Production of 1,3-propanediol from glycerol using a new isolate Klebsiella sp. AA405 carrying low levels of virulence factors

Ying Li, Xi-Zhen Ge and Ping-Fang Tian

ABSTRACT
Klebsiella pneumoniae is a promising industrial species for the production of chemicals, yet its opportunistic pathogenicity restrains real-world applications. In this work, we identified a novel isolate designated Klebsiella sp. AA405 which carried low levels of virulence factors. No capsule, fimbriae and flagella were observed around colonies. Genome alignment between Klebsiella sp. AA405 and other 186 Klebsiella species revealed 3421 homologous genes. Notably, the endotoxin of Klebsiella sp. AA405 was only half of E. coli BL21. This strain matched well with common cloning and expression vectors and was sensitive to antibiotics. More importantly, this strain metabolized diverse carbon sources. Under micro-aerobic conditions, it produced 51.66 g/L 1,3-propanediol, 3.82 g/L 3-hydroxypropionic acid and 6.86 g/L 2,3-butanediol in a 5 L bioreactor using glycerol as the sole carbon source. Overall these results indicate that Klebsiella sp. AA405 is a promising strain for the production of bulk chemicals.

ARTICLE HISTORY
Received 15 September 2016
Accepted 23 May 2017

KEYWORDS
Klebsiella pneumoniae; virulence factor; endotoxin; 1,3-propanediol; 2,3-butanediol

Introduction
Biorefinery has garnered much attention due to depletion of oil reserves and deterioration of environment. To date, series of chemicals, including citric acid, succinic acid, lactic acid and ethanol, have been produced through large-scale microbial fermentation [1–3]. Among recently reported industrial species, Klebsiella pneumoniae is of great attractiveness due to the innate ability to produce chemicals and efficient utilization of glucose and glycerol. When glucose is supplied as carbon source, K. pneumoniae can synthesize a panel of value-added chemicals, including 2,3-butanediol (2,3-BDO), acetoin and pyrroloquinoline quinon (PQQ) [4,5]. The titers and productivities can be enhanced by reprogramming metabolic flux because biosynthesis pathways have been well documented [6,7]. On the other hand, when glycerol is provided as carbon source, K. pneumoniae turns to generate a range of bulk chemicals, including 1,3-propanediol (1,3-PDO), 3-hydroxypropionic acid (3-HP) and 1-butanol [8–10]. In K. pneumoniae, glycerol metabolism is mediated by the dha regulon [11]. Compared with glucose-utilizing pathways, glycerol-based biosynthesis is more appealing because of inexpensive glycerol, which is the major byproduct of biodiesel industry [12]. For biological production of 1,3-PDO, 3-HP and 2,3-BDO, K. pneumoniae outperforms E. coli owing to its striking biochemical attributes, including fast growth, extraordinary capability to metabolize glycerol, and particularly the innate ability to synthesize vitamin B12, which is the cofactor of glycerol dehydratase [9,11]. Despite the aforementioned advantages of K. pneumoniae, its pathogenicity to human restricts real-world applications [13]. To our knowledge, at least four aspects contribute to pathogenicity. The first is fimbriae and flagella, as both participate in bacterial movement and adhesion to human cells [14–16]. The spatial proximity between K. pneumoniae and human cells is necessary for subsequent infection and immunity. The second aspect for pathogenicity is the capsule by which bacteria survive in harsh conditions [17]. The third pathogenic factor is the endotoxin or lipopolysaccharides (LPS) in cell wall. When bacteria are decomposed, LPS is movable and can elicit immune reactions of human cells [18,19]. The fourth is bacterial insensitivity to antibiotics. Namely, bacteria evolve insensitivity to cope with the immune reactions of host cells. The underlying mechanisms are multifaceted, including the recruitment of resistance genes, overproduction of capsule and deficiency of porin proteins in cytomembrane [20]. Presumably, it is hard to circumvent all virulence factors.
In addition to pathogenicity, other aspects also affect industrial applications of *K. pneumoniae*. The first is the compatibility between *K. pneumoniae* and expression vectors. The second is the cost of fermentation medium, which is particularly important for large-scale fermentation. Ideally, strains can efficiently metabolize low-cost carbon sources such as xylose, cellulose or even food waste. Besides, strains are expected to grow aggressively on medium because cell growth usually contributes to the production of desired chemicals. Apart from compatibility and medium cost, the inherent capacity of strains to produce wanted chemicals is highly desirable. In this case, gene manipulation of codon optimization is not required. For example, *lactobacillus* species is regarded as an ideal host strain for the production of lactic acid because it can naturally synthesize a large quantity of lactic acid. Similarly, *K. pneumoniae* is a promising host for production of 1,3-PDO and 2,3-BDO because it harbours native synthesis pathways. Presumably, if gene transformation is tractable, native host strains might outperform model micro-organisms (e.g. *E. coli* and *Saccharomyces cerevisiae*) in overproduction of desired metabolites.

