One Health Advances

ARTICLE Open Access

A novel depolymerase specifically degrades the K62-type capsular polysaccharide of *Klebsiella pneumoniae*

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Abstract

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is emerging as an imminent threat to worldwide public health because of its high level of antimicrobial resistance, which can result in severe and challenging-to-treat infections. The capsular polysaccharide (CPS) of bacteria is well acknowledged as a crucial virulence factor that shields *K. pneumoniae* from the host's innate immune system. Polysaccharide depolymerase, encoded by bacteriophages, can hydrolyze the CPS of *K. pneumoniae* and may be a promising approach for treating *K. pneumoniae* infections. In this study, we identified a novel K62-type capsule depolymerase (K62-Dpo30) from the *K. pneumoniae* phage SH-KP2492. We demonstrated that the K62-Dpo30 depolymerase could specifically degrade the CPS of K62-type *K. pneumoniae* strains and promote the susceptibility of K62-type *K. pneumoniae* strains to serum and neutrophil killing. Furthermore, our findings highlight the potential of the K62-Dpo30 depolymerase as a reliable *K. pneumoniae* capsular typing tool.

Keywords *Klebsiella pneumoniae*, Bacteriophage, Capsule depolymerase

Introduction

Klebsiella pneumoniae is a gram-negative pathogenic bacterium responsible for a substantial number of bacterial infections acquired from hospitals or communities. This bacterium is capable of inducing severe infections such as bacteremia, urinary tract infections, and pneumonia, particularly among immunocompromised people. Additionally, it can also colonize the skin, nasopharynx, and gut of healthy individuals [1]. Notably, the global incidence of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) has substantially increased in recent years, primarily attributed to the inappropriate use of antibiotics [2, 3]. The situation in China is particularly alarming; the resistance rates of *K. pneumoniae* to meropenem and imipenem significantly increased from 2.9% and 3.0%, respectively, in 2005 to 24.2% and 22.6% in 2022, as reported by the CHINET Antimicrobial Surveillance Network [4, 5]. Coupled with the rising mortality associated with CRKP infections [6–8], this underscores the necessity of finding efficacious alternative treatments to manage CRKP infections.

Immune strategies targeting bacterial surface polysaccharides have been suggested as a promising alternative strategies for addressing antibiotic-resistant *K. pneumoniae* infections by reducing the reliance on antibiotic usage [1, 9]. The capsule polysaccharide (CPS) is the extracellular polysaccharide matrix of *K. pneumoniae*. It functions as a major defense structure against the innate immune system of the host, and is considered a pivotal

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virulence factor [10]. The capsule plays a critical role in protecting strains from opsonization and phagocytosis by neutrophils and macrophages, as well as evading antimicrobial peptides and complement-mediated killing. Recent studies have demonstrated the efficacy of vaccines and monoclonal antibodies (mAbs) specifically targeting the CPS in preventing and treating *K. pneumoniae* infections in several animal models [9, 11–13]. The efficacious development of K-based *K. pneumoniae* vaccines necessitates a comprehensive understanding of distinct K-types.

To date, more than 130 capsule locus types, commonly referred to as K types, have been reported [14]. Traditional serotyping is limited by the extensive manufacture of capsule-specific serum, which is time-consuming and labor-intensive [15–17]. Several other genotyping techniques for capsule type determination, including *cps* PCR restriction fragment length polymorphism (PCR–RFLP) analysis and *cps* gene-specific PCR amplification, have been proposed [18–20]. Among these methods, capsule-specific *wzi* PCR-based genotyping has been widely used for *K. pneumoniae* K typing in epidemiological investigations, because of its swiftness and technical simplicity [19, 21]. Nevertheless, this approach is incapable of detecting alterations in the CPS structure resulting from mutations in capsule biosynthesis genes [22]. Phage-encoded capsule depolymerase is considered a viable agent for capsule typing because it directly degrades the specific CPSs [23]. Our previous research has shown that depolymerase-based typing is equally dependable as the conventional serological capsular typing method [22, 24]. To date, numerous capsule depolymerases that specifically targeting K1-3, K5, K7-8, K11, K13, K19-23, K25, K27, K30, K35, K37, K47, K51, K56, K57, K63-64, K69 and KN1-N5 have been identified [23] and some of their typing capabilities have been demonstrated.

An analysis of globally available genomic data revealed that K1, K2, K5, K16, K23, K27, K28, K54, K62, and K64 represent the ten foremost prevalent K-types across six different countries [1]. In China, the most common K-types found in CRKP strains were K47 and K64 [6, 25]. Furthermore, certain specific regions showed a high incidence of K2, K15, K19, K21, K24, K28, K36, K62, KN2, KN5 and other K-types [3, 26–28]. The characteristics and potential functions of depolymerases targeting the majority of these prevalent K-types strains have been reported. Specifically, we focused on the prevalence of K62-type *K. pneumoniae*, which has emerged as the second most common K-type among CRKP strains in a children's hospital in Shanghai, China [26]. Moreover, the K62-type *K. pneumoniae* strain emerged as one of the five most prevalent serotypes in Taiwan, and exhibited significant resistance to a multitude of antibiotics in a two-decade seroepidemiological study [29]. This highlights the importance of thoroughly examining and resolving the challenges posed by K62 strains in certain regions of China.

