



## LETTER

# Isolation and characterization of a lytic bacteriophage $\phi$ Kp-lyy15 of *Klebsiella pneumoniae*

Dear Editor,

Bacteriophages (phages) are viruses that specifically infect and kill bacteria. They are ubiquitous throughout all environments that bacteria inhabit. Following their discovery by F.W. Twort in 1915 and F. d'Herele in 1917, bacteriophages were recognized as potential agents to treat bacterial diseases and phage therapy has been used in clinical practice ever since. However, with the invention of penicillin and other antibiotics, phage therapy was largely given up by most Western countries, with the exception of Russia, Georgia and Poland. Recently, the increasing incidence of antibiotic resistance and untreatable bacterial infections has led researchers to repurpose these bacteria-specific viruses as important agents against pathogenic bacteria, in human and veterinary medicine, food industry, and agriculture.

*Klebsiella pneumoniae* is a facultative anaerobic, gram-negative, rod-shaped bacterium that is very common in hospital environments. This organism is an important causative agent of nosocomial and opportunistic infection in humans, such as pneumonia, urinary tract infections, bacteremia, and intra-abdominal infections (Podschun and Ullmann, 1998; Fang et al., 2007). Infections caused by *K. pneumoniae* are frequently life threatening in infants, the elderly and immunocompromised individuals (Ko et al., 2002). In addition, extensive use of broad-spectrum antibiotics in hospitalized patients has facilitated the development of multidrug-resistance due to the production of extended-spectrum  $\beta$ -lactamases (Lin et al., 2007). The declining efficacy of antibiotic therapies leads to the necessary development of novel therapeutics for *K. pneumoniae* infections (Kumari et al., 2011; Hung et al., 2011). The goals of this study were to isolate phages that were lytic against *K. pneumoniae* isolates, and characterize their biological features to evaluate their potential as therapeutic agents to fight *K. pneumoniae* infections.

Here 25 clinical isolates of *K. pneumoniae*, previously isolated at Department of Clinical Laboratory, the third affiliated hospital of Jilin University, were used for phage isolation and identification (Table 1). Phage isolation was conducted as described previously (Yang et al., 2010; Parasion et al., 2012). Purified phage particle morphology was examined by transmission electron microscope

(TEM) of negatively stained preparations. The extracted phage DNA was subjected to restriction digestion with nine restriction enzymes (*EcoR* I, *EcoR* V, *Xho* I, *Nde* I, *Pst* I, *Bgl* II, *Hind* III, *Xba* I, and *Not* I) and analyzed by agarose gel electrophoresis. Multiplicity of infection (MOI) for *K. pneumoniae* was determined by mixing bacteria and phages at ratios ranging from  $10^{-4}$  to  $10^2$  PFU/CFU in tubes containing  $10^8$  CFU/mL *K. pneumoniae*. The tubes were incubated at 37 °C for 4 h, and the number of plaques on double-layer solid medium was calculated. One-step growth experiments were carried out according to a standard method (Hyman and Abedon, 2009).

To determine the proteins of phage particles, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as previously described (Boulanger, 2009). Resistance to different pH values at 37 °C was carried out as previously documented (Merabishvili et al., 2014). Phage thermal stability of phage at different temperatures (50, 60, 70 and 80 °C) was determined by incubating the phage ( $10^7$  PFU/mL) at the indicated temperature for 30 and 60 min in nutrient broth (pH 7.0); and then calculating the surviving phages. The host range of isolated phages was measured using spot test on 25 *K. pneumoniae* clinical strains, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

Table 1. Lytic activity of  $\phi$ Kp-lyy15 against various *Klebsiella* strains.

Bacterial species, strain tested	Sensitivity to $\phi$ Kp-lyy15*
<i>Klebsiella pneumoniae</i>	
K1, K2, K15, K17, K18, K19	+
K31, K36, K62, K67, K81	+
K3, K5, K6, K9, K11, K12, K13	–
K20, K21, K22, K26, K27, K28, K30	–
<i>Escherichia coli</i> ATCC 25922	–
<i>Pseudomonas aeruginosa</i> ATCC 27853	–

\*: Strains were grown in LB medium at 37 °C. Spots were divided into two categories: Clear zone (+) and no reaction (–).

