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ORIGINAL RESEARCH PAPER

Non-capsulated mutants of a chemical-producing *Klebsiella pneumoniae* strain

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Abstract
Objectives To investigate the outcomes of capsule lost on cell transformation efficiency and chemicals (1,3-propanediol, 2,3-butanediol, and 2-ketogluconic acid) production by *Klebsiella pneumoniae*.
Results The *cps* gene cluster showed low sequence homology with pathogenic strains. The *wza* is a highly conserved gene in the *cps* cluster that encodes an outer membrane protein. A non-capsulated mutant was constructed by deletion of *wza*. Phenotype studies demonstrated that non-capsulated cells were less buoyant and easy to sediment. The transformation efficiency of the non-capsulated mutant reached 6.4×10^5 CFU μg^{-1} DNA, which is 10 times higher than that of the wild strain. 52.2 g 1,3-propanediol L^{-1}, 30.7 g 2,3-butanediol L^{-1}, and 175.9 g 2-ketogluconic acid L^{-1} were produced by non-capsulated mutants, which were 10–40% lower compared to wild strain. Furthermore, viscosities of the three fermentation broths decreased to approximately 1.3 cP from the range of 1.8–2.2 cP.
Conclusions Non-capsulated *K. pneumoniae* mutants should allay concerns regarding biological safety, improve transformation efficiency, lower viscosity, and subsequently ameliorate the financial burden of the downstream process of chemicals production.

Keywords 2,3-Butanediol · Capsule · 2-Ketogluconic acid · *Klebsiella pneumoniae* · 1,3-Propanediol

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Introduction

Klebsiella pneumoniae is crucial to the biotechnology industry, producing valuable chemicals such as 1,3-propanediol, 2,3-butanediol, 2-ketogluconic acid, and 3-hydroxypropionic acid. Along with other members of the *Enterobacteriaceae* family, *K. pneumoniae* is able to form a polysaccharides outer-capsule, which plays a critical role in protecting *K. pneumoniae* from phagocytic host cells (Schembri et al. 2004). The composition of the capsular polysaccharide is very much strain-dependent, and at least 78 serological types, based on capsule antigens, have been assigned to *Klebsiella* spp. (Pan et al. 2008).

In addition to these physiological functions, the capsule also hinders DNA penetration by electroporation, leading to low transformation efficiency and limiting genetic studies (Fournet-Fayard et al. 1995). In researching chemical production by *K. pneumoniae*, the capsular polysaccharide was found to increase broth viscosity, which impeded filtration, and added to financial burden of downstream processing (Guo et al. 2010).

Capsular polysaccharide synthesis occurs through complicated interactions involving enzymes encoded in gene clusters. To date, the genetic and biosynthetic pathways of *K. pneumoniae* capsule expression have yet to be fully characterized. The first *K. pneumoniae cps* gene cluster to be cloned was a serotype K2 strain, in which 19 open reading frames (ORF) were identified (Arakawa et al. 1995). Six genes located at the 5′ end (*galF*, *acidPPc*, *wzi*, *wza*, *wzb*, and *wzc*) and a single gene (*gnd*) located at the 3′ end were found to be highly conserved among *K. pneumoniae* species (Brisse et al. 2013; Rahn et al. 1999). The functions of a number of *cps* genes have been characterized in a serotype K1 strain (Ho et al. 2011).

Klebsiella pneumoniae CGMCC 1.6366 (TUAC01) was isolated for 1,3-propanediol production (Hao et al. 2008). An efficient gene replacement system in *K. pneumoniae* CGMCC 1.6366 has been exploited, and several mutants have been constructed (Wei et al. 2012). In the current study, the highly conserved *wza* gene of *K. pneumoniae* was deleted, creating a non-capsulated mutant, and the phenotype, transformation efficiency, chemical production of the non-capsulated mutant were investigated.

Materials and methods

Bacterial strains, plasmids, and primers

The bacterial strains and plasmids are listed in Table 1. The PCR primers are listed in Table 2.

Determination of capsular serological type

The serological type was determined by *wzi* gene sequencing as per the method of Brisse et al. (2013). The *wzi* gene encodes an outer membrane protein involved in capsular attachment to the cell's surface, and is highly conserved in *K. pneumoniae* species.

Construction of *K. pneumoniae* CGMCC 1.6366-derived mutants

Klebsiella pneumoniae and *Escherichia coli* were cultured in Luria–Bertani (LB) medium at 37 °C. When required, the medium was supplemented with ampicillin (50 μg mL^{-1}), kanamycin (50 μg mL^{-1}), apramycin (50 μg mL^{-1}), or streptomycin (25 μg mL^{-1}).

