Efficient 2,3-butanediol production from whey powder using metabolically engineered *Klebsiella oxytoca*

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**Abstract**

**Background:** Whey is a major pollutant generated by the dairy industry. To decrease environmental pollution caused by the industrial release of whey, new prospects for its utilization need to be urgently explored. Here, we investigated the possibility of using whey powder to produce 2,3-butanediol (BDO), an important platform chemical.

**Results:** *Klebsiella oxytoca* strain PDL-0 was selected because of its ability to efficiently produce BDO from lactose, the major fermentable sugar in whey. After deleting genes *pox*, *pta*, *frdA*, *ldhD*, and *pflB* responding for the production of by-products acetate, succinate, lactate, and formate, a recombinant strain *K. oxytoca* PDL-K5 was constructed. Fed-batch fermentation using *K. oxytoca* PDL-K5 produced 74.9 g/L BDO with a productivity of 2.27 g/L/h and a yield of 0.43 g/g from lactose. In addition, when whey powder was used as the substrate, 65.5 g/L BDO was produced within 24 h with a productivity of 2.73 g/L/h and a yield of 0.44 g/g.

**Conclusion:** This study demonstrated the efficiency of *K. oxytoca* PDL-0 for BDO production from whey. Due to its non-pathogenicity and efficient lactose utilization, *K. oxytoca* PDL-0 might also be used in the production of other important chemicals using whey as the substrate.

**Keywords:** Whey, Lactose, *Klebsiella oxytoca* PDL-0, 2,3-Butanediol, Metabolic engineering
Sugarcane bagasse hydrolysate, and kenaf core biomass have been used in fermentative production of BDO [17–19]. Several BDO-producing microorganisms can use fermentable sugars, including glucose, xylose, fructose, and lactose as the sole carbon source for growth [20–23]. However, these strains exhibit unsatisfactory fermentative performance in BDO production when lactose is used as the carbon source. For example, *Klebsiella oxytoca* NRRL-B199 can use the mixture of glucose and galactose as substrate for growth and produce BDO as its main product. Nevertheless, BDO was present in a low concentration and the strain produced acetate as the major product in the fermentation broth with lactose [24, 25].

Production of BDO using whey as the substrate can enhance the economic feasibility of BDO fermentation and facilitate resource utilization of the pollutant whey. Therefore, it is critical to identify a suitable microbial strain with BDO production potential using lactose and whey. In this study, we cultured *Klebsiella pneumonia* ATCC 15380, *Enterobacter cloacae* SDM, *Bacillus licheniformis* DSM13, *K. oxytoca* PDL-0, and *Escherichia coli* BL21-pETRABC in fermentation broths with lactose as the carbon source. *K. oxytoca* PDL-0 exhibited the best performance in lactose utilization and BDO production. Next, byproduct-producing genes in *K. oxytoca* PDL-0, including *pox*, *pta*, *frdA*, *ldhD*, and *pflB*, were knocked out to improve the efficiency of BDO production from lactose. Finally, high production of BDO from whey powder was achieved through fed-batch fermentation using the recombinant strain (Fig. 1).

**Results and discussion**

**Selection of *K. oxytoca* PDL-0 for BDO production from lactose**

To select a strain for efficient BDO production from whey, we first assessed strains that can utilize lactose and produce BDO. *K. pneumonia*, *E. cloacae*, *B. licheniformis*, and *K. oxytoca* can produce BDO from glucose [16]. *E. coli* BL21-pETRABC carrying the BDO pathway gene cluster from *E. cloacae* can also efficiently bio-transform glucose into BDO [26]. In the present study, we first compared the ability of *K. pneumonia* ATCC 15380, *E. cloacae* SDM, *B. licheniformis* DSM13, *K. oxytoca* PDL-0, and *E. coli* BL21-pETRABC to produce BDO from lactose; results are shown in Fig. 2.