Given that *K. pneumoniae* is a promising industrial strain despite pathogenicity, we therefore sought to screen a novel *Klebsiella* species with low pathogenicity. To this end, large amounts of soil samples were collected from relatively uncontaminated sites. Glycerol was added into medium as the sole carbon source to isolate *Klebsiella* species. After multiple rounds of purification and identification by PCR, a novel *Klebsiella* species was acquired and the genome was sequenced. Morphological observation with scanning electron microscope (SEM), investigation of the compatibility between vector and recipient host, and assay of endotoxin content were determined whether the screened species harboured low levels of virulence factors compared with previously reported *Klebsiella* species. Finally, fermentation using different substrates was to examine the production of bulk chemicals.

### Materials and methods

**Isolation and identification of klebsiella species**

All samples were collected from relatively uncontaminated river and soil to acquire a novel *Klebsiella* species containing low levels of virulence factors. Each solid sample (10 mg) was diluted with 100 μL sterile ddH2O in a 1.5 mL Eppendorf tube. After oscillation, Eppendorf tubes were incubated for 10 min, and the supernatant was added into 4 mL glycerol medium at 30 ºC and shaken at 200 rpm. For liquid sample, 5 μL supernatant was added into 4 mL medium. The glycerol-containing medium included 3.4 g/L K2HPO4•3H2O, 1.3 g/L KH2PO4, 4 g/L (NH4)2SO4, 0.5 g/L MgSO4•7H2O, 0.1 g/L CaCO3 and 20 g/L glycerol. Since glycerol was the sole carbon source in medium, only the microbes able to consume glycerol would survive. After successive cultivation of 10 generations, the strains were grown in LB solid medium for isolation. The colonies devoid of visible capsule were retained for further identification through 16S rRNA sequencing. 16S rRNA was amplified with primers 27F and 1492R, and DNA fragment was sequenced in Beijing BioMed Co., Ltd. Multiple sequence alignments were performed using Clustal W program [21]. Phylogenetic tree was constructed using neighbour-joining and maximum-likelihood method in MEGA 5 [22].

SEM of HITACHI S4700 was recruited for morphological observation. The samples were treated according to the reported method [23]. Briefly, samples were fixed by 2.5% glutaraldehyde for 3 h, and washed with sodium cacodylate buffer. After dehydration, samples were subjected to critical point drying and mounted on stubs. After gold coating, the samples were subjected to SEM analysis. To explore genetic background, *de novo* genome sequencing was carried out in Beijing YuanQuanYiKe Co., Ltd. The sequencing results were deposited in GenBank.