Klebsiella pneumoniae Δwza was constructed according to a previously described method (Wei et al. 2012). Briefly, the *wza* gene and flanking sequences of *K. pneumoniae* were amplified by PCR using the primer pair wza-s1/wza-a1. The PCR product was ligated with pMD18-T simple vector to generate pMD18-T-wza. Linear DNA with 39 and 40 nt homologous extensions flanking the apramycin resistance gene *aac(3)IV* were amplified from plasmid pIJ773 using the primer pair wza-FRT-s1/wza-FRT-a1. pMD18-T-Δwza was constructed by replacing the *wza* in plasmid pMD18-T-wza with the *aac(3)IV* cassette using the Red recombination system in *E. coli*. pMD18-T-Δwza was then used as a template for PCR preparation of linear DNA containing *aac(3)IV* with 500-bp homologous regions at either end. The linear DNA was transformed into *K. pneumoniae*/red, which hosts the plasmid pDK6-red. Homologous recombination between the linear DNA and the chromosome was facilitated by Red recombinase, and led to *wza* deletion in *K. pneumoniae* CGMCC 1.6366. The mutant was isolated on apramycin plates, and the primer pair Test773 and wza-s were used for PCR confirmation.

Klebsiella pneumoniae ΔbudAΔwza was constructed from *K. pneumoniae* Δwza. Chromosomal *budA* of *K. pneumoniae* Δwza was replaced with the *aadA* cassette, generating a *budA* and *wza* deletion strain. The *aadA* cassette was amplified from pMD18-T-ΔbudA by PCR using primers budA-s and budA-a.

Phenotype observation

Klebsiella pneumoniae Δwza and *K. pneumoniae* CGMCC 1.6366 were cultured in LB medium at 37 °C for 12 h. The culture broth was centrifuged 10,000×*g* for 10 min, and the supernatant discarded. The pellet was washed twice with deionized water and resuspended in the same volume of water. The cells

Table 1 Bacterial strains and plasmids

Strain	Relevant genotype and description	Reference or source
Strains		
E. coli DH5α	Host of plasmid	Lab stock
K. pneumoniae CGMCC 1.6366	wild type, Amp^r	Hao et al. (2008)
K. pneumoniae/red	*K. pneumoniae* CGMCC 1.6366, pDK6-red	Wei et al. (2012)
K. pneumoniae Δwza	*K.pneumoniae* CGMCC 1.6366, Δwza	This work
K. pneumoniae ΔbudA	*K.pneumoniae* CGMCC 1.6366, ΔbudA	Wei et al. (2013)
K. pneumoniae ΔbudAΔwza	*K.pneumoniae* CGMCC 1.6366, Δwza, ΔbudA	This work
Plasmids		
pDK6	Kan^r, $lacI^Q$, *tac*, 5.1 kb	Lab stock
pDK6-red	Kan^r, carries λ-Red genes, 7.1 kb	Wei et al. (2012)
pMD18-T-ΔbudA	Amp, St^r, carries ΔbudA	Wei et al. (2013)
pIJ773	$Apra^r$, *aac(3)IV* with FRT sites, 4334 bp	Lab stock
pMD18-T simple	Amp^r, TA cloning vector, 2692 bp	Takara
pMD18-T-wza	Amp, carries wza, 4.6 kb	This work
pMD18-T-Δwza	Amp, $Apra^r$, carriesΔwza	This work

Table 2 Oligonucleotides used for PCR

Primers	Nucleotide sequence (5′–3′)
wza-s1	CTGAGGATAACCAGCGCTGGAGTAC
wza-a1	CTTCTTCGCTCTTGCGATAAGGGTC
wza-s	ATCTGGAAGCTCATGATACACGGAC
Test773	GCAAATACGGCATCAGTTACC
wza-FRT-s1	CAGCCTGCGCAAGAACGTGGTCG AGCTGCCGGACAGCGAATTCCG GGGATCCGTCGACC
wza-FRT-a1	ACCAGAGGAGCTGTCGTCACATAT ACGATATCGTATGGTTTGTAGGCT GGAGCTGCTTC
budA-s	GAAGATCAGAACATCGCCAGA
budA-a	CTCTGATGGACCTGCTTCGCCTTAT
budA-s1	GCCGCATGGAACATGGATG
Test778	AGAATCTCGCTCTCTCCAGGGGAAG

were pelleted by centrifugation at 4000×*g* for 10 min, and the phenotypes were observed.