All five strains were cultured in M9 medium supplemented with 5 g/L yeast extract and ~40 g/L lactose for 48 h. *B. licheniformis* DSM13 is the only strain that cannot consume lactose. *E. cloacae* SDM and *E. coli* BL21-pETRABC could grow well and utilize ~30 g/L lactose within 48 h, but only accumulated about 2 g/L BDO. *K. pneumonia* ATCC 15380 and *K. oxytoca* PDL-0 can completely consume ~40 g/L lactose within 36 h and 18 h, and produce BDO from lactose with a yield of 0.21 g/g and 0.30 g/g lactose, respectively (Additional file 1: Fig. S1 and Fig. 2d). Considering the fact that *K. oxytoca* PDL-0 belongs to Risk Group 1 [15] and produces BDO from lactose with a higher yield than other strains, this strain was selected for further study in successive experiments.

**Inactivation of by-product pathways in *K. oxytoca* PDL-0**

*Klebsiella oxytoca* PDL-0 produced BDO as its major fermentative product during lactose fermentation in a shaking flask culture. However, only 56% of theoretical yield (0.293 vs 0.526 g/g) was observed (Fig. 3). BDO is produced by a fermentative pathway known as the mixed acid-BDO pathway in *K. oxytoca* [7, 15]. Acetate (1.57 g/L), succinate (1.14 g/L), lactate (1.34 g/L), and...
formate (0.27 g/L) were also detected as by-products in the fermentation broth (Fig. 3).

In *K. oxytoca* PDL-0, the formation of acetate, succinate, lactate, and formate is catalyzed by *pto*, *pox*, *frdA*, *ldhD*, and *pflB*, respectively [27]. To achieve higher BDO yield, these genes were successively deleted in strain *K. oxytoca* PDL-0 (Fig. 1). Effects of these gene deletions on growth, lactose consumption, by-product accumulation, and BDO production were studied in M9 medium supplemented with 5 g/L yeast extract and ~40 g/L lactose. As shown in Fig. 3a, b, deletion of these by-product pathways in *K. oxytoca* PDL-0 had no effect on lactose consumption but did slightly increase growth. Accumulation of by-products, including acetate (0.23 g/L), succinate (0.70 g/L), lactate (0.11 g/L), and formate (0 g/L), was markedly decreased due to deletion of *pto*, *pox*, *frdA*, *ldhD*, and *pflB* (Fig. 3c). The final strain, *K. oxytoca* PDL-K5, exhibited high concentration (16.0 g/L) and yield (0.36 g/g lactose) of BDO (Fig. 3d, e) and low by-product generation (Fig. 3c).

**Performance of recombinant strain in 1-L batch fermentation**

The effects of inactivation of by-product pathways on BDO production were further studied through batch fermentation in a 1-L fermenter. The strains *K. oxytoca* PDL-0 and *K. oxytoca* PDL-K5 were cultured in a fermentation medium containing corn steep liquor powder as a nitrogen source and ~40 g/L lactose as carbon source. As shown in Fig. 4a, b, *K. oxytoca* PDL-0 consumed 42.75 g/L lactose and produced 15.26 g/L BDO...
with a yield of 0.36 g/g at 12 h, while *K. oxytoca* PDL-K5 consumed 39.29 g/L lactose and produced 17.65 g/L BDO with a yield of 0.45 g/g. Thus, the recombinant strain *K. oxytoca* PDL-K5 demonstrates advantages over wild type in both concentration and yield of BDO.

**Utilization of lactose for BDO production in fed-batch fermentation**

To achieve higher product concentration, we performed fed-batch fermentation using strain *K. oxytoca* PDL-K5 with initial lactose concentration of ~100 g/L. Fermentation medium containing corn steep liquor was used in
a 7.5-L fermenter. As shown in Fig. 5a, 173.2 g/L lactose was consumed and 74.9 g/L BDO was produced within 33 h. The productivity was 2.27 g/L/h and the yield was 0.43 g/g lactose. The final concentration of the major by-product succinate was 0.82 g/L and there was no formate production throughout the fermentation process (Additional file 1: Fig. S2a).