This study describes a newly isolated bacteriophage  $\phi$ Kp-lyy15 specifically infecting *K. pneumoniae* isolates and its biological features. The *Klebsiella* bacteriophages described in publications belong to *Podoviridae*, *Myoviridae* and *Siphoviridae* families (Ackermann, 2001; Kumari et al., 2010). Our results showed that phage  $\phi$ Kp-lyy15 has an

icosahedral head 50 nm in diameter and a filamentous tail 120 nm in length. Thus, the bacterial virus was classified as a representative of *Siphoviridae* families (Figure 1A and 1B). The phage genome is presented by double-stranded DNA that is digested with the restriction endonucleases *EcoR* V, *Nde* I, and *Bgl* II (Figure 1C), and

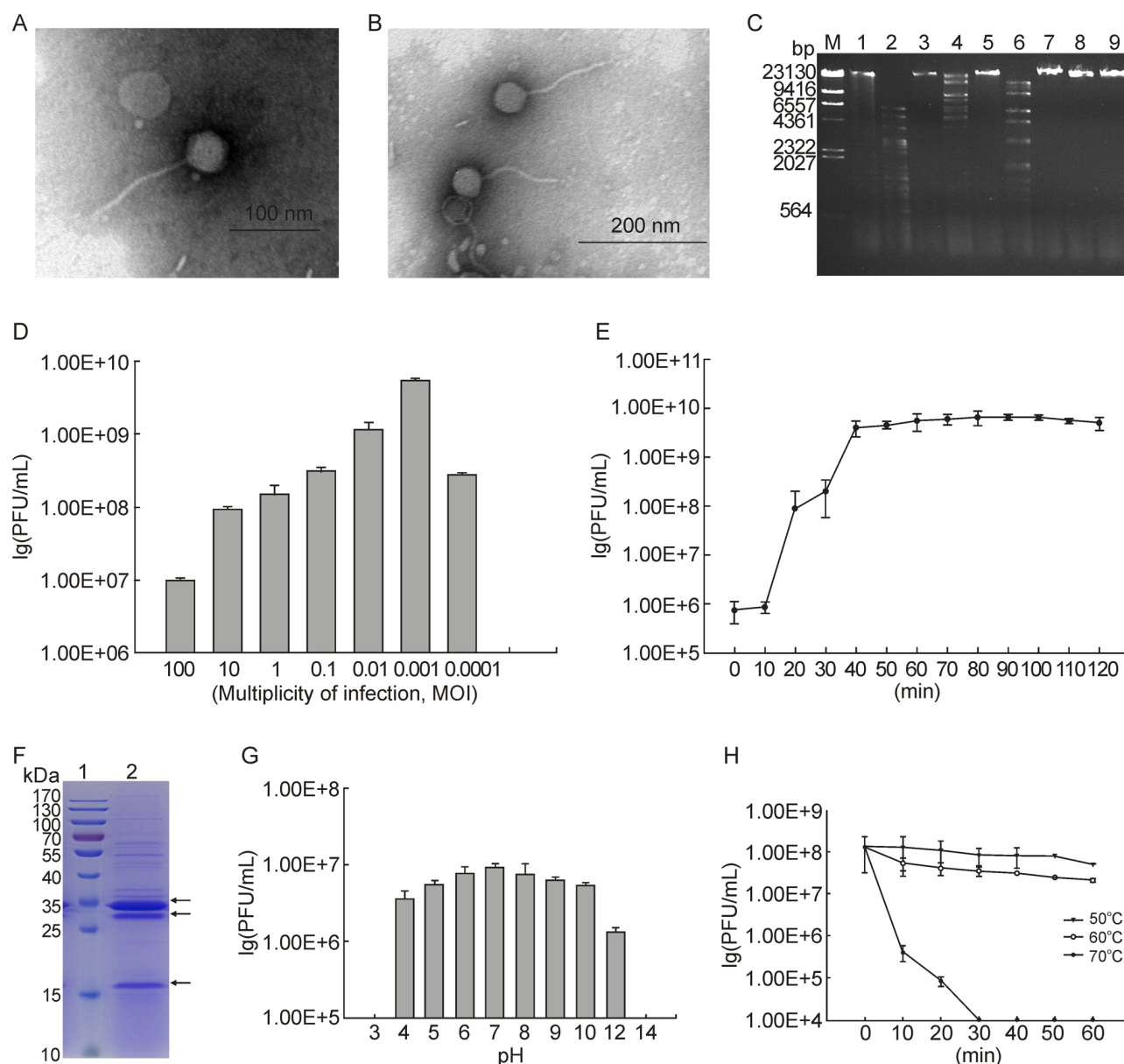


Figure 1. (A, B) Morphology of phage  $\phi$ Kp-lyy15 as revealed by TEM. (A) Scale bar=100nm, (B) Scale bar=200nm. (C) Restriction of phage genomic DNA. M: DNA marker; Lanes 1–9: Digestion of DNA with *EcoR* I, *EcoR* V, *Xho* I, *Nde* I, *Pst* I, *Bgl* II, *Hind* III, *Xba* I, and *Not* I respectively. (D) The MOI experiment. (E) One-step growth experiments. Latent phase from 0 to 10 min; Rise phase from 10 to 40 min, and Plateau phase from 40 to 120 min. (F) SDS-PAGE of phage protein. Lane 1: PageRuler Prestained Protein Ladder #26617 (Thermo), Lane 2: Phage  $\phi$ Kp-lyy15 ( $10^9$  PFU/mL), arrows indicate major protein bands. (G) pH stability tests of phage  $\phi$ Kp-lyy15. (H) Thermal stability tests of phage  $\phi$ Kp-lyy15.