### Testing of sensitivity to antibiotics and compatibility with vectors

Towards industrial applications, we investigated the sensitivity of strain to antibiotics and compatibility with common vectors. Prior to *Klebsiella* sp. inoculation, kanamycin (25 μg/mL), ampicillin (50 μg/mL), chloramphenicol (34 μg/mL), tetracycline (30 μg/mL) and carbenicillin (50 μg/mL) were individually mixed with LB solid medium. The plates were incubated at 37 ºC for 24 h. The sensitivity to antibiotics was determined by counting colony number. The LB solid plate without antibiotic was used as the control. Vectors including pUC19, pET-28a, pBR322, pLysS and pCP20 that harbour different replicons and antibiotic markers were electro-transformed into *Klebsiella* species. The colonies were analysed by PCR and the vectors were extracted. The strains harbouring vectors were considered compatible with the corresponding vectors.

The endotoxin (lipopolysaccharide) content of newly isolated *Klebsiella* species was measured and compared with that of *E. coli* BL21 (DE3). Same amount of *Klebsiella* species and *E. coli* cells were taken out by measuring OD600 after cultivation in LB medium for 12 h. The *Klebsiella* species and *E. coli* cells were harvested by centrifugation and washed twice with sterile 50 mmol/L PBS...
buffer at pH 7.0. Next, the cells were sonicated (80 W, working 3 s, pause 2 s, 50 times) and the resultant solution was centrifuged at 17,000 rpm for 15 min. The endotoxin was measured in Beijing Center for Physical and Chemical Analysis using Tachypleus Amebocyte Lysate reaction method.

**Carbon distribution analysis**

In addition to glucose and glycerol, other inexpensive carbon sources including sucrose, lactose, starch and cracked food waste were individually recruited as carbon sources to examine carbon utilization by newly isolated strain. The strain was grown in LB medium at 37 °C for 150 rpm and the air was supplied at 0.4 vvm. The temperature was 37 °C and pH value was maintained at 7.0 by adding 5 mol/L NaOH. Initial glycerol concentration was 40 g/L and residual glycerol was maintained at 10 g/L. When glucose or sucrose was applied as carbon source, the initial concentration of carbon source was 40 g/L and further supplementation was not necessary, because glucose and sucrose were consumed less than glycerol. Dissolved oxygen was monitored automatically. Samples were taken out every 6 h to examine cell concentrations and metabolite titers.

**Analytical methods**

Cell concentrations were measured by using microplate reader at 600 nm with 200 μL fermentation broth added in a cuvette. The metabolites 3-HP, lactic acid and acetic acid were determined by high performance liquid chromatography (HPLC) system (Shimazu, Kyoto, Japan) equipped with a C18 column and a SPD-20A UV detector at 210 nm. Column temperature was 25 °C and mobile phase was 0.05% phosphoric acid at a flow rate of 0.8 mL/min. 1,3-PDO, glycerol, glucose, sucrose and 2,3-butanediol (2,3-BDO) were quantitatively analysed by HPLC (Shimazu, Japan) equipped with a column of Aminex HPX-87H Ion Exclusion particles (300 mm × 7.8 mm, Bio-Rad, Hercules, CA, USA) using a differential refractive index detector. The column was maintained at 65 °C and mobile phase was 5 mmol/L sulphuric acid (in Milli-Q water) at 0.6 mL/min. Residual glycerol concentration was measured every 3 h by a titration method with NaIO4 (for control of glycerol). All samples were filtered through 0.22-μm membrane filter.

**Results and discussion**

**Isolation and characterization of isolates**

To obtain a Klebsiella species with low pathogenicity, the colonies devoid of visible capsule were screened, given that most virulence factors reside in capsule area. Briefly, the colonies manifesting clear boundary were retained and subjected to further identification. After successive purification and characterization, a strain designated Klebsiella sp. AA405 was acquired. Alignment of 16S rRNA sequences revealed high homology between Klebsiella sp. AA405 and K. pneumoniae, and a phylogenetic tree was constructed (Figure 1, Supplementary Table 1). Similar to E. coli BL21, Klebsiella sp. AA405 demonstrated faint colony with clear boundary (Figure 2(A)). For further morphological observation, Klebsiella sp. AA405 was immobilized with glutaraldehyde and analysed with SEM. As shown in Figure 2(B, C), Klebsiella sp. AA405 was a rod-shaped bacterium, and neither fimbriae nor flagella was observed, indicating low pathogenicity relative to species harbouring fimbriae and flagella. Collectively, Klebsiella sp. AA405 is morphologically different from other Klebsiella species, especially the cell surface where virulence factors reside.