Utilization of whey powder for BDO production in fed-batch fermentation
Fed-batch fermentation using *K. oxytoca* PDL-K5 with whey powder as the carbon source was also conducted. After 24 h of fermentation, 65.5 g/L BDO was obtained from 148.3 g/L lactose (Fig. 5b). The productivity and yield of BDO were 2.73 g/L/h and 0.44 g/g, respectively. The major by-products in the final fermentation broth were acetate and lactate, which were found at concentrations of 3.24 g/L and 0.38 g/L, respectively (Additional file 1: Fig. S2b). During fermentation, agitation and airflow were set at 400 rpm and 1vvm, respectively, and dissolved oxygen was uncontrolled. Acetoin started to accumulate at the end of fermentation and feeding more whey powder into the fermentation system did not increase BDO production. Dissolved oxygen has a profound impact on the distribution of BDO and its dehydrogenation product, acetoin. Since BDO biosynthesis occurs under microaerobic conditions [28, 29], fine-tuning the dissolved oxygen through an automatic control system might provide the optimal microaerobic condition to further increase BDO production.

Several microbial strains have been screened to produce BDO from whey or lactose. However, as shown in Table 1, the final concentration and yield of BDO produced by wildtype isolates were relatively low. For example, Vishwakarma tried to use strain *K. oxytoca* NRRL-13-199 for BDO production from whey. After the addition of 50 mM acetate, 8.4 g/L BDO was acquired with a yield of 0.365 g/g lactose [30]. Barrett et al. studied...
production of BDO from whey by *K. pneumoniae* ATCC 13882 [23]. After 60 h of fermentation, 19.3 g/L BDO was produced from whey with a productivity of 0.32 g/L/h. Ramachandran et al. obtained a concentration of 32.49 g/L BDO from lactose by using *K. oxytoca* ATCC 8724; however, the yield (0.207 g/g lactose) and productivity (0.861 g/L/h) of BDO were still unsatisfactory [31]. In a previous work, *Lactococcus lactis* MG1363 was metabolically engineered to produce BDO from residual whey permeate, and a final titer of 51 g/L BDO was acquired [32]. Exogenous antibiotics were needed for the maintenance of two plasmids, pJM001 and pLP712, which carry the genes needed for BDO production and metabolism of lactose, respectively. To make bio-based BDO production from whey more economically efficient and environment-friendly, BDO production without antibiotic addition to the fermentation system for the maintenance of plasmids should be initiated. In this work, *K. oxytoca* PDL-K5 was metabolically engineered to efficiently produce BDO from lactose in whey powder through deleting *pox*, *pta*, *frdA*, *ldhD*, and *pflB*. Using whey powder as the carbon source, the recombinant strain *K. oxytoca* PDL-K5 can produce 65.5 g/L BDO (Table 1). Compared with other strains used for BDO production from whey, the engineered strain has significant production advantages, such as high product concentration (65.5 g/L), high productivity (2.73 g/L/h), and lack of a need for unnecessary exogenous antibiotics.

Recently, lactose or whey have been used to produce various biochemicals, e.g., ethanol [33], butanol [34], lactic acid [35], citric acid [36], poly(3-hydroxybutyrate) (PHB) [37], and gluconic acid [38], through endogenous or exogenous biosynthetic pathways. However, because of the low utilization efficiency of lactose in these chassis cells, it is difficult to produce the target chemicals with high productivity and high yield [34, 36]. Ahn et al. constructed a fermentation strategy with a cell-recycle membrane system for the production of PHB from whey [37]. A high consumption rate of lactose (7.67 g/L/h) was acquired using this complicated fermentation strategy. The engineered strain *K. oxytoca* PDL-K5 in this study had the ability to efficiently transform lactose in whey powder into BDO with relatively high yield (0.44 g/g) and high consumption rate of lactose (6.18 g/L/h). This work provides a suitable method for BDO production as well as whey utilization (Fig. 6). Considering its excellent