In this research, we isolated a bacteriophage from K62-type *K. pneumoniae* strains and identified its capsule-specific depolymerase. We provided evidence to illustrate the specific degradation of K62-type CPS by this depolymerase, which subsequently increased the susceptibility of K62-type *K. pneumoniae* strains to serum and neutrophil killing. In addition, this depolymerase exhibited potential as a dependable and promising agent for K-typing.

Results

Characterization of phage SH-KP2492

Through co-cultivation with the host K62-type *K. pneumoniae* strain 2492, the bacteriophage SH-KP2492 was effectively obtained from hospital sewage. The phage generated clear plaques accompanied by translucent halos on the double-layered agar plates when infecting the host strain 2492. Notably, the halo size progressively increased over time (Fig. 1A), suggesting the existence of a bacteriophage polysaccharide depolymerase originating from phage SH-KP2492. Transmission electron microscopy (TEM) revealed an icosahedral head measuring 60 ± 5 nm in diameter and a spidery tail measuring 200 ± 5 nm in length of the phage (Fig. 1B), which demonstrated that the phage was classified as the family Siphoviridae.

Whole-genome sequencing and annotation of phage SH-KP2492

Based on the results of whole-genome sequencing, the genome of the phage consisted of 48,383 bp and exhibited a G + C content of 50.32%. Using BLASTn, we discovered that SH-KP2492 was highly similar to the *K. pneumoniae* siphophage phage Shelby (GenBank accession number: MK931445) in the NCBI database, with a query coverage rate of 91% and a percentage identity of 96.77%. Furthermore, BLASTp revealed that SH-KP2492 contained 78 open reading frames (ORFs), which could be classified into four categories including hypothetical proteins (n = 37), DNA packaging and morphology-related proteins (n = 18), DNA replication/recombination/modification proteins (n = 16), and host lysis proteins (n = 7) (Fig. 2A). Previous research has shown that bacteriophage depolymerases are predominantly found in the tail spike or tail fiber [30]. Using NCBI-BLASTp and HHpred, the protein encoded by *ORF30* (designated K62-Dpo30) was predicted to be the tail fiber protein (Fig. 2A). It was observed to possess a domain with a β-helix structure within the central region (368 to 503 aa) and displayed significant similarity to pectate lyase [30]

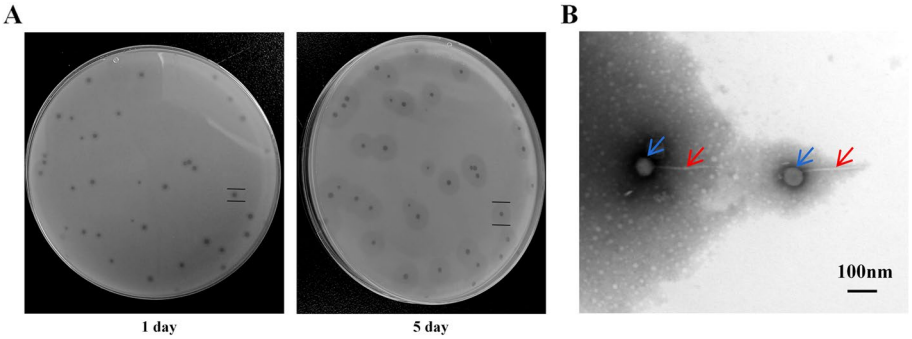

Fig. 1 Characterization of the host phage SH-KP2492. **A** The bacteriophage SH-KP2492 infected the host *K. pneumoniae* strain 2492 on the double-layer LB agar, and halos around the plaque were observed on day 1 and day 5. **B** TEM images of the host phage SH-Kp2492. The phage icosahedral head and spidery tail are indicated by blue and red arrows, respectively. Scale bar: 100 nm

(Fig. 2B). Based on these characteristics, K62-Dpo30 had a high possibility of being a bacteriophage polysaccharide depolymerase.

Depolymerization activity of the K62-Dpo30

The pSUMO3 vector was used for the cloning of the phage SH-KP2492-*ORF30* gene to determine the depolymerase activity of K62-Dpo30. The recombinant depolymerase K62-Dpo30 was subsequently expressed in *Escherichia coli* BL21 strain and further purified using a Ni–NTA column. The purity of the recombinant K62-Dpo30 protein (83 kDa) was confirmed by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 3A). Various concentrations of the purified K62-Dpo30 protein (from 1.7 mg/mL to 0.1 mg/mL) were used to assess the depolymerization of the polysaccharide via a spot assay. We observed clear halos at a concentration of 0.21 mg/mL of K62-Dpo30 protein (Fig. 3B). The Size Exclusion Chromatography-High Performance Liquid Chromatography (SEC-HPLC) analysis revealed that the K62-CPS group (depolymerase-untreated) exhibited a peak at approximately 2 min retention time, while the peak corresponding to K62-CPS disappeared after incubation with K62-Dpo30 depolymerase at either 37 °C or 25 °C for 30 min, indicating that the K62-Dpo30 protein could depolymerize the K62-type CPS of host strain 2492 (Fig. 3C). The degradation of K62-type CPS by the K62-Dpo30 protein was further confirmed through SDS-PAGE and alcian blue staining (Fig. 3D).