Capsule contributes largely to pathogenicity [24]. There are at least three modes of action. The first is bacterial adhesion to human cells, which is mediated by the polysaccharides surrounding the cell membrane [25]. The second is the physical barrier of the capsule, by which bacteria cope with harsh conditions such as antibiotics and phagocyte cells [26]. The third is capsule which serves as an antigen and trigger immunoreaction [27]. Apart from the capsule, both fimbriae and flagella also act as virulence factors and are responsible for the adhesion and motion [14,28]. In fact, capsule, fimbriae and flagella are ubiquitous in pathogenic...
micro-organisms and make up the majority of virulence factors [28–31].

To better understand *Klebsiella* sp. AA405, its genome was sequenced. Results showed that a total of 97 scaffolds contained 5473 542 nucleotides, and DNA GC content was 57.27%. Genome annotation revealed 5001 protein-coding genes and 113 non-coding genes, including 102 tRNA coding genes, five 23S rRNA coding genes, two 16S rRNA coding genes and four 5S rRNA coding genes. All data were deposited in NCBI (BioSample: SAMN04094116; Bioproject: PRJNA295946; WGS: LKKX00000000). Genome alignment between *Klebsiella* sp. AA405 and 186 *Klebsiella* genomes was performed via blat program in Python. A total of 3421 homologous genes were found in 187 species. Namely, the 3421 genes were relatively conserved in *Klebsiella* genera. The 3421 homologous genes accounted for 68.4% of the entire genes in *Klebsiella* sp. AA405 (Supplementary Table 1). Considering this percentage (68.4%) is higher than that of other species, we speculate that *Klebsiella* sp. AA405 genome could be minimized.

**Plasmid compatibility, antibiotic sensitivity and endotoxin content**

For genetically engineered strains, vectors are critical for overexpression of key enzymes and reallocation of metabolic flux. Thus, we investigated the compatibility between *Klebsiella* sp. AA405 and common vectors. We found that *Klebsiella* sp. AA405 matched well with vectors pET-28a, pBR322, pUC19 and pLysS, but not with pKD46 and pCP20 (Table 1). This is because the latter

![Figure 1](image1.png)

**Figure 1.** Neighbour-joining phylogenetic tree based on 16S rRNA sequences demonstrating the relationship of strain *Klebsiella* sp. AA405 with other species of *Klebsiella* genera and *E. coli*.

![Figure 2](image2.png)

**Figure 2.** Scanning electron microscope (SEM) analysis of *Klebsiella* sp. AA405. (A) Bacterial colonies after 12 h cultivation. (B and C) SEM analysis of *Klebsiella* sp. AA405.
Table 1. Antibiotic sensitivity and plasmid compatibility of Klebsiella sp. AA405.

| Antibiotics       | Plasmids      |
|-------------------|---------------|
| Kanamycin         | pET-28a (+)   |
| Chloramphenicol   | pLysS (+)     |
| Tetracycline      | pBR322 (+)    |
| Ampicillin        | pUC19 (+)     |
| Carbenicillin     | pCP20 (−)     |

Note: For antibiotics, +: sensitive; −: insensitive.
For plasmids, +: compatible; −: incompatible.

two vectors harbour the temperature-sensitive replicon of plasmid pSC101. The compatibility with common vectors indicates that Klebsiella sp. AA405 could be a genetically tractable host strain. In contrast, the incompatibility with vector pKD46 (carrying lambda Red recombinases) implies that developing Red recombination system in Klebsiella sp. AA405 is challenging.