Transformation of *K. pneumoniae* by electroporation

The preparation of electrocompetent cells and the conditions used for electroporation were as previously described (Wei et al. 2012). Ethylenediaminetetraacetic acid (EDTA)-treated electrocompetent cells were prepared by adding 0.7 mM EDTA to the cell culture when the OD600 reached 0.2. Plasmid pDK6 was used to transform cells for transformation efficiency determination. All experiments were conducted in triplicate.

Medium and culture conditions

The fermentation medium and culture conditions used for 1,3-propanediol production were as previously described (Hao et al. 2008).

The composition of the fermentation medium used for 2,3-butanediol production is as follows: 50 g glucose L^{-1}, 4 g corn steep liquor L^{-1}, 5 g $(NH_4)_2SO_4$ L^{-1}, 3 g sodium acetate L^{-1}, 0.4 g KCl L^{-1}, and 0.1 g $MgSO_4$ L^{-1}. For the seed culture, 250 mL flasks containing 50 mL LB broth were incubated in a rotary shaker at 37 °C and 200 rpm overnight. The 50 mL of seed culture was inoculated into a 5 L bioreactor (BIOSTAT-A plus Sartorius) with a working volume of 3 L. The air supplement, agitation, and culture temperatures were 4 L min^{-1}, 250 rpm, and 37 °C, respectively. The culture pH was maintained at pH 6 by the automated addition of 10 M NaOH. 600 g glucose L^{-1} solution was fed into the bioreactor when the glucose level in the medium decreased to 20 g L^{-1}.

The composition of the fermentation medium used in 2-ketogluconic acid production was as described previously (Wei et al. 2013). A two-stage fermentation strategy was used in 2-ketogluconic acid production. During the first stage of fermentation (4 h), the culture was maintained at pH 7.0, and agitated at 500 rpm. Upon completion of the first fermentation stage, the pH was adjusted to pH 5.0, and the bioreactor was agitated at 800 rpm. NH_3 solution was used to maintain pH.

All fermentation experiments were conducted in triplicate.

Analysis of biomass, substrate, and metabolic products

Biomass concentration was determined at 600 nm using UV-visible spectroscopy system (Beckman DU730, USA). Glucose, glycerol, 1,3-propanediol, 2,3-butanediol, and 2-ketogluconic acid concentrations were measured by HPLC as previously described (Hao et al. 2008; Wei et al. 2013).

Fermentation broth viscosity measurements

Fermentation broth viscosity was measured with a Brookfield viscometer at 30 °C and 100 rpm. The viscosity of the supernatant was also measured. Briefly, the fermentation broth was centrifuged at 10,000 × g for 10 min, the supernatant recovered, and the viscosity of the supernatant determined as for fermentation broth.

Results

Gene type and serological type of the capsule

The *cps* gene cluster of *K. pneumoniae* CGMCC 1.6366 (GenBank accession no: KJ128966) is 24862 bp and contains 20 ORFs (Fig. 1). In addition to the seven highly conserved genes (*galF*, *acidPPc*, *wzi*, *wza*, *wzb*, *wzc*, and *gnd*) of *K. pneumoniae*, six genes (*wcaF*, *gmd*, *wcaG*, *wcaH*, *wcaI*, and *wcaJ*) were found to have high homology with alleles of *K. pneumoniae* NTUH K-2044. The remaining seven genes (*orf7*, *orf8*, *orf9*, *orf10*, *orf11*, *orf12*, and *orf19*) did not exhibit high homology with any sequence in GenBank. The functions of these genes were predicted from the protein sequence alignment in GenBank as follows: *orf7*, polysaccharide biosynthesis protein; *orf8*, a putative pyruvyltransferase; *orf9*, a putative O-antigen polymerase; *orf10*, *orf11*, *orf12*, three glycosyl transferase-GTB-type superfamily proteins; and *orf19*, a putative acetylase.

The capsular serological type of *K. pneumoniae* CGMCC 1.6366 was determined using *wzi* gene sequencing. The alignment of the *wzi* gene of *K. pneumoniae* CGMCC 1.6366 with 135 alleles of *Klebsiella* spp. presented by Brisse et al. (2013) demonstrated that *K. pneumoniae* CGMCC 1.6366 has a unique *wzi* sequence, which clusters closely with alleles of *K. oxytoca* strain wzi26-K27 and *K. terrigena* strain wzi68. wzi26-K27 strain was of serological type K27, while no serological type was assigned to strain wzi68.