K62-Dpo30 enhanced the susceptibility of strain 2492 to serum killing

The CPS of *K. pneumoniae* is a critical virulence factor that confers resistance to serum killing. To assess the impact of depolymerase on bacterial mortality in serum, *K. pneumoniae* strain 2492 was pre-incubated with K62-Dpo30 protein or K64-ORF41 protein [22] (a depolymerase that specifically targets K64-type CPS), after which the survival rate was calculated after incubation with 75% baby rabbit serum. We discovered that the bacterial survival rate decreased in K62-Dpo30-treated cells compared with K64-ORF41-treated cells in the presence of rabbit serum, while there was no notable difference in the presence of heat-inactivated rabbit serum or PBS (Fig. 4A). These data demonstrated that K62-Dpo30 depolymerase could increase the susceptibility of the K62-type strain to serum.

K62-Dpo30 increased the susceptibility of strain 2492 to neutrophil killing

Neutrophils are essential killer cells that eliminate bacterial infections as part of the innate immune response. Therefore, we evaluated the effect of K62-Dpo30 on depolymerase activity in the HL-60 cell line in the presence of 10% baby rabbit serum or heat-inactivated rabbit serum to perform a neutrophil-killing assay. The *K. pneumoniae* strain 2492 was pre-incubated with the K62-Dpo30 protein, K64-ORF41 protein or PBS and then incubated with cells for 45 min. The results showed no statistically significant difference among the groups when HL60 cells were treated with heat-inactivated serum. The group treated with K62-Dpo30 exhibited a markedly lower bacterial survival rate than the K64-ORF41 and PBS groups when incubated with cells in the presence of normal rabbit serum (Fig. 4B), which demonstrated that the polysaccharide depolymerase can significantly improve the susceptibility of the K62-type strain to neutrophil killing in the presence of 10% baby rabbit serum.

Bacteriophage SH-KP2492 and K62-Dpo30 depolymerase specifically target K62-Type *K. pneumoniae*

We analysed the bacterial lysis spectrum of SH-KP2492 on thirty-seven strains using a phage spot test to