Considering that antibiotic resistance genes usually reside in vectors, we investigated the sensitivity of Klebsiella sp. AA405 to several antibiotics. As described in Table 1, the growth of Klebsiella sp. AA405 was retarded by common antibiotics including kanamycin, chloramphenicol, tetracycline, ampicillin and carbenicillin. Despite sensitivity to antibiotics, Klebsiella sp. AA405 usually grows faster than E. coli. As shown in Figure 3, the cell dry weight of Klebsiella sp. AA405 in LB medium reached 1.3 g/L at 12 h, which was 30% more when compared to E. coli BL21. This finding implies a shorter fermentation time of Klebsiella sp. AA405 relative to E. coli.

In addition to aforementioned compatibility with vectors and sensitivity to antibiotics, Klebsiella sp. AA405 contained less endotoxin (4.75E+08 Eu/g cell dry weight) than E. coli BL21 (Figure 3). Since E. coli BL21 has been allowed for large-scale fermentation, Klebsiella sp. AA405 should also be permitted for industrial applications upon further genetic modification. Although Klebsiella species harbour endotoxin which may serve as antigen to elicit immunoreaction of host cells [18,19], the expanding synthetic biology methods raise the possibility of attenuating virulence factors.

Fermentation is sometimes hampered by the microbial resistance to antibiotics. The reasons may involve at least three aspects. The first is the antibiotic resistance genes in chromosome or plasmids, which cause microbial insensitivity to antibiotics. For example, β-lactamase confers resistance to penicillin [32]. The second is thick capsule or lipopolysaccharide in bacteria, which intercepts antibiotics on their way to microbial cells [33]. The third is the lack of porin in bacterial plasma membrane. In this case, antibiotics cannot enter bacteria. Relying on these mechanisms, bacteria are resistant to antibiotics and thus survive [34]. For Klebsiella sp. AA405, no capsule was observed around cell surface, and only one β-lactamase gene was found in Klebsiella sp. AA405 genome. The copy number was much fewer than other K. pneumoniae species (Supplementary Table 2). The above findings indicate low pathogenicity of Klebsiella sp. AA405.

**Utilization of carbon sources**

In this study, glycerol, glucose and sucrose were used as the carbon sources of Klebsiella sp. AA405 and the major metabolites were analysed by HPLC. As shown in Figure 4, Klebsiella sp. AA405 produced 9.69 g/L 1,3-PDO in shake flask when glycerol was carbon source. Interestingly, 1,3-PDO was not detected when glucose and sucrose were provided. This finding may indicate that glycerol instead of glucose or sucrose is appropriate for the biosynthesis of 1,3-PDO. Consistent with this finding, 1,3-PDO was found to be the major metabolite in glycerol reduction pathway [11]. Considering that K. pneumoniae synthesizes 2,3-BDO in glycerol oxidation pathway, we anticipated that Klebsiella sp. AA405 might also
generate 2,3-BDO. As expected, when glucose, glycerol and sucrose were supplied, *Klebsiella* sp. AA405 produced 5.87, 1.75 and 6.04 g/L 2,3-BDO, respectively. Interestingly, when lactose, starch and food waste were individually used as carbon sources, 1,3-PDO and 2,3-BDO were far less than produced when glycerol, glucose or sucrose was utilized. This might be ascribed to the long pathways from lactose, starch and food waste to desired chemicals 1,3-PDO and 2,3-BDO. Overall these results indicated that *Klebsiella* sp. AA405 harboured a *dha* regulon similar to that in *K. pneumoniae*.