Phenotype of *K. pneumoniae* Δwza

Klebsiella pneumoniae Δwza and *K. pneumoniae* ΔwzaΔbudA were constructed as described in the section of Materials and methods. A greater volume of water is retained when the capsule is attached to the cell surface, affecting cell density. Cell suspensions of *K. pneumoniae* Δwza and *K. pneumoniae* CGMCC 1.6366 post-centrifugation are presented in Fig. 2.

Figure 2 clearly illustrates that the majority of cells remained in suspension for *K. pneumoniae* CGMCC 1.6366. In contrast, the majority of cells for *K. pneumoniae* Δwza had settled to the bottom of the centrifuge tube. These results indicate that capsule formation was significantly diminished in *K. pneumoniae* Δwza.

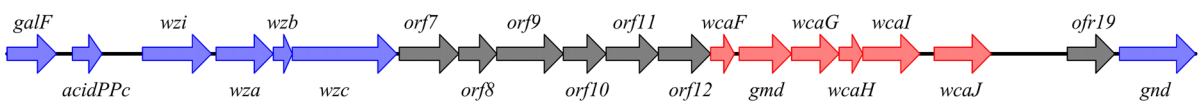

Fig. 1 The *cps* gene cluster of *K. pneumoniae* CGMCC 1.6366. Genes highly conserved (blue), genes have alleles (red), remaining genes (black)

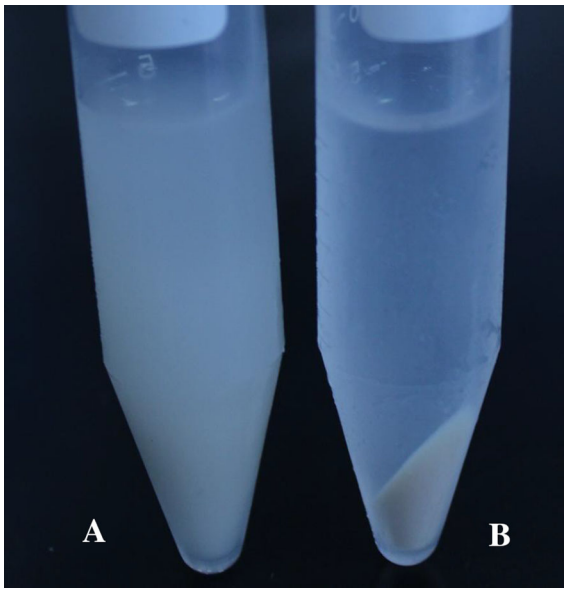

Fig. 2 Cell suspensions of *K. pneumoniae* CGMCC 1.6366 (*A*) and *K. pneumoniae* Δwza (*B*) post-centrifugation

Capsule effect on the efficiency of transformation by electroporation

Klebsiella pneumoniae Δwza, *K. pneumoniae* CGMCC 1.6366, and EDTA-treated *K. pneumoniae* CGMCC 1.6366 electrocompetent cells were transformed with pDK6. The transformation efficiency was determined, and the results shown in Fig. 3.

Transformation efficiency of pDK6 into *K. pneumoniae* CGMCC 1.6366 was 5.5×10^4 CFU μg^{-1} DNA. The addition of EDTA to electrocompetent cells increased the transformation efficiency to 2.6×10^5 CFU μg^{-1} DNA, while the transformation efficiency of the *wza* mutant was 6.4×10^5 CFU μg^{-1} DNA. Deletion of the *wza* gene resulted in diminished/annihilated capsule formation, which removed the barrier to DNA penetration, and consequently improved transformation efficiency.

Capsule effect on 1,3-propanediol production

Klebsiella pneumoniae CGMCC 1.6366 and *K. pneumoniae* Δwza were cultured under micro-aerobic conditions, with glycerol as a carbon source, for 1,3-propanediol production, in which the formation of 1,3-propanediol and cell growth were measured at regular intervals for 34 h (Fig. 4).