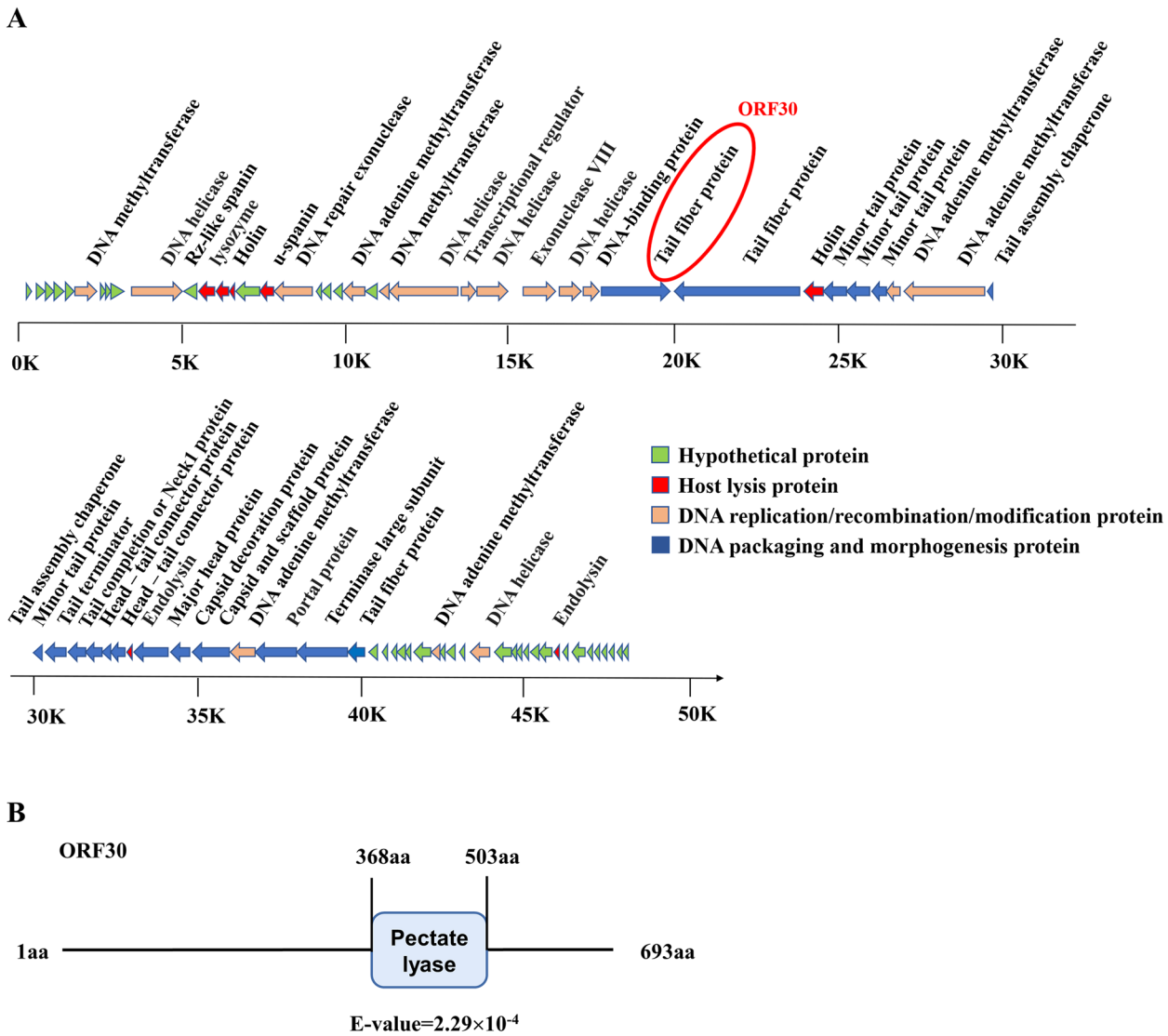

Fig. 2 Genomic analysis of phage SH-KP2492. **A** Gene map of host phage SH-KP2492. A total of 78 predicted ORFs are displayed in different colors. Among them, the gene *ORF30* circled in red, encodes the tail fiber protein and is anticipated to function as a polysaccharide depolymerase. **B** Bioinformatic analysis of the predicted depolymerase K62-Dpo30. The tail fiber protein was a 692 aa protein and was predicted to have one domain: pectate lyase domain (residues 368–503) utilizing BLASTp and HHpred

determine the relationship between SH-KP2492 and bacterial K types. A total of twenty-three K62-type strains exhibited plaques and halos, whereas two K62-type strains (17–349 and 18–448) lacked plaques or halos (Table 1). These two K62-type strains without plaques or halos were appeared as translucent colonies and were confirmed to be nonencapsulated *K. pneumoniae* strains by uronic acid assay (Fig. 5A). The results of synthetic gene sequencing CPS revealed that that IS (IS*Kpn26* or IS*1A*) insertion destroyed the initiating glycosyltransferase gene (*wbaP*) in strains 17–349 and 18–448, respectively (Fig. 5B). The remaining twelve non-K62-type strains exhibited no plaques or halos (Table 1). Collectively, these results suggest that phage SH-KP2492 specifically targets the K62-type *K. pneumoniae* strains.

Furthermore, to evaluate the specific depolymerizing activity of K62-Dpo30, the purified K62-Dpo30 was spotted separately onto double-layer LB plates containing thirty-seven different *K. pneumoniae* strains. The translucent halo was observed for all

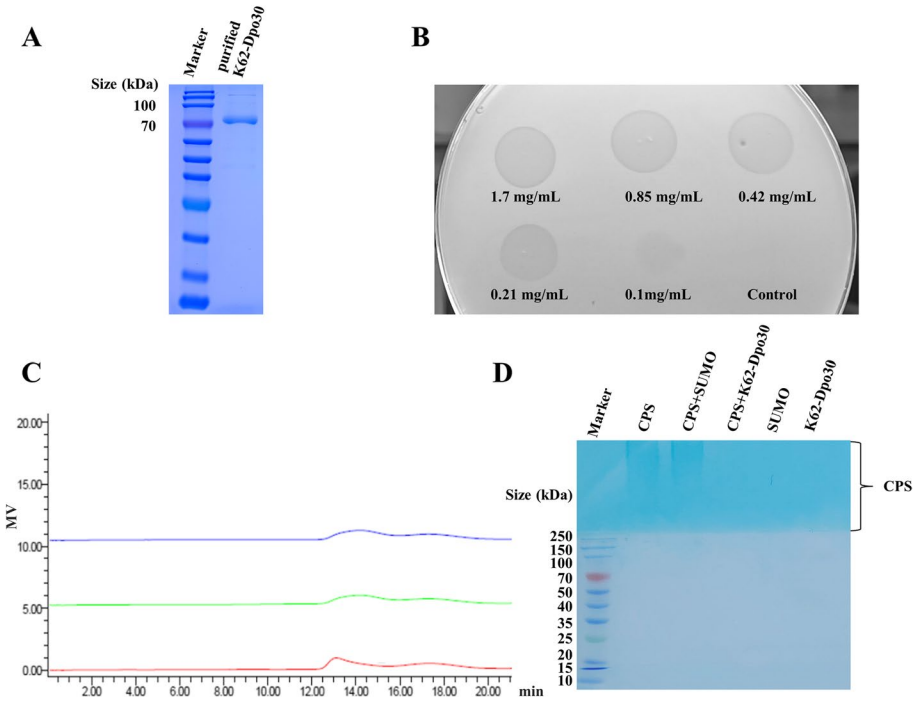

Fig. 3 Expression and depolymerization activity of K62-ORF30 depolymerase. Purified K62-Dpo30 protein was determined by 10% SDS-PAGE gel and Coomassie blue staining. **B** The polysaccharide depolymerization activity of K62-Dpo30 protein was examined through the spot assay utilizing various concentrations (ranging from 0.1 mg/mL to 1.7 mg/mL) of K62-Dpo30 protein. SUMO protein was employed as a negative control. **C** The impact of K62-Dpo30 depolymerase on K62-CPS was assessed by SEC-HPLC. The K62-CPS was incubated with either 10μg/mL K62-Dpo30 protein for 30 min at 37 °C (blue line), or at 25 °C (green line), or with 10μ g/mL SUMO protein for 30 min at 37 °C (red line). **D** The effect of K62-Dpo30 on K62-CPS was analyzed by Alcian blue staining. K62-CPS was incubated with or without K62-ORF30 or SUMO protein for 30 min at 25 °C

