# Electronic supplementary information

## Energy- and cost-effective non-sterilized fermentation of 2,3-butanediol by an engineered *Klebsiella pneumoniae* OU7 with anti-microbial contamination system

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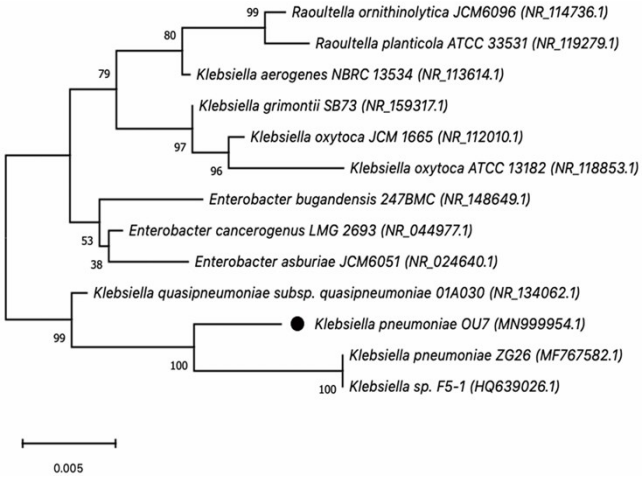



**Fig. S1.** Phylogenetic analysis of *K. pneumoniae* OU7 and its representative homologous species based on 16S rRNA sequences. ● indicated *K. pneumoniae* OU7 of this study.

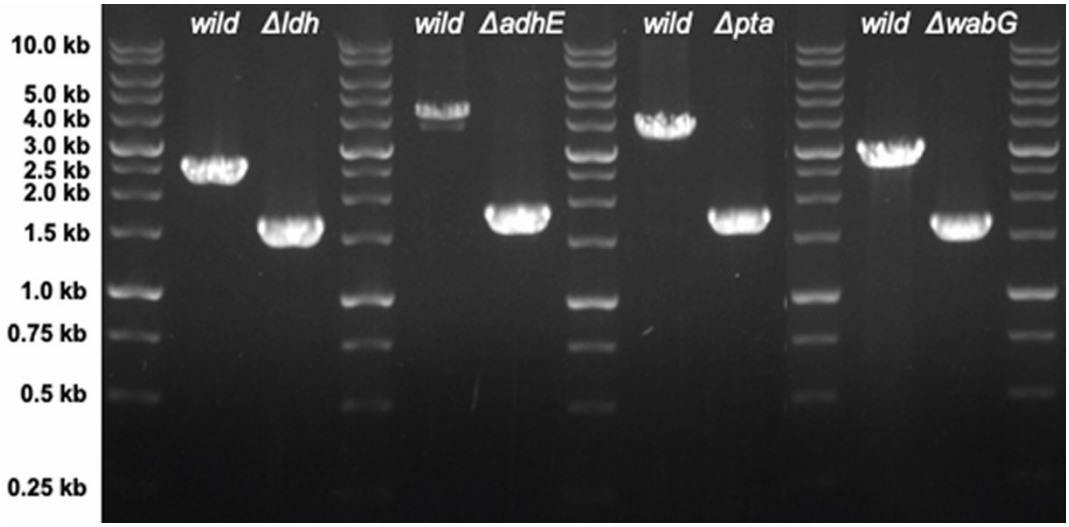
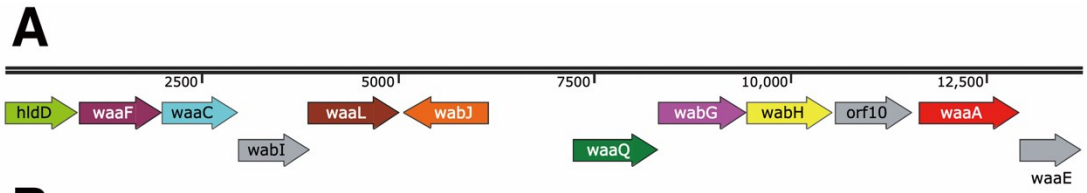



