | 47901 | 599          | −              | −                                             |

*Continued*
to determine the attachment site for phage phiAxp-2 on the cell surface of A. xylosoxidans (Fig. 7a,b). Phage adsorption to LPS-deficient A. xylosoxidans cells was inhibited, indicating that phage-specific adhesion is mediated by LPS (Fig. 7b). These results were confirmed with a phage inactivation assay performed with pure LPS isolated from strain A22732. These experiments showed a direct correlation between the LPS concentration and the inhibition of viral particle infectivity (Fig. 7c), and approximately 12.5 μg/ml LPS inhibited the activity of 2.8 × 10^3 pfu phiAxp-2. LPS of E. coli 0111:B4 was used as the negative control and showed no phage-inactivating capacity compared with A. xylosoxidans LPS, indicating that A. xylosoxidans LPS is the specific receptor for phage phiAxp-2.

Concluding remarks. In this study, we have characterized a Siphoviridae phage that infects the important nosocomial pathogen A. xylosoxidans. Genomic data are an important resource with which to study and engineer phages to control specific bacterial species15, and advances in phage genomic characterization have made phage therapy more feasible in terms of both its logistics and safety10. A combination of genomic sequencing and a morphological analysis showed that phiAxp-2 is a member of the family Siphoviridae, and is related to the previously sequenced phage AH2. The most striking feature to emerge from a comparative analysis of the phage genome was the extensive mosaic structure of phiAxp-2, which contains different segments with distinct evolutionary histories. The results of this comparative analysis indicate that the left half of the phiAxp-2 genome has a similar genomic structure to partial genomic sequences of AH2. A simple general explanation is that horizontal genetic exchange has played a dominant role in shaping these genomic architectures. Gene modules are exchanged using host- or phage-encoded recombination machinery16. This analysis provides an important contribution to the field of bacteriophage genomics and a foundation upon which to extend our understanding of the natural predecessors of A. xylosoxidans. Further clarification of the functions of the unique hypothetical phage proteins identified may provide new insight into the mechanisms of genome evolution. Identification of the receptor molecules of phages provides crucial insight into the early stages of infection17. Our results show that phage phiAxp-2 recognizes LPS as its primary receptor for adsorption. Further studies of this phage will be useful in understanding the role of phages in evolution and bacterial lifestyles.

### Table 2. Achromobacter phage phiAxp-2 gene annotations.

| ORFs | Strand | Start | End  | Length (aa)* | aa identity (%) | Function |
|------|--------|-------|------|--------------|----------------|----------|
| orf57 | −      | 47963 | 48052 | 49           | —              | —        |
| orf58 | −      | 48055 | 48309 | 85           | —              | —        |
| orf59 | −      | 48309 | 48671 | 120          | —              | —        |
| orf60 | −      | 48674 | 48946 | 272          | —              | —        |
| orf61 | −      | 48943 | 49611 | 222          | —              | —        |
| orf62 | −      | 49608 | 49889 | 93           | —              | —        |
| orf63 | −      | 49876 | 51153 | 2293         | —              | —        |
| orf64 | −      | 51153 | 51472 | 321          | —              | —        |
| orf65 | −      | 51474 | 51764 | 290          | —              | —        |
| orf66 | −      | 51860 | 52297 | 433          | —              | —        |
| orf67 | +      | 52792 | 53001 | 269          | —              | —        |
| orf68 | +      | 53072 | 54028 | 956          | —              | —        |
| orf69 | +      | 54041 | 54736 | 295          | —              | —        |
| orf70 | +      | 54761 | 55336 | 587          | —              | —        |
| orf71 | +      | 55333 | 55644 | 311          | —              | —        |
| orf72 | +      | 55858 | 56799 | 942          | —              | —        |
| orf73 | +      | 56802 | 57029 | 230          | —              | —        |
| orf74 | +      | 57026 | 57144 | 142          | —              | —        |
| orf75 | +      | 57153 | 57476 | 323          | —              | —        |
| orf76 | +      | 57481 | 57762 | 281          | —              | —        |
| orf77 | +      | 57988 | 58239 | 251          | —              | —        |
| orf78 | +      | 58236 | 58466 | 230          | —              | —        |
| orf79 | +      | 58480 | 58920 | 440          | —              | —        |
| orf80 | +      | 59004 | 59153 | 150          | —              | —        |
| orf81 | +      | 59168 | 59296 | 128          | —              | —        |
| orf82 | +      | 59301 | 59789 | 500          | —              | —        |
| orf83 | +      | 59808 | 60353 | 545          | —              | —        |
| orf84 | +      | 60358 | 60711 | 353          | —              | —        |
| orf85 | +      | 60720 | 61406 | 686          | —              | —        |
| orf86 | +      | 61465 | 61638 | 173          | —              | —        |