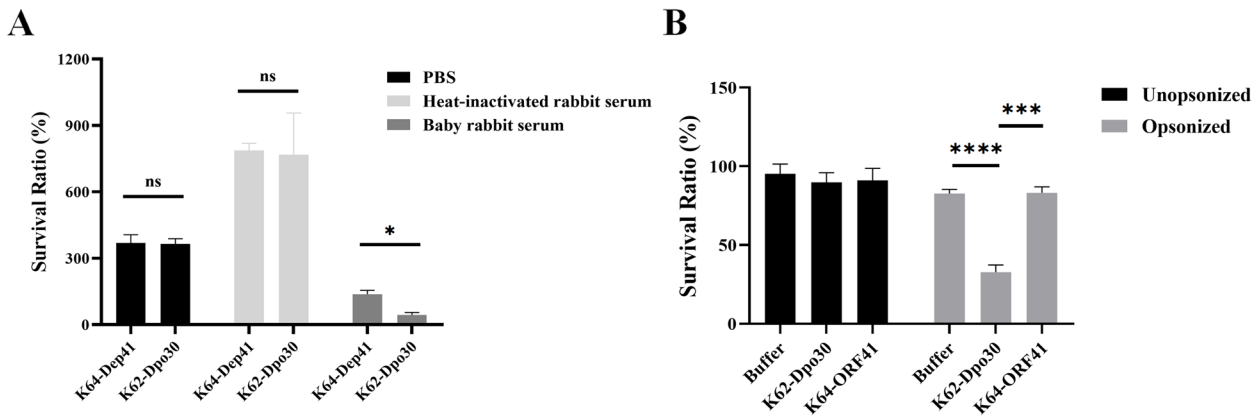

Fig. 4 Impact of the K62-Dpo30 on bacterial vulnerability to serum killing and neutrophil killing. **A** Serum-killing assay was conducted by pre-incubating the strain 2492 with K62-Dpo30 or K64-ORF41 protein for 1 h at 37 °C. Subsequently, the strain was incubated with 75% rabbit serum, heat-inactivated rabbit serum or PBS for 2 h at 37 °C. **B** Neutrophil killing assay was performed by incubating the strain 2492 with K62-Dpo30, K64-ORF41 or PBS with HL60 cells for 45 min at 37 °C. HL60 cells were pre-mixed with 10% baby rabbit serum or heat-inactivated rabbit serum. The percent survival was determined as the number of surviving bacteria relative to the initial inoculum. Data are presented as the mean ± SEM and represent by the three separate experiments. Two-tailed unpaired Student's t-test was used for statistical analysis. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ****$p < 0.0001$

K62-type encapsulated *K. pneumoniae* strains, but not for K62-type nonencapsulated strains or other K-type strains (Table 1). This finding indicated that the K62-Dpo30 protein specifically targets K62-CPS.

Table 1 The activity spectra of phage SH-KP2492 and K62-Dpo30

Isolate No	K genotype	SH-KP2492		K62-Dpo30
		Plaques	Halos	
2492[1c], 6159[a], 6236[a], 6550[a], 7285[a], 8368[a], 16–18[b], 16–19[b], 16–202[b], 17–87[b], 17–173[b], 17–175[b], 17–177[b], 17–351[b], 18–61[b], 18–65[b], 18–195[b], 19–177[b], 19–178[b], 19–199[b], 19–514[b],19–867[b], 19–872[b]	KL62	+	+	+
17–349[b], 18–448[b]	KL62	-	-	-
5174[a], 7512[a]	KL1	-	-	-
5146[a], 20–1143[b]	KL2	-	-	-
16–312[b], 20–445[b]	KL64	-	-	-
18–81[b], 19–249[b]	KL47	-	-	-
18–199[b], 18–201[b]	KL19	-	-	-
20–991[b], 5054[a]	KL57	-	-	-

The capsular genotypes of strains were identified by wzi genotyping. -, no lysis; +, showed plaques or halos

[a] Strains were isolated from Renji Hospital affiliated with Shanghai Jiao Tong University School of Medicine