**Fig. S2.** Analysis of PCR fragments to confirm *ldhA*, *adhE*, *pta* and *wabG* disruption.




B

| Gene | Protein | Gene ID |
|---|---|---|
| *hldD* | ADP-L-glycero-D-manno-heptose-6-epimerase | MT786467 |
| *waaF* | heptosyltransferases II | MT786468 |
| *waaC* | heptosyltransferases I | MT786469 |
| *waaQ* | heptosyltransferases III | MT786470 |
| *wabI* | outer-core Kdo transferase/glycosyltransferase | MT786471 |
| *waaL* | O-antigen ligase | MT786472 |
| *wabJ* | outer-core heptosyltranferase | MT786473 |
| *wabG* | Glycosyltransferase | MT786474 |
| *wabH* | 4-alpha-N-acetyl-galactosaminyltransferase | MT786475 |
| *orf10* | putative glycosyltransferase | MT786476 |
| *waaA* | 3-deoxy-D-manno-octulosonic-acid transferase (KDO transferase) | MT786477 |
| *waaE* | inner-core glucosyltransferase | MT786478 |

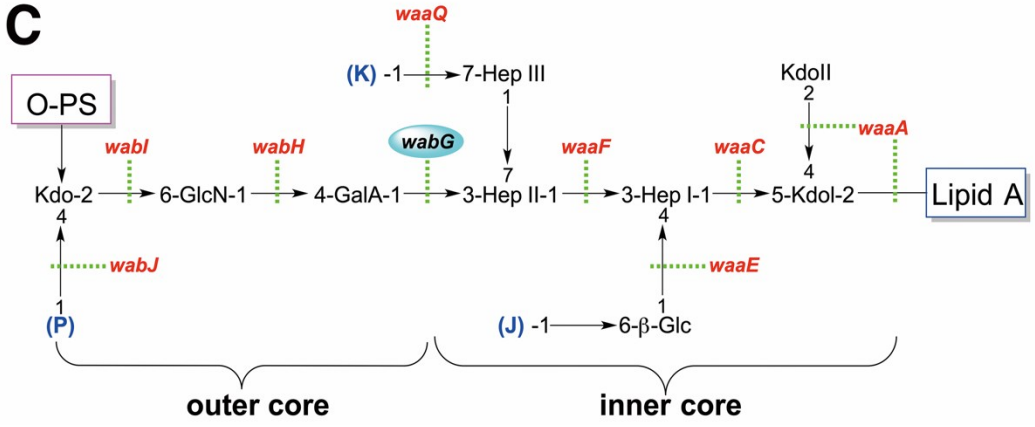



**Fig. S3.** (A) Genetic organization of the core oligosaccharide (OS) biosynthetic gene cluster of the *K. pneumoniae* OU7. (B) Gene annotations of the core OS biosynthetic gene cluster. (C) The LPS structure of the *K. pneumoniae* OU7. Depending on the *K. pneumoniae* strain, residues J and K could be H or GalA, and residue P could be H or Hep. The dashed lines indicate the known or predicted gene products involved in the indicated linkages.