our future research will focus on further detailed analyses of the entire  $\phi$ Kp-lyy15 genome to understand the genetic characteristics of this bacteriophage. As shown in Figure 1D, the group with an MOI of  $10^{-3}$  yielded the highest production of phage progeny ( $5 \times 10^9$  PFU/mL). One-step growth tests were carried out to determine the latent time and phage burst size of  $\phi$ Kp-lyy15. The latent period, defined as the time interval between the adsorption and the beginning of the first burst, was 10 min, and the burst size was 65 PFU/infected cell (Figure 1E). In this study, the burst size was equivalent to the number of phage at plateau phase ( $6.5 \times 10^9$  PFU/mL) divided by the initial number of host cells ( $1.0 \times 10^8$  CFU/mL).

Purified phage particles were subjected to SDS-PAGE to quantify the number of structural phage proteins. Three major protein bands and at least 12 minor protein bands were detected, with molecular weights ranging from approximately 15 to 170 kDa (Figure 1F). The most predominant polypeptide band of approximately 35 kDa presumably corresponded to a major capsid protein.  $\phi$ Kp-lyy15 stability was investigated at different pHs and temperatures. The results showed that no apparent effect on  $\phi$ Kp-lyy15 was observed after 1 hour of incubation at pH values ranging from 5 to 10. However, the phage dramatically lost its activity at pHs lower than 3 and higher than 13 (Figure 1G). The thermal stability test shown in Figure 1H indicates that phage  $\phi$ Kp-lyy15 was relatively heat stable, the  $5.1 \times 10^7$  PFU/mL and  $2.1 \times 10^7$  PFU/mL titers of active phages were detected after 60 min incubation at 50 °C and 60 °C, respectively. Furthermore, the phage titer quickly dropped at 70 °C, and no active phages were detected after 30 min.

The main aim of this study was the identification of a lytic bacteriophage with potential for therapeutic use. Therefore, the phage's lytic activity and host specificity were tested against 25 strains *K. pneumoniae* strains. The phage was shown to specifically infect and lyse 44% (11 of 25) of *K. pneumoniae* strains by forming clear zones (Table 1). Two other gram-negative microorganisms, *E. coli* and *P. aeruginosa*, were insensitive to the phage. In contrast, the phage KP36 belonging to *Siphoviridae* exhibited lower lytic activity against *K. pneumoniae* strains (7%–15%) as documented in a recent publication (Kesik-Szeloch et al., 2013).

In conclusion, the lytic bacteriophage  $\phi$ Kp-lyy belonging to the *Siphoviridae* family specific for *K. pneumoniae* was isolated and characterized.  $\phi$ Kp-lyy displayed a

short latent period, stability to a wide pH range, high thermal resistance, and lytic activity toward a relatively broad range of *K. pneumoniae* isolates. Thus, phage  $\phi$ Kp-lyy should be considered as a candidate for inclusion in phage cocktails to control *K. pneumoniae*-associated nosocomial infections.

## FOOTNOTES

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## REFERENCES

- Ackermann HW. 2001. Arch Virol, 146: 843–857.
- Boulanger P. 2009. In: Bacteriophages: Method and Protocols, Clokie MR, Kropinski AM (eds). New York: Humana Press. pp227–238.
- Fang CT, Lai SY, Yi WC, et al. 2007. Clin Infect Dis, 45: 284–293.
- Hung CH, Kuo CF, Wang CH, et al. 2011. Antimicrob Agents Chemother, 55: 1358–1365.
- Hyman P, Abedon ST. 2009. In: Bacteriophages: Method and Protocols, Clokie MR, Kropinski AM (eds.), New York: Humana Press, pp175–202.
- Kesik-Szeloch A, Drulis-Kawa Z, Weber-Dabrowska B, et al. 2013. Virol J, 10: 100.
- Ko WC, Paterson DL, Sagnimeni AJ, et al. 2002. Emerg Infect Dis, 8: 160–166.
- Kumari S, Harai K, Chhibber S. 2011. J Med Microbiol, 60: 205–210.
- Kumari S, Harjai K, Chhibber S. 2010. Folia Microbiol, 55: 221–227.
- Lin JC, Siu LK, Fung CP, et al. 2007. J Clin Microbiol, 45: 266–269.
- Merabishvili M, Vandenheuvel D, Kropinski AM, et al. 2014. PloS One, 9: e104853.
- Parasion S, Kwiatek M, Mizak L, et al. 2012. Curr Microbiol, 65: 284–289.
- Podschun R, Ullmann U. 1998. Clin Microbiol Rev, 11: 589–603.
- Yang H, Liang L, Lin S, et al. 2010. BMC Microbiol, 10: 131.