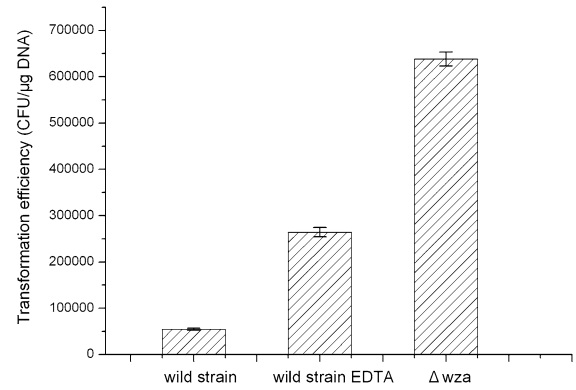

Fig. 3 Transformation efficiency of *K. pneumoniae* Δwza, *K. pneumoniae* CGMCC 1.6366 (wild strain), and EDTA-treated *K. pneumoniae* CGMCC 1.6366. The transformation experiments were done in triple and the error bars represent the standard deviation

Klebsiella pneumoniae CGMCC 1.6366 produced a maximum of 52.2 g 1,3-propanediol L^{-1} at 34 h, while *K. pneumoniae* Δwza produced only 47.9 g L^{-1}. Similar growth curves were observed between the two strains with *K. pneumoniae* CGMCC 1.6366 achieved its greatest cell dry weight (CDW) 3.69 g L^{-1} at 24 h, and *K. pneumoniae* Δwza CDW 3.6 g L^{-1} at 21 h.

Capsule effect on 2,3-butanediol production

Klebsiella pneumoniae CGMCC 1.6366 and *K. pneumoniae* Δwza were cultured under micro-aerobic conditions, with glucose as a carbon source, for 2,3-butanediol production. The results are presented in Fig. 5.

Klebsiella pneumoniae CGMCC 1.6366 produced a maximum of 51.2 g 2,3-butanediol L^{-1} at 34 h; *K. pneumoniae* Δwza produced 30.7 g L^{-1}. Throughout the duration of this experiment, cell growth for *K. pneumoniae* Δwza was less than the wild strain. The greatest cell dry weights were 3.24 and 4.65 g L^{-1} for *K. pneumoniae* Δwza and *K. pneumoniae* CGMCC 1.6366, respectively.

Capsule effect on 2-ketogluconic acid production

Klebsiella pneumoniae ΔbudA can't synthesize 2,3-butanediol, but 2-ketogluconic acid accumulates to a high level. Therefore, a *budA* and *wza* double gene deletion strain *K. pneumoniae* ΔbudAΔwza was constructed and used to evaluate 2-ketogluconic acid

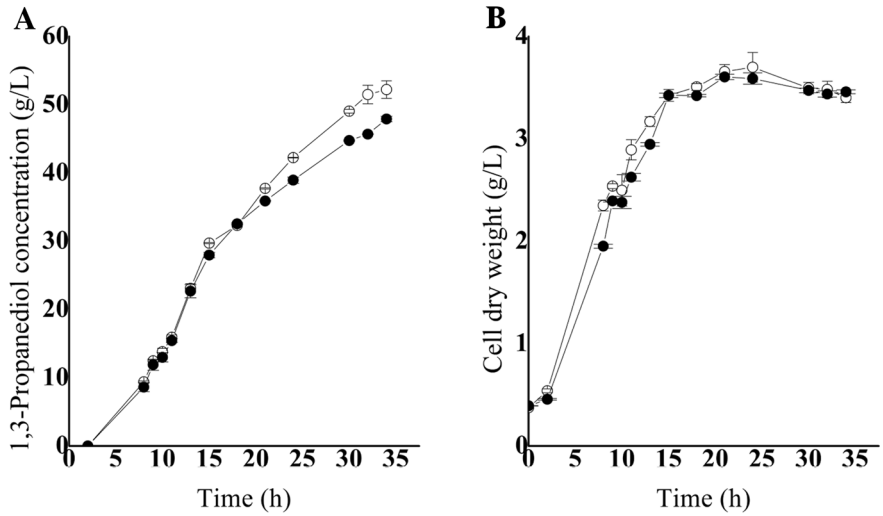

Fig. 4 1,3-Propanediol production by *K. pneumoniae* CGMCC 1.6366 and *K. pneumoniae* Δwza. **a**: 1,3-propanediol formation; **b**: Cell growth. *K. pneumoniae* CGMCC 1.6366 (open circle); *K. pneumoniae* Δwza (filled circle). The fermentations were done in triple and the error bars represent the standard deviation