| Strain                  | Substrate                  | Method                                                                 | Concentration (g/L) | Yield (g/g) | Productivity (g/L/h) | References |
|-------------------------|----------------------------|------------------------------------------------------------------------|---------------------|-------------|----------------------|------------|
| *Bacillus polymyxa* ATCC 1232 | Cheese whey               | Wild-type                                                             | 5.5                 | 0.25        | 0.03                 | [22]       |
| *K. pneumoniae* NCIB 8017    | Rennet whey permeate       | Wild-type                                                             | 7.5                 | 0.46        | 0.08                 | [42]       |
| *K. oxytoca* NRRL-13-199     | Whey                      | Wild-type, adding 50 mM acetate                                       | 8.4                 | 0.365       | –                    | [30]       |
| *Enterobacter aerogenes* 3889 | Whey                      | Wild-type, using neutralized acid whey with 50 mM acetate             | 15.1                | –           | 0.24                 | [23]       |
| *K. pneumoniae* ATCC 13882   | Whey                      | Wild-type, using unsterilized acid whey and adjusting pH to 6.5       | 19.3                | –           | 0.32                 | [23]       |
| *Lactococcus lactis* mL001   | Residual whey permeate (lactose) | Deletion of *ldh*, *ldhB*, *ldhX*, *pta*, *adhE*, *burBA*, overexpression of *bdh* and lactose utilizing pathway in *L. lactis* MG1363 | 51                  | 0.47        | 1.46                 | [32]       |
| *K. oxytoca* PDL-K5          | Whey powder               | Deletion of *pox*, *pta*, *frdA*, *ldhD*, *pflB* in *K. oxytoca* PDL-0 | 65.5                | 0.44        | 2.73                 | This study |
| *K. pneumoniae* KG1          | Lactose                   | Wild-type                                                             | 4.38                | 0.33        | 0.365                | [21]       |
| *K. oxytoca* NRRL-B199 with nonviable cells of *Kluyveromyces lactis* CBS 683 | Lactose | Wild-type, co-immobilization by adhesion of β-galactosidase in nonviable cells of *K. lactis* with *K. oxytoca* | 14.3                | 0.29        | 0.80                 | [25]       |
| *K. oxytoca* ATCC 8724       | Lactose                   | Wild-type                                                             | 32.49               | 0.207       | 0.861                | [31]       |
| *K. oxytoca* PDL-K5          | Lactose                   | Deletion of *pox*, *pta*, *frdA*, *ldhD*, *pflB* in *K. oxytoca* PDL-0 | 74.9                | 0.43        | 2.27                 | This study |
characteristics of non-pathogenicity (Risk Group 1) and efficient lactose utilization, *K. oxytoca* PDL-0 may be a promising chassis for production of various chemicals from whey through metabolic engineering. For example, acetoin, the oxidized precursor of BDO, might be produced through increasing dissolved oxygen levels and deleting 2,3-butanediol dehydrogenases responsible for BDO production from acetoin [39].

**Conclusions**

In this study, the ability of *K. oxytoca* PDL-0 to metabolize lactose and produce BDO was identified. Then, byproduct pathways encoding genes in *K. oxytoca* PDL-0 were knocked out to improve the yield of BDO. The engineered strain *K. oxytoca* PDL-K5 was able to utilize whey powder as the substrate for high production of BDO. The fermentative process developed here is a promising alternative method for both biotechnological production of BDO and whey utilization. In addition, other important chemicals may also be produced from whey using metabolically engineered *K. oxytoca* PDL-0, which has the characteristics of efficient lactose utilization.

**Methods**

**Enzymes and chemicals**

FastPfu DNA polymerase was purchased from TransGen Biotech (Beijing, China) and T4 DNA ligase from Thermo Scientific (Lithuania). Restriction enzymes were purchased from TaKaRa Bio Inc. (Dalian, China). Polymerase chain reaction (PCR) primers were provided by Tsingke Biology Co., Ltd (QingDao, China). Racemic acetoin and BDO was purchased from Apple Flavor & Fragrance Group (Shanghai, China) and ACROS (The Kingdom of Belgium), respectively. Whey powder with a lactose content of 77% was purchased from KuoQuan Biotech (Shandong, China). All other chemicals were of analytical grade and commercially available.

**Bacterial strains, plasmids and culture medium**

The strains and plasmids used in this study are listed in Table 2. All engineered strains used in this work are based on *K. oxytoca* PDL-0 and its derivatives. *E. coli* S17-1 was used to hold and amplify plasmids as well as for conjugation with *K. oxytoca*. The plasmid pKR6Kcm was used for gene knockout in *K. oxytoca* [27].