To further validate the presence of *dha* regulon in *Klebsiella* sp. AA405, fed-batch cultivation was performed in a 5 L bioreactor (Baoxing, China) containing 3 L fermentation medium. When glucose was used as carbon source, this strain produced 14.37 g/L 2,3-BDO and 8.64 g/L succinic acid at 48 h (Figure 5(B)). When sucrose was provided, this strain synthesized 12.29 g/L 2,3-BDO (Figure 5(C)) and 13.44 g/L lactic acid at 48 h. The lactic acid level was much higher than that of using glucose as carbon source. Remarkably, when glycerol was used as carbon source, this strain produced up to 51.66 g/L 1,3-PDO and 3.82 g/L 3-HP at 48 h (Figure 5(A)), with 49% of glycerol conversion rate. Fortunately, the byproducts lactic acid, acetic acid and formic acid only accounted for a small portion of the overall metabolic flux. It is clear that glycerol is an appropriate carbon source for the production of 1,3-PDO. Overall, these results indicate that *Klebsiella* sp. AA405 can utilize diverse carbon sources and guarantees industrial production of chemicals aforementioned.

As mentioned, *K. pneumoniae* can naturally synthesize 1,3-PDO when glycerol is consumed as carbon source. The *dha* regulon governs parallel glycerol oxidation and reduction pathways, where glycerol is consumed and 1,3-PDO is generated [11]. It has been reported that wild-type *K. pneumoniae* produced 50–60 g/L 1,3-PDO in bioreactor [8,35,36], and the yield of 1,3-PDO on glycerol reached 50%. In the present study, *Klebsiella* sp. AA405

![Figure 5](image-url)

**Figure 5.** Fed-batch cultivation of *Klebsiella* sp. AA405 with different carbon sources in a 5 L bioreactor. (A) glycerol; (B) glucose; (C) sucrose.
generated 51.66 g/L 1,3-PDO in 48 h, and the conversion ratio from glycerol to 1,3-PDO was 49%. Intriguingly, Klebsiella sp. AA405 generated less 2,3-BDO compared with other Klebsiella species when glucose was carbon source [37,38].

Although glucose is a commonly used carbon source in fermentation, over-consumption of glucose may cause food crisis. Therefore, alternative carbon sources are required. In this study, Klebsiella sp. AA405 was shown to consume glycerol, glucose, sucrose, lactose and food waste. Since K. pneumoniae can also metabolize glycerol, glucose and sucrose [4,37,39], we reasoned that Klebsiella sp. AA405 might have similar genetic background with K. pneumoniae. Compared with glycerol, other carbon sources led to excessive byproducts including lactic acid and acetic acid. Hence, glycerol seems to be suitable for Klebsiella sp. AA405. Since glycerol is the major byproduct in biodiesel industry, Klebsiella sp. AA405 may be a competitive strain for glycerol-based biosynthesis of 1,3-PDO, 2,3-BDO, 3-HP, polyhydroxyalkanoates, or beyond.

Conclusion

We isolated a novel strain named Klebsiella sp. AA405. No capsule, fimbriae and flagella were observed around bacterial surface. The endotoxin level was only half of that in E. coli BL21. This strain matched well with the common cloning and expression vectors and was sensitive to a series of antibiotics. Importantly, this strain could metabolize different carbon sources. In a 5 L bioreactor, this strain produced 51.66 g/L 1,3-PDO, 3.82 g/L 3-HP and 6.86 g/L 2,3-BDO using glycerol as the sole carbon source. Overall, these results indicate that Klebsiella sp. AA405 is a promising strain for the production of bulk chemicals.

Disclosure statement

None.

Funding

This work was supported by grants from National High Technology Research and Development Program (863 Program) [grant number 2015AA021003]; National Basic Research Program of China (973 Program) [grant number 2012CB725200]; National Natural Science Foundation of China [grant number 21276014], [grant number 21476011]; Fundamental Research Funds for the Central Universities [grant number YS1407]; Innovation Capability Enhancement Program for universities Governed by Beijing Municipal Commission of Education [grant number PXM2015_014 209_000 010]; Beijing Natural Science Foundation [grant number 1164030].