* Amino acids.
Methods
Bacterial strains, bacteriophage, and media. A 16S rDNA sequence analysis was used to identify the host bacterium. The multidrug-resistant *A. xylosoxidans* A22732 strain was used as an indicator for phage screening of sewage samples. The samples were centrifuged at 12,000 × g for 10 min to remove the solid impurities, the supernatants were filtered through a 0.22 μm pore-size membrane filter to remove bacterial debris. The filtrates were mixed with *A. xylosoxidans* culture to enrich the phage at 37 °C. Following enrichment, the culture was centrifuged at 10,000 × g for 10 min, and then the supernatant was filtered with a 0.22 μm pore-size membrane filter to remove the residual bacterial cells. The filtrate (0.1 ml) was mixed with 0.3 ml of *A. xylosoxidans* culture and 3 ml of molten top soft nutrient agar (0.75% agar), which was then overlaid onto solidified base nutrient agar (1.5% agar). Following incubation for 6 h, clear phage plaques were picked from the plate18. The phage titer was determined with the double-layered method. Luria–Bertani (LB) broth or LB agar was used to culture the bacterium. The host range of the phage was tested against 17 clinical strains from our microorganism center with standard spot tests19.

Electron microscopy. Phage particles were allowed to adsorb onto a carbon-coated copper grid before they were negatively stained with 2% phosphotungstic acid (pH 7.0). After the grid was dried at room temperature, it was examined under a Philips TECNAI-10 transmission electron microscope.
One-step growth curve. For the one-step growth curve analysis, A. xylosoxidans cells (optical density at 600 nm [OD₆₀₀] = 0.5) were infected with phage phiAxp-2 at a multiplicity of infection of 0.0001. The bacteriophage was allowed to adsorb for 5 min at 37 °C. The mixture was then centrifuged at 12,000 × g for 30 s to remove any unadsorbed phage particles, and the resultant pellet was resuspended in 5 ml of LB medium. The samples were incubated at 37 °C and collected every 20 min for 300 min.

Stability studies. A temperature-controlled incubator was used to determine the stability of phiAxp-2 at different pHs or temperatures, and in the presence of disinfectants or ions. Briefly, a 1.5 ml tube containing a filter-sterilized phage sample was incubated at a specified temperature or pH. After the desired treatment, the tube was cooled slowly and placed in an ice-water bath, and the samples were assayed to determine the surviving plaque-forming units (pfu). To test the phage sensitivity to disinfectants and ions, a known amount of phage was incubated with different concentrations of disinfectants or ions. The plates were then overlain with liquefied soft agar (LB with 0.75% [w/v] agar) containing the host cells and incubated at 37 °C for 12 h.

Genome sequencing and assembly. The purified phage DNA was sequenced with an Illumina HiSeq 2500 sequencer. The sequence reads were filtered to remove low-quality sequences and trimmed to remove adapter sequences, and the filtered sequences were assembled. The final assembled sequence was compared with current protein and nucleotide databases (http://www.ncbi.nlm.nih.gov/) using the Basic Local Alignment Search Tool (Blast) software. BlastP was used to determine the similarity of the deduced phage proteins to proteins in the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov). Simulation of the restriction enzyme mapping of the phiAxp-2 genome sequence was carried out using the software package DNAStar. The phiAxp-2 DNA was digested by selected restriction endonucleases (HindIII, purchased from New England Biolabs, Ipswich, MA, USA). For a reaction system of 20 μL, 10 units of the restriction endonuclease and 300 ng of phiAxp-2 DNA were used. The mixture was incubated at 37 °C for 180 min and then used to perform agarose gel electrophoresis. Agarose gel electrophoresis was subsequently performed to separate the restriction fragments. The CLC Main Workbench, version 6.1.1 (CLC bio, Aarhus, Denmark) was used to annotate the
Identification of the phage receptor. The receptor properties of phiAxp-2 were determined as described previously. Briefly, A. xylosoxidans A22732 cultures were treated with sodium acetate (50 mM, pH 5.2) containing 100 mM IO₄⁻ at room temperature for 2 h (protected from light) or proteinase K (0.2 mg/ml; Promega) at 37 °C for 3 h to determine whether proteinase K or periodate destroys the phage receptor. A phage adsorption assay was then performed, as previously described. LB medium was used as the nonadsorbing control in each assay, and the phage titer in the control supernatant was set to 100%. Each assay was performed in duplicate and repeated twice.

Phage inactivation by LPS. LPS was extracted from A. xylosoxidans using an LPS extraction kit from Intron Biotechnology (17144; Boca Scientific, Boca Raton, FL, USA), according to the manufacturer’s instructions. A control containing LPS from Escherichia coli (O111:B4, purchased from Sigma-Aldrich, Inc. (L2630; Sigma, USA), was used as the negative control to ensure that any effect was specific to A. xylosoxidans LPS. Phage inactivation by LPS was tested as previously described.

Nucleotide sequence accession number. The nucleotide sequence of phiAxp-2 phage reported in this article has been deposited in the GenBank database as accession number KT321316.

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Author Contributions
E.L. and Z.Y. did the experiments and contributed equally to this study as joint first authors. Y.M., H.L., W.L. and X.W. analyzed the data. R.Z., A.J. and J.Y. provided the bacterial strains. X.Z. designed the experiments, managed the project and wrote the article.

Additional Information
Competing financial interests: The authors declare no competing financial interests.