Luria–Bertani (LB) medium was used for the cultivation of all the strains used. The M9 minimal medium [40] supplemented with 5 g/L yeast extract and 40 g/L lactose was used in shake flasks experiments for selection of the efficient BDO producing strain. The selection medium for single exchange strains of *K. oxytoca* was M9 minimal medium supplemented with 20 g/L sodium citrate and 40 µg/mL chloramphenicol. The selection medium for double exchange strains of *K. oxytoca* was solid LB medium supplemented with 15% sucrose.

**Knockout the genes of *K. oxytoca* PDL-0**

The primers used for knockout of byproduct-producing genes in *K. oxytoca* PDL-0 are listed in Additional file 1: Table S1. Vector isolation, restriction enzyme digestion, agarose gel electrophoresis, and other DNA manipulations were carried out using standard protocols [41]. Knockout mutants of *K. oxytoca* PDL-0 were generated via allele exchange using the suicide plasmid pKR6Kcm [27]. The left and right flanking sequences were amplified from *K. oxytoca* PDL-0 and then ligated through
PCR to get Δpox fragment using primer pairs PΔpox.f (EcoRI)/PΔpox.r (overlap) and PΔpox.f (overlap)/PΔpox.r (BamHI), respectively. The gel-purified Δpox fragments were ligated to the pKR6KCm digested with EcoRI and BamHI. The resulting plasmid was designated pKDΔpox and introduced into E. coli S17-1. Then, a three-step deletion procedure was applied to select the Δpox mutant after conjugating the pKDΔpox in K. oxytoca PDL-0 as described previously [27]. The pta, frdA, ldhD, and pflB mutants of strain K. oxytoca PDL-0 were generated by using the same procedure and primers listed in Additional file 1: Table S1.

### Batch and fed-batch fermentations

Batch fermentations were conducted in a 1-L bioreactor (Multifors 2, Infors AG, Switzerland) with 0.8 L of medium. The seed culture was inoculated (10%, v/v) into the fermentation medium containing 8.27 g/L corn steep liquor powder (CSLP); 4.91 g/L (NH4)2HPO4; 3 g/L sodium acetate; 0.4 g/L KCl; 0.1 g/L MgSO4; 0.02 g/L FeSO4·7H2O; 0.01 g/L MnSO4·7H2O and 40 g/L lactose. The cultivation was carried out at 37 °C, stirring at 400 rpm, airflow at 1.0vvm and initial pH of 7.0. When pH dropped to 6.0, it was maintained at this level by automatic addition of 4 M H3PO4 or 5 M NaOH. Fed-batch fermentation was carried out in a 7.5-L fermenter (BioFlo 310, NBS, USA) containing 5 L of medium and the cultivation condition was the same as 1-L fermenter except that the initial concentration of lactose was about 100 g/L. Alternatively, 130 g/L whey powder was fed into the fermentation broth to make the initial concentration of lactose at about 100 g/L. Solid lactose or whey powder was fed in the fermenter when residual lactose concentration was reduced to about 20 g/L.

### Analytical methods

The optical density (OD) was measured at 600 nm using a spectrophotometer (V5100H, Shanghai Metash Instruments Co., Ltd, China) after an appropriate dilution. The concentrations of lactose and other by-products were detected by high performance liquid chromatography (HPLC) in an Agilent 1100 series, equipped with a Aminex HPX-87H column (300 × 7.8 mm; Bio-Rad, USA) and a refractive index detector [40]. The mobile phase was 10 mM H2SO4 at a flow rate of 0.4 mL/min at 55 °C. The concentrations of acetoin and BDO were analyzed by gas chromatography (GC) (Shimadzu, GC2014c) using a capillary GC column (AT. SE-54, inside diameter, 0.32 mm; length, 30 m, Chromatographic Technology Center, Lanzhou Institute of Chemical Physics, China). Prior to GC analysis, the sample was extracted by ethyl acetate with isoamyl alcohol as the internal standard. Nitrogen was used as the carrier gas for GC analysis. The temperature of both the injector and the detector was 280 °C, the column oven was maintained at 80 °C for