[b] Strains were isolated from Huashan Hospital affiliated with Fudan University

[c] Host strain of SH-KP2492

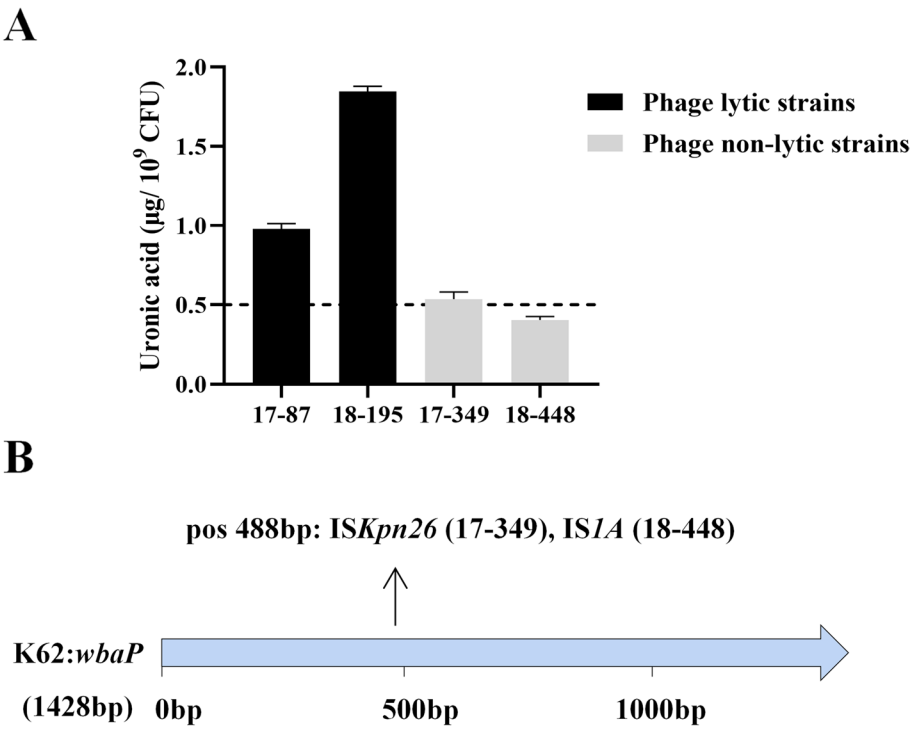

Fig. 5 The capsular polysaccharide of phage SH-KP2492 non-lytic K62 strains. **A** Uronic acid assay was performed on phage lytic strains (17–87 and 18–195) and phage non-lytic strains 17–349 and 18–448. Strains 17–349 and 18–448 had a significantly lower amount of polysaccharide capsule uronic acid than strains 17–87 and 18–195. Data are shown as mean ± SEM. **B**. The *wbaP* gene variants in phage non-lytic K62 strains. Mutations found in the capsule biosynthesis gene *wbaP* in nonencapsulated isolates

Discussion

Because of their significant morbidity and mortality, CRKP infections represent a major public health threat worldwide [7, 31]. Additionally, the prevalence of carbapenem-resistant hypervirulent *K. pneumoniae* (CR-HvKP), which is associated with both hyperresistance and hypervirulence, has rapidly increased

worldwide, particularly in China [26], posing a major threat to public safety.

Recently, phage-derived depolymerase has been considered as one of the promising alternatives to prevent *K. pneumoniae* infection [32] due to its strong specificity [22, 24, 33, 34] in degrading the resistance structure (CPS) [30] of *K. pneumoniae* against innate immunity. Currently, more than 34 unique depolymerases have been discovered, each with a hydrolytic function specific to different types of capsules [24]. However, there is limited reported documentation on K62-specific capsular depolymerases. Although Yi-Jiun Pan and colleagues have used K62-specific capsule depolymerase to identify K62-type *K. pneumoniae* strains [3], the sequence and biological properties of the K62-specific depolymerase have not been published. Hence, this study aimed to identify a novel K62-type *K. pneumoniae*-specific depolymerase (K62-Dpo30). We elucidated the effectiveness of K62-Dpo30 in inhibiting the infection of K62-type *K. pneumoniae* strains and the potential application of this depolymerase.

Considering that CPS is a critical virulence factor in *K. pneumoniae* as it can protect *K. pneumoniae* from complement-mediated killing [35, 36] and phagocytosis by macrophages and neutrophils [37, 38]. Therefore, we measured the antibacterial impact of depolymerase K62-Dpo30 on the serum and neutrophils. Our data showed that K62-Dpo30 depolymerase could decrease the survival rate of the K62-type strain in 75% rabbit serum and promote the neutrophil killing to the K62-type strain, indicating that the degradation of the capsule by K62-Dpo30 facilitates the killing of *K. pneumoniae* by the host defense system.

Our study revealed that the K62 depolymerase typing results were basically identical with the *wzi* genotyping results in K62 *K. pneumoniae* strains except for two strains. Further sequencing analysis indicated that these two strains were non-encapsulated strains and had IS*Kpn26* or IS*1A* insertions in the *wbaP* gene. The *wbaP* gene was located in the cps gene cluster and encoded the initial glycosyltransferase, which was essential for capsule biosynthesis in *K. pneumoniae* strains [39]. The disruption of the *wbaP* gene could result in the loss of the capsule [40], as confirmed by the quantification of uronic acid in the capsule of K62 strains. Capsular typing using K62-specific depolymerase was found to be a more dependable method compared to *wzi* genotyping for detecting the disrupted capsular polysaccharide structure [22, 24].

Conclusions

In conclusion, we proved that the K62-specific depolymerase K62-Dpo30 could effectively degrade the CPS of K62-type *K. pneumoniae* strains. This depolymerase could promote the susceptibility of K62 *K. pneumoniae* strains to serum and neutrophils. Moreover, this approach has the potential to serve as a more reliable approach for determining the capsular type.

Materials and Methods

Strain collection and identification

A total of thirty-seven *K. pneumoniae* strains were obtained from two hospitals in Shanghai, China (Renji Hospital affiliated with Shanghai Jiao Tong University School of Medicine and Huashan Hospital affiliated with Fudan University), and identified by MALDI-TOF MS (bioMérieux, France). The *wzi* sequencing method was utilized to determine the capsular types of these strains [19], of which twenty-five were K62 type, and the others were K1, K2, K64, K47, K19 and K57 type. The host bacterium *K. pneumoniae* strain 2492 is a K62 -type CRKP strain resistant to various antibiotics, including amikacin, ciprofloxacin, gentamicin, imipenem, meropenem, tigecycline and trimethoprim-sulfamethoxazole.