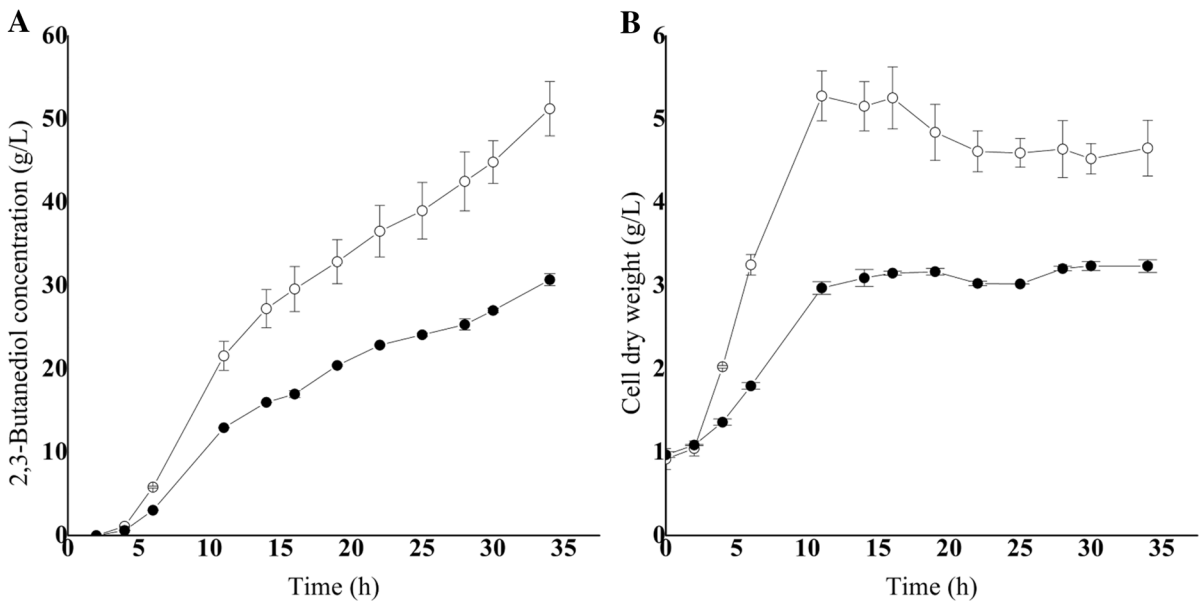

Fig. 5 2,3-Butanediol production by *K. pneumoniae* CGMCC 1.6366 and *K. pneumoniae* Δwza. A: 2,3-butanediol formation; B: Cell growth. *K. pneumoniae* CGMCC 1.6366 (open circle); *K. pneumoniae* Δwza (filled circle). The fermentations were done in triple and the error bars represent the standard deviation

productivity. The two strains were cultured under aerobic conditions, with glucose as a carbon source, for 2-ketogluconic acid production. The fermentations proceeded through a two-stage strategy and the results are shown in Fig. 6.

The total amounts of 2-ketogluconic acid produced by *K. pneumoniae* ΔbudA and *K. pneumoniae* ΔbudAΔwza at 30 h culture were 175.9 and 158.5 g L^{-1}, respectively. Cell growth for *K. pneumoniae* ΔbudAΔwza was slower compared with *K. pneumoniae* ΔbudA.

Fermentation broth viscosities

The viscosities of 1,3-propanediol, 2,3-butanediol, and 2-ketogluconic acid fermentation broths were measured on continuous fermentation (Table 3).