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### Table 2 Strains and plasmids used in this study

| Strain or plasmid | Characteristic(s) | References or sources |
|-------------------|-------------------|-----------------------|
| **Strain**        |                   |                       |
| *Escherichia coli* S17-1 | recA, pro, thi, conjugative strain able to host λ-pir-dependent plasmids | [43] |
| *Enterobacter cloacae* SDM | Wild-type | [12] |
| *E. coli* BL21-pETRABC | *E. coli* BL21 (DE3) harboring pET-RABC | [26] |
| *Klebsiella pneumonia* ATCC 15380 | Wild-type | ATCC |
| *Bacillus licheniformis* DSM13 | Wild-type | DSMZ |
| *Klebsiella oxytoca* PDL-0 | Wild-type | CCTCC M 2016184 |
| *K. oxytoca* PDL-K1 | *K. oxytoca* PDL-0 with deletion of *pox* | This study |
| *K. oxytoca* PDL-K2 | *K. oxytoca* PDL-0 with deletion of *pox* and *pta* | This study |
| *K. oxytoca* PDL-K3 | *K. oxytoca* PDL-0 with deletion of *pox*, *pta*, and *frdA* | This study |
| *K. oxytoca* PDL-K4 | *K. oxytoca* PDL-0 with deletion of *pox*, *pta*, *frdA*, and *ldhD* | This study |
| *K. oxytoca* PDL-K5 | *K. oxytoca* PDL-0 with deletion of *pox*, *pta*, *frdA*, *ldhD*, and *pflB* | This study |
| **Plasmid**       |                   |                       |
| pKR6K<sub>cm</sub> | Cmr<sup>+</sup>, gene replacement vector derived from plasmid pK18mob<sub> sacB</sub>, R6K origin, Mob<sup>+</sup> sac<sub>B</sub>, and the Km<sup>+</sup> resistance was replaced by Cmr<sup>+</sup> | [27] |
| pKDΔpox | pKR6K<sub>cm</sub> derivative, carries a 580 bp deletion of *pox* | This study |
| pKDΔpta | pKR6K<sub>cm</sub> derivative, carries a 1152 bp deletion of *pta* | This study |
| pKDΔfrdA | pKR6K<sub>cm</sub> derivative, carries a 720 bp deletion of *frdA* | This study |
| pKDΔldhD | pKR6K<sub>cm</sub> derivative, carries a 386 bp deletion of *ldhD* | This study |
| pKDΔpflB | pKR6K<sub>cm</sub> derivative, carries a 1150 bp deletion of *pflB* | This study |
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Authors’ contributions
CG, CL and CM designed this study. WM, YZ, MC and WZ conducted the research. WM, YZ, MC, CY and PX analyzed the data. CG, CM, PX and WM wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article and its additional file.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References
1. Carvalho F, Prazeres AR, Rivas J. Cheese whey wastewater: characterization and treatment. Sci Total Environ. 2013;445:6385–96.
2. Domingos JMB, Martinez GA, Scoma A, Fracaccio S, Kerckhof FM, Boon N, Reis MAM, Fava F, Bertin L. Effect of operational parameters in the continuous anaerobic fermentation of cheese whey on titers, yields, productivities, and microbial community structures. ACS Sustain Chem Eng. 2017;5:1400–7.
3. Macwan SR, Dahbi BK, Parmar SC, Apamathi KD. Whey and its utilization. Int J Curr Microbiol Appl Sci. 2016;5:134–55.
4. Prazeres AR, Carvalho F, Rivas J. Cheese whey management: a review. J Environ Manag. 2012;108:48–68.
5. Asunis F, De Gioannis G, Ispato M, Muntoni A, Poletti A, Pomi R, Rossi A, Spiga D. Control of fermentation duration and pH to orient biochemicals and biofuels production from cheese whey. Bioresearch Technol. 2019;289:121722.
6. Ma C, Wang A, Qin J, Li L, Ai X, Jiang T, Tang H, Xu P. Enhanced 2,3-butanediol production by Klebsiella pneumoniae SDM. Appl Microbiol Biotechnol. 2009;82:49–57.