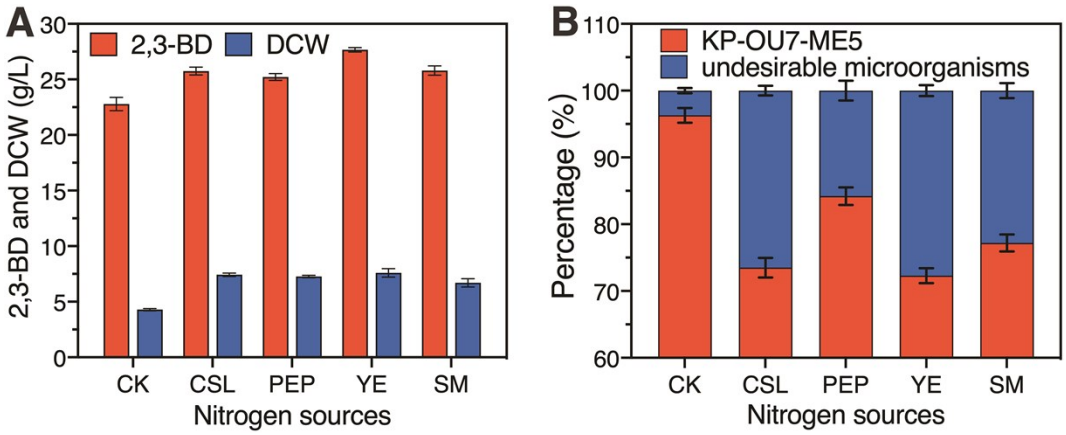



**Fig. S4.** (A) Effect of different nitrogen sources at the concentration of 5 g/L on KP-OU7-ME5 growth and 2,3-BD synthesis. (B) Quantitative comparison of KP-OU7-ME5 and undesirable microorganisms in non-sterilized auxotrophic MOPS medium (5 g/L different nitrogen sources were added). CK: control; CSL: corn steep liquor; PEP: peptone; YE: yeast extract; SM: soybean meal. Error bars are given showing standard deviations for $n$ = 3.

**Table S1. Primers used in this study.**

| Primers | Sequence (5'-3') [a] |
|---|---|
| **Δ*ldhA* construction** | |
| LdhA-UF | caagcttcttctagaggtaccACACGTTCGACCAGTGAGTGAA (*Kpn*I) |
| LdhA-UR | tgctgactgcagattgcccaAAGACTTTTCTCCAGTGATTATAACGTC |
| LdhA-DF | agtcttTGGGCAATCTGCAGCAGG |
| LdhA-DR | catgaattcccgggagagctcTAGTGAACCCGTTCAGCAGGC (*Sac*I) |
| **Δ*adhE* construction** | |
| adhE-UF | caagcttcttctagaggtaccCTGATCATCGACATCGCGATG (*Kpn*I) |
| adhE-UR | ccagctcagctGATCACTTTGTCTTCAACGATGCC |
| adhE-DF | aaagtgatcAGCTGAGCTGGGTATTCCGAA |
| adhE-DR | catgaattcccgggagagctcCGTACCGTCGTTTATACCGCTG (*Sac*I) |
| **Δ*pta* construction** | |
| pta-UF | caagcttcttctagaggtaccATCATCTTCCATCTGCACGACA (*Kpn*I) |
| pta-UR | gggaagatgaacacggtATGATCTCTTCCATCAGCACATCTT |
| pta-DF | catACCGTGTTCATCTTCCCGG |
| pta-DR | catgaattcccgggagagctcAGCACTCAATGATAATCAGGTTATTGC (*Sac*I) |
| **Δ*wabG* construction** | |
| wabG-UF | caagcttcttctagaggtaccGATGCAATGGCAGCTCATTCA (*Kpn*I) |
| wabG-UR | gcatcgGCCAGTACCTTTTTCGCCG |
| wabG-DF | aaaaaggtactggcCGATGCGCATCTTATTTGTGAT |
| wabG-DR | catgaattcccgggagagctcACCACATTGCCGAATCCTTCG (*Sac*I) |

[a] Restriction sites are underlined, and the restriction enzymes are indicated in parentheses.

**Table S2. Enzymatic assays of the different *K. pneumoniae* OU7 strains.**

| Enzyme activity (U/mg protein) [a] | KP-OU7 | KP-OU7-ME1 | KP-OU7-ME2 | KP-OU7-ME3 |
|---|---|---|---|---|
| Lactate dehydrogenase (LDH) | 3.17± 0.18 | 0.23 ± 0.04 | 0.27± 0.03 | 0.31± 0.02 |
| Alcohol dehydrogenase (ADH) | 2.45 ± 0.23 | 2.53 ± 0.24 | 0.07 ± 0.02 | 0.06 ± 0.01 |
| Phosphotransacetylase (PTA) | 2.74 ± 0.31 | 2.08 ± 0.27 | 2.14 ± 0.19 | 0.13 ± 0.02 |

[a] Data from three separate experiments were stated as the mean ± SD ($n$ = 3).