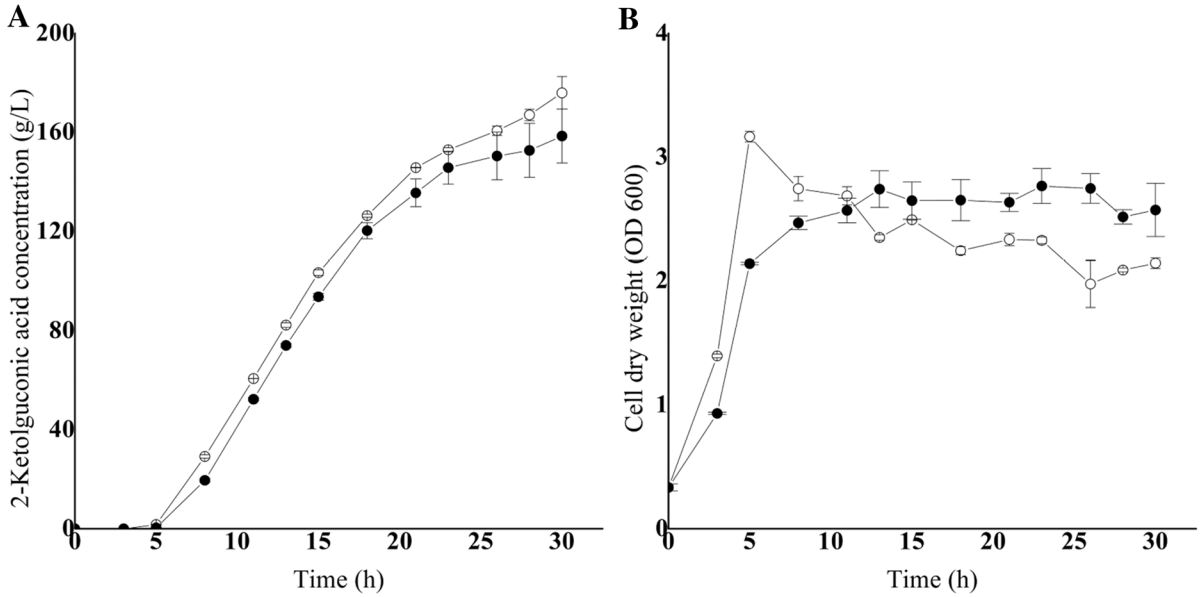

Fig. 6 2-Ketogluconic acid production by *K. pneumoniae* ΔbudA and *K. pneumoniae* ΔbudAΔwza. **a** 2-Ketogluconic acid formation; **b** Cell growth. *K. pneumoniae* ΔbudA (open circle); *K. pneumoniae* ΔbudAΔwza (filled circle). The fermentations were done in triple and the error bars represent the standard deviation

Table 3 Viscosities of 1,3-propanediol, 2,3-butanediol, and 2-ketogluconic acid fermentation broths

	K. pneumoniae CGMCC 1.6366	*K. pneumoniae* Δwza
Viscosities of 1,3-propanediol broths (cP)		
Broths	1.93	1.26
Supernatants	1.61	1.13
Viscosities of 2,3-butanediol broths (cP)		
Broths	2.12	1.22
Supernatants	1.64	1.05
	K. pneumoniae ΔbudA	*K. pneumoniae* ΔbudAΔwza
Viscosities of 2-ketogluconic broths (cP)		
Broths	1.85	1.39
Supernatants	1.79	1.24

The viscosities of fermentation broths are dependent on solute concentration; the higher the concentration of capsular polysaccharide in the broth, the greater the viscosity. As shown in Table 3, the viscosities of the fermentation broths and supernatants of *K. pneumoniae* CGMCC 1.6366 or *K. pneumoniae* ΔbudA were consistently high. These results suggested that in addition to the cell surface, some of the capsular polysaccharide produced was dissolved in the fermentation broth. A reduction in capsular polysaccharide formation for the *wza* mutants (*K. pneumoniae* Δwza and *K. pneumoniae* ΔbudAΔwza) lowered the viscosities of fermentation broths; the viscosities were 150% that of water [water viscosity is 0.80 cP at 30 °C (Dean 1999)].

Discussion

Klebsiella pneumoniae is an important industrial bacterium, used in the production of many chemicals. Unfortunately, public perception associates *K.*

pneumoniae with pneumonia, and its use, therefore, prompts safety concerns from the public. The pathogenic mechanism of this infectious disease is yet to be deciphered (Struve et al. 2005). The capsule has been identified as a virulence factor of *K. pneumoniae*, and capsular serotypes K1 and K2 are considered the predominant virulent strains (Chuang et al. 2006). Meanwhile, the capsule is not equivalent to pathogenicity. *K. pneumoniae* 342 is a nitrogen-fixing endophyte and has capsule, and mouse models have proved that the pathogenicity was attenuated in the strain (Fouts et al. 2008). Seven genes and a section of non-coding sequence in the *cps* gene cluster of *K. pneumoniae* CGMCC 1.6366 don't share high sequence homology with nucleotide sequences of any allele in GenBank. Collectively, these sequences comprise 37.5% of the total length of the *cps* gene cluster. Serological typing indicated that *K. pneumoniae* CGMCC 1.6366 closely aligned with wzi26-K27 and wzi68 strains; non-*K. pneumoniae* species of genus *Klebsiella*. These results indicated critical divergences between *K. pneumoniae* CGMCC 1.6366 and pathogenic strains.