References

[1] Nielsen J. Yeast cell factories on the horizon. Science. 2015;349:1050–1051.
[2] John RP, Nampoothiri KM, Pandey A. Fermentative production of lactic acid from biomass: an overview on process developments and future perspectives. Appl Microbiol Biotechnol. 2007;74:524–534.
[3] Ni Y, Sun Z. Recent progress on industrial fermentative production of acetone-butanol-ethanol by Clostridium acetobutylicum in China. Appl Microbiol Biotechnol. 2009;83:415–423.
[4] Jung MY, Mazumdar S, Shin SH, et al. Improvement of 2,3-butanediol yield in Klebsiella pneumoniae by deletion of the pyruvate formate-lyase gene. Appl Environ Microbiol. 2014;80:6195–6203.
[5] Meulenberg JJM, Sellink E, Riegman NH, et al. Nucleotide sequence and structure of the Klebsiella pneumoniae pqq operon. Mol Gen Genet. 1992;232:284–294.
[6] Weckslor SR, Stoll S, Tran H, et al. Pyrroloquinoline quinone biogenesis: demonstration that PqqE from Klebsiella pneumoniae is a radical S-adenosyl-L-methionine enzyme. Biochemistry. 2009;48:10151–10161.
[7] Kim B. Enhanced 2,3-butanediol production in recombinant Klebsiella pneumoniae via overexpression of synthesis-related Genes. J Microbiol Biotechnol. 2012;22:1258–1263.
[8] Chen X, Zhang DJ, Qi WT, et al. Microbial fed-batch production of 1,3-propanediol by Klebsiella pneumoniae under micro-aerobic conditions. Appl Microbiol Biotechnol. 2003;63:143–146.
[9] Huang Y, Li Z, Shimizu K, et al. Co-production of 3-hydroxypropionic acid and 1,3-propanediol by Klebsiella pneumoniae expressing aldH under microaerobic conditions. Bioresour Technol. 2013;128:505–512.
[10] Wang M, Fan L, Tan T. 1-Butanol production from glycerol by engineered Klebsiella pneumoniae. RSC Adv. 2014;4:57791–57798.
[11] Celinska E. Debottlenecking the 1,3-propanediol pathway by metabolic engineering. Biotechnol Adv. 2010;28:519–530.
[12] Fan X, Burton R, Zhou Y. Glycerol (byproduct of biodiesel production) as a source for fuels and chemicals – mini review. Open Fuel Energy Sci J. 2010;3:17–22.
[13] Kang CI, Kim SH, Park WB, et al. Bloodstream infections due to extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae: risk factors for mortality and treatment outcome, with special emphasis on antimicrobial therapy. Antimicrob Agents Chemother. 2004;48:4574–4581.
[14] Struve C, Bojer M, Krogfelt KA. Characterization of Klebsiella pneumoniae type 1 fimbriae by detection of phase variation during colonization and infection and impact on virulence. Infect Immun. 2008;76:4045–4065.
[15] O’Toole R, Milton DL, Horstedt P, et al. RpoN of the fish pathogen Vibrio (Listonella) anguillarum is essential for flagellum production and virulence by the waterborne but not intraperitoneal route of inoculation. Microbiology. 1997;143:3849–3859.
[16] Kleta S, Nordhoff M, Tedin K, et al. Role of F1C fimbriae, flagella, and secreted bacterial components in the inhibitory effect of probiotic Escherichia coli Nissle 1917 on atypical enteropathogenic E. coli infection. Infect Immun. 2014;82:1801–1812.
[17] Goller CC, Seed PC. Revisiting the *Escherichia coli* polysaccharide capsule as a virulence factor during urinary tract infection. Virulence. 2010;1:333–337.

[18] Cortes G, Borrell N, Astorza B, et al. Molecular analysis of the contribution of the capsular polysaccharide and the lipopolysaccharide O side chain to the virulence of *Klebsiella pneumoniae* in a murine model of pneumonia. Infect Immun. 2002;70:2583–2590.

[19] Izquierdo L, Coderch N, Pique N, et al. The *Klebsiella pneumoniae* wabG gene: role in biosynthesis of the core lipopolysaccharide and virulence. J Bacteriol. 2003;185:7213–7221.