Bacteriophage isolation and phage activity spectrum analysis

The bacteriophage SH-KP2492 was isolated as previously described [41]. Briefly, we isolated the phage from the sewage samples by utilizing the host bacterium *K. pneumoniae* strain 2492. After incubating with the sewage at 37 °C overnight, the supernatant was filtered through a 0.22 μm sterile filter (JET BIOFIL, China, Cat. No. FPE204013) following centrifugation. The filtrates containing the phages were then dropped onto a double-layer LB plate containing strain 2492. Subsequently, the phage was purified and amplified as previously reported [24]. The solution was then stored in SM buffer, which consisted of 8 mM $MgSO_4 7H_2O$, 50 mM Tris–HCl and 100 mM NaCl, with a pH of 7.5.

The phage spot assay against 37 strains of *K. pneumoniae* was also conducted to analyze the activity spectrum of SH-KP2492. Briefly, 5 μL of purified phage was spotted onto double-layer LB agar of log phase bacterial cultures and incubated for 12 h at 37 °C.

Genomic DNA Sequencing of Phage SH-KP2492

We extracted the genomic DNA of phage SH-KP2492 as previously described [22]. Briefly, we incubated the purified phage SH-KP2492 with 1 μg/mL deoxyribonuclease I (Beyotime Biotechnology, China, Cat. No. D7073) and 10 μg/mL Ribonuclease A (Beyotime Biotechnology,

China, Cat. No. ST578) at 37 °C for 1.5 h, followed by the addition of 25 mol/L ethylenediaminetetraacetic acid (Sigma-Aldrich, United States, Cat. No. E9884). The phage genomic DNA was then extracted through the lambda bacteriophage Genomic DNA Rapid Extraction Kit (Aidlab, China, Cat. No. DN2201). Genome sequencing was performed at Shanghai Personalbio Biotechnology Co. Ltd through the Illumina HiSeq 3000 platform (Shanghai, China). The genome was assembled by the SOAP denovo2 software. ORFs were predicted by GeneMark and annotated by PHASTER (http://phaster.ca/) and NCBI-BLASTP (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Cloning, expression and purification of K62-Dpo30

We used PCR to amplify the *ORF30* gene, which encodes the depolymerase from phage SH-KP2492 with primers K62-ORF30-F (5'-CAGCAGCAGACGGGAGGATCC ATGGCACTATACAGAGAAGGC-3') and K62-ORF30-R (5'-CTCGAGTGCGGCCGCAAGCTTTTAAACAGG ACGTAAGTTCTC-3'). Then we inserted the amplied DNA into the pSUMO3 expression vector. Subsequently, the recombinant plasmid was transformed into *Escherichia coli* BL21 strain. The K62-ORF30 protein was expressed after induction with 0.1 mM isopropyl-β-D-thiogalactopyranoside (Thermo Scientific, United States, Cat.No.34060) and suspended in lysis buffer, which consists of 10% glycerol, 0.25 M NaCl, and 20 mM Tris–HCl (pH = 8.0). Finally, the depolymerase ORF30 (named K62-Dpo30) was purified on a Ni–NTA column (GE Healthcare, United States), treated with SUMO protease (LifeSensors, United States) and analyzed via SDS-PAGE.

The depolymerase activity of K62-Dpo30

To test the depolymerase activity of K62-Dpo30, a total of 5 μL of the purified K62-Dpo30 depolymerase (ranging from 0.1 mg/mL to 1.7 mg/mL) or SUMO protein was added to a double-layer LB plate containing *K. pneumoniae* strain 2492 and incubated at 37 °C for 12 h. The depolymerase activity of K62-Dpo30 was observed by forming semi-clear halo zones. The sensitivity of other *K. pneumoniae* strains to K62-Dpo30 enzyme (0.21 mg/mL) was also determined via a spot assay.

Purification of the K62-CPS

The K62-CPS purification process was carried out following a previously described method with some modifications [24]. In summary, strain 2492 was cultured in Tryptone Soy Broth medium (Macklin, China, Cat. No. T770421) for 5 days at 37 °C. Then, 10 mL of culture was incubated with 60 μL of 37% formaldehyde solution (Sigma-Aldrich, United States, Cat. No. 1039991000) at 100 rpm for 1 h, and incubated with 1 M NaOH (Sinopharm Chemical Reagent, China, Cat. No. 10019718) for 3 h at room temperature. The mixture was then centrifuged for 1 h at 4 °C. The resulting supernatant was filtered using a 0.22 μm filter (JET BIOFIL, China, Cat. No. FPE204013) and then dialyzed in ddH_2O for an extended period. Subsequent addition of 0.5% w/v cetyltrimethylammonium bromide (Sigma-Aldrich, United States, Cat. No. 219374) induced polysaccharide precipitation. $CaCl_2$-driven dissociation at various concentrations preceded supernatant centrifugation. Selective precipitation with ethanol (Sinopharm Chemical Reagent, China, Cat. No. 10009218), followed by NaCl washing, was performed. Subsequent steps included Capto adhere chromatography post-ultrafiltration (30 kDa membrane). Ultimately, the K62-CPS was filtered, dried, and weighed after dialysis.