A highly conserved block of genes, *wzi-wza-wzb-wzc*, have been identified in the *cps* gene cluster of *E. coli* and *Klebsiella* spp. *wza* encodes an outer membrane protein, *wzb* encodes a cytoplasmic phosphatase and *wzc* encodes an ATP-binding protein. These proteins represent a common translocation-surface assembly pathway for cell surface polysaccharides (Rahn et al. 1999). Since *wza* is a highly conserved gene in *cps* gene clusters, it was targeted for deletion, and a non-capsulated mutant *pneumoniae* Δwza was generated. The *rmpA2* gene encodes an activator for capsular polysaccharide synthesis. This gene was deleted to produce a non-capsulated variant of *K. pneumoniae* CG43S3, which sedimented much faster than its parental strain (Lai et al. 2003). This result is in agreement with the current investigation on *K. pneumoniae* Δwza. *K. pneumoniae* J2B strain has reduced lipopolysaccharide formation (Arasu et al. 2011), and has similar sedimentation properties to *K. pneumoniae* Δwza constructed in this study.

A *wza* deletion mutant of *K. pneumoniae* NTUH K2044 underwent mucoviscosity loss and a significant decrease in virulence (Ho et al. 2011), which was also observed in *K. pneumoniae* Δwza. The *uge* gene encodes UDP galacturonate 4-epimerase, and the mutations in the *uge* gene affect polysaccharide and lipopolysaccharide processing, resulting in capsule loss. *uge* mutants have been shown to be completely avirulent in two different animal models (Regue et al. 2004). Therefore, *K. pneumoniae* Δwza appears to be a safer strain for use in industrial applications.

The transfer of exogenous DNA into cells is essential for genetic and molecular biology studies, and electroporation is a common method practiced in many laboratories worldwide. The electroporation method was developed using *E. coli* K12. However, transformation rate using this method for wild strains is quite low. Transformation of *K. pneumoniae* 21 with pBR328 using electroporation resulted in 10^4 transformants μg^{-1} DNA (Fournet-Fayard et al. 1995). The transformation rate for *K. pneumoniae* CGMCC 1.6366 with pDK6 obtained in the current study was of the same order of magnitude. The exact mechanism for DNA uptaken by electroporation is not entirely clear, but plasmid DNA must breach the bacterial membrane. The capsule acts as a barrier to plasmid DNA penetration by electroporation. The addition of chemicals such as EDTA, which reduced capsule formation, enhanced transformation efficiency to 10^8 transformants μg^{-1} DNA in *K. pneumoniae* 21 (Fournet-Fayard et al. 1995). For *K. pneumoniae* CGMCC 1.6366, the transformation efficiency increased to 2.64×10^5 CFU μg^{-1} DNA with the inclusion of EDTA. The *wza* mutant had the highest transformation efficiency.

1,3-Propanediol, 2,3-butanediol, and 2-ketogluconic acid are all valuable chemicals produced by *K. pneumoniae*. An approximate 10% decrease in 1,3-propanediol and 2-ketogluconic acid productivity was observed for the non-capsulated mutant when compared with *wza* wild strains. The rates of cell growth during 1,3-propanediol and 2-ketogluconic acid production were similar for mutant and *wza* wild strains. This is in agreement with studies conducted on *cps* mutants of *K. pneumoniae* NTUH K2044, whereby the authors proposed that the capsule was important for pathogenicity but not for growth (Ho et al. 2011).

Unfortunately, 2,3-butanediol productivity of *K. pneumoniae* Δwza was markedly lower than that of the wild strain. There is no overlap between the 2,3-butanediol synthesis pathway and the *cps* cluster, hence the mechanism for the decrease in 2,3-butanediol productivity observed for the *wza* mutant is difficult to deduce. However, it was shown in other research that the yield of 2,3-butanediol, which was

52.4 g L^{-1}, with the non-capsulated strain was close to the yield of this study. (Rathnasingh et al. 2012).

In the downstream processing of 1,3-propanediol and 2,3-butanediol fermentation broths, cells, and other solid impurities must be removed from the broth to obtain a clear liquid for further purification processes (Hao et al. 2006; Xiu and Zeng 2008), and same applies to 2-ketogluconic acid broth. Filtration techniques, especially membrane filtration, are commonly used for clarifying fermentation broth. The viscosity of the broth is a key parameter in the filtration process. Viscosity has a positive relationship with energy consumption, and a negative relationship with membrane flux (Van and Zydney 2001). The capsule polysaccharide produced in the broth greatly contributes to viscosity and impedes filtration. Fermentation broths produced by *K. pneumoniae* Δwza and *K. pneumoniae* ΔbudAΔwza had low viscosities; a desirable quality for filtration.

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