[20] Campos MA, Vargas MA, Regueiro V, et al. Capsule polysaccharide mediates bacterial resistance to antimicrobial peptides. Infect Immun. 2004;72:7107–7114.

[21] Thompson JD, Higgins DG, Gibson TJ, et al. Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 1994;22:4673–4680.

[22] Tamura K, Peterson D, Peterson N, et al. MEGAS: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011;28:7231–7239.

[23] Sanchez-Andrea I, Knittel K, Amann R, et al. Quantification of Tinto River sediment microbial communities: importance of sulfate-reducing bacteria and their role in attenuating acid mine drainage. Appl Environ Microbiol. 2012;78:4638–4645.

[24] Chen L, Valentine JL, Huang C, et al. Outer membrane vesicles displaying engineered glycotopes elicit protective antibodies. Proc Natl Acad Sci USA. 2016;113:E3609–E3618.

[25] Kachlany SC, Levery SB, Kim JS, et al. Structure and carbohydrate analysis of the exopolysaccharide capsule of *Pseudomonas putida* G7. Environ Microbiol. 2001;3:774–784.

[26] Emdy L. Virulence factors of uropathogenic *Escherichia coli*. Int J Antimicrob Agents. 2003;22:29–33.

[27] Frosch M, Gorgen I, Boulnois GJ, et al. NZB mouse system for production of monoclonal antibodies to weak bacterial antigens: isolation of an IgG antibody to the polysaccharide capsules of *Escherichia coli* K1 and group B meningococci. Proc Natl Acad Sci USA. 1985;82:1194–1198.

[28] Giron JA, Torres AG, Freer E, et al. The flagella of enteropathogenic *Escherichia coli* mediate adherence to epithelial cells. Mol Microbiol. 2002;44:361–379.

[29] Connell I, Agace W, Klemm P, et al. Type 1 fimbrial expression enhances *Escherichia coli* virulence for the urinary tract. Proc Natl Acad Sci USA. 1996;93:9827–9832.

[30] Hsu CR, Lin TL, Pan YJ, et al. Isolation of a bacteriophage specific for a new capsular type of *Klebsiella pneumoniae* and characterization of its polysaccharide depolymerase. PLoS One 2013;8:e70092.

[31] Gunn JS, Bakaletz LO, Wozniak DJ. What’s on the outside matters: the role of the extracellular polymeric substance of gram-negative biofilms in evading host immunity and as a target for therapeutic intervention. J Biol Chem. 2016;291:12538–12546.

[32] Yong D, Toleman MA, Giske CG, et al. Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob Agents Chemother. 2009;53:5046–5054.

[33] Clements A, Gaboriaud F, Duval JF, et al. The major surface-associated saccharides of *Klebsiella pneumoniae* contribute to host cell association. PLoS One. 2008;3:e3817.

[34] Turner KL, Cahill BK, Dilello SK., et al. Porin loss impacts the host inflammatory response to outer membrane vesicles of *Klebsiella pneumoniae*. Antimicrob Agents Chemother. 2016;60:1360–1369.

[35] Zhang Y, Li Y, Du C, et al. Inactivation of aldehyde dehydrogenase: a key factor for engineering 1,3-propanediol production by *Klebsiella pneumoniae*. Metab Eng. 2006;8:578–586.

[36] Zhao YN, Chen G, Yao SJ. Microbial production of 1,3-propanediol from glycerol by encapsulated *Klebsiella pneumoniae* SDM. Appl Microbiol Biotechnol. 2009;82:49–57.

[37] Ji XJ, Huang H, Du J, et al. Development of an industrial medium for economical 2,3-butanediol production through co-fermentation of glucose and xylose by *Klebsiella oxytoca*. Bioresour Technol. 2009;100:5214–5218.

[38] Zhang H, Obias V, Gonyer K, et al. Production of polyhydroxyalkanoates in sucrose-utilizing recombinant *Escherichia coli* and *Klebsiella* strains. Appl Environ Microb. 1994;60:1198–1205.