Quantification of uronic acid content in encapsulated or nonencapsulated K62 *K. pneumoniae* strains

The CPSs of encapsulated (17–87 and 18–195) or nonencapsulated (17–349 and 18–448) *K.pneumoniae* were extracted as previously described [42]. Specifically, the bacterial culture mixture was harvested overnight ($OD_{600} = 5$). Then, we mixed 500 μL of bacterial suspension with 100 μL of 1% zwittergent 3–14 detergent (Sangon Biotech, China, Cat. No. A610552) in 100 mM citric acid buffer at pH 2.0, and heated the mixture for 30 min at 55 °C with intermittent mixing. Following centrifugation, we collected 400 μL of supernatant, mixed it with absolute ethanol (final concentration 80%) and incubated it for 30 min at 4 °C. Subsequently, the CPS of these strains was extracted after centrifugation. Finally, we dried the CPS and dissolved it overnight in ddH_2O. The uronic acid content of CPS was quantified according to a previously described method [43]. In brief, we mixed the CPS specimen with 500 μL of 0.0125 M sodium tetraborate (Sigma-Aldrich, United States, Cat. No. 221732) in concentrated sulfuric acid. Subsequently, the resulting mixture was boiled at 100 °C for 15 min and cooled to ambient temperature. Then, we added 20 μL of 0.125% carbazole in absolute ethanol to the mixture, further incubated for 15 min at 100 °C and similarly cooled the mixture to room temperature. Finally, we detected the absorbance at 530 nm for each sample. The uronic acid content was directly correlated with the OD value using a standard curve of D-glucuronic acid.

SEC-HPLC

We utilized SEC-HPLC to validate the degradation activity of the recombinant depolymerase K62-Dpo30 against the K62 polysaccharide. In short, we dissolved the purified K62-CPS in 50 mM Na_2HPO_4, treated with 10 μg/mL K62-Dpo30 or ddH_2O for 30 min at 37 °C, and

finally discontinued the reaction by incubating for 5 min at 100 °C. Then, we used the Waters HPLC equipment (Waters, United States) to analyze the resulting reaction mixture.

TEM

We dropped the phage SH-KP2492 onto copper grids containing carbon-coated Formvar films. After 30-min air drying, the phage specimens were negatively stained with 2% phosphotungstic acid solution (Solarbio, China, Cat. No. G1870). Finally, the images were acquired with a Hitachi H-9500 transmission electron microscope (Tokyo, Japan).

Alcian blue staining

To assess the effectiveness of the K62-Dpo30 in degrading K62-CPS, we conducted the SDS-PAGE [44] and Alcian blue staining [23]. We separated the CPSs by 10% SDS-PAGE and rinsed the gel three times using the fix and wash buffer, consisting of 10% acetic acid, 25% ethanol in ddH_2O. Ultimately, we stained the gel with 0.125% Alcian blue at 50 °C for 15 min, and destained it with the fix and wash buffer at room temperature overnight.

Serum resistance assay

We conducted a serum resistance assay following the previously described methodology [24, 45]. Bacteria were incubated with K62-Dpo62 depolymerase (final concentration 100 μg mL^{-1}) or K64-ORF41 depolymerase [22] (final concentration 100 μg mL^{-1}) for 1 h, then incubated with 75% baby rabbit serum, heat-inactivated rabbit serum or PBS at 37 °C for 2 h. Finally, the survival rate of bacteria was calculated by the serial dilution agar plating method.

Neutrophil killing assay

The HL-60 human promyelocytic cell line was utilized to conduct the neutrophil killing assay as previously described [22, 46]. First, HL-60 cells were differentiated into neutrophils using 0.8% N, N-dimethylformamide for 5 days. *K. pneumoniae* was pre-incubated with K62-Dpo30 (final concentration 100 μg/mL), K64-ORF41 protein (final concentration 100 μg/mL) or buffer (10% glycerol, 0.25 M NaCl, 20 mM Tris–HCl) at 37 °C for 1 h. A total of 4×10^5 differentiated HL-60 cells were subsequently infected with *K. pneumoniae* at the multiplicity of infection of 1:400 for 45 min in the presence of 10% (vol/vol) rabbit serum or heat-inactivated rabbit serum while rotating at 700 rpm. Survival bacteria were then counted by serial dilution on LB agar plates.

Statistical analyses

Statistical analyses were performed utilizing GraphPad Prism. Data are shown as the mean ± SEM. Two-tailed unpaired Student's t-test was used for statistical analysis in serum resistance assay and neutrophil killing assay. P value < 0.05 was considered to indicate statistical significance.

Acknowledgements

We thank the Core Facility of Basic Medical Sciences, Shanghai Jiao Tong University School of Medicine for helping the imaging of transmission electron microscopy.

Authors' contributions

P.H. conceived and supervised the study; P.H. and Y.P. designed the experiments and wrote the manuscript; Y.P. and H.C. performed the experiments and analyzed the data; R.M., Y.W. and H.L. helped with experiments; A.W. and K.H. helped with data analysis; J.Y. took part in the editing of the manuscript. All authors read and approved the final manuscript.

Funding

This work was funded by the the National Key Research and development Project of China (grant no. 2022YFA1304300) and National Natural Science Foundation of China (grant no. 81971896).

Availability of data and materials

The complete genome sequence of bacteriophage SH-KP2492 has been deposited in NCBI database (GenBank accession: OR700190). Data are available from the corresponding author on request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

The data presented in this manuscript have not been previously reported by the authors nor are they under consideration for publication elsewhere. All authors have contributed to, reviewed, and approved the final, submitted version of the manuscript.

Competing interests

All authors declare that they have no competing interests.

Received: 16 October 2023 Revised: 17 January 2024 Accepted: 21 January 2024
Published online: 16 February 2024

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