7. Cho S, Kim T, Woo HM, Lee J, Kim Y, Um Y. Enhanced 2,3-butanediol production by optimizing fermentation conditions and engineering Klebsiella oxytoca M1 through overexpression of acetoin reductase. PLoS ONE. 2015;10:e0138109.
8. Jantama K, Poliyam P, Khunnonkwao P, Chan S, Sangproo M, Khor K, Jantama SS, Kanchanatawee S. Efficient reduction of the formation of by-products and improvement of production yield of 2,3-butanediol by a combined deletion of alcohol dehydrogenase, acetate kinase-phosphotransacetylase, and lactate dehydrogenase genes in metabolically engineered Klebsiella oxytoca in mineral salts medium. Metab Eng. 2015;30:16–26.
9. Ge Y, Li K, Li L, Gao C, Zhang L, Ma C, Xu P. Contracted but effective: production of enantiopure 2,3-butanediol by thermophilic and GRAS Bacillus licheniformis. Green Chem. 2016;18:4693–703.
10. Haider J, Harvianto GR, Qyyum MA, Lee M. Cost- and energy-efficient butanol-based extraction-assisted distillation designs for purification of 2,3-butanediol for use as a drop-in fuel. ACS Sustain Chem Eng. 2018;6:14901–10.
11. Cheng KK, Liu Q, Zhang JA, Li JP, Xu JM, Wang GH. Improved 2,3-butanediol production from corn cob acid hydrolysate by fed-batch fermentation using Klebsiella oxytoca. Process Biochem. 2010;45:613–6.
12. Wang A, Xu Y, Ma C, Gao C, Li L, Wang Y, Tao F, Xu P. Efficient 2,3-butanediol production from cassava powder by a crop-biomass-utilizer, Enterobacter cloacae subsp. dissolvens SDM. Appl Microbiol Biotechnol. 2018;102:e40442.
13. Li L, Li K, Wang Y, Chen C, Xu Y, Zhang L, Han B, Gao C, Tao F, Ma C, Xu P. Metabolic engineering of Enterobacter cloacae for high-yield production of enantiopure (2R,3R)-2,3-butanediol from lignocellulose-derived sugars. Metab Eng. 2015;28:19–27.
14. Feng J, Gu Y, Yan PF, Song C, Wang Y. Recruiting energy-conserving sucrose utilization pathways for enhanced 2,3-butanediol production in Bacillus subtilis. ACS Sustain Chem Eng. 2017;5:11221–5.
15. Moon SK, Kim DK, Park JM, Min J, Song H. Development of a semi-continuous two-stage simultaneous saccharification and fermentation process for enhanced 2,3-butanediol production by Klebsiella oxytoca. Lett Appl Microbiol. 2018;66:300–5.
16. Song CW, Park JM, Chung SC, Lee SY, Song H. Microbial production of 2,3-butanediol for industrial applications. J Ind Microbiol Biotechnol. 2019;46:1583–601.
17. Saratade GD, Jung MY, Oh MK. Reutilization of green liquor chemicals for pretreatment of whole rice waste biomass and its application to 2,3-butanediol production. Bioresearch Technol. 2016;205:90–6.
18. Um J, Kim DG, Jung MY, Saratade GD, Oh MK. Metabolic engineering of Enterobacter aerogenes for 2,3-butanediol production from sugarcane bagasse hydrolysate. Bioresearch Technol. 2017;249:1567–74.
19. Saratade RG, Shin HS, Ghodake GS, Kumar G, Oh MK, Saratade GD. Combined effect of inorganic salts with calcium peroxide pretreatment for kenaf core biomass and their utilization for 2,3-butanediol production. Bioresearch Technol. 2018;258:26–32.
20. Song CW, Rathnasingh C, Park JM, Lee J, Song H. Isolation and evaluation of Bacillus strains for industrial production of 2,3-butanediol. J Microbiol Biotechnol. 2018;28:409–17.
21. Guo XW, Zhang YH, Cao CH, Shen T, Wu MY, Chen YF, Zhang CY, Xiao DG. Enhanced production of 2,3-butanediol by overexpressing acetalactate synthase and acetoin reductase in Klebsiella pneumoniae. Biotechnol Appl Biochem. 2014;61:707–15.
22. Speckman RA, Collins EB. Microbial production of 2,3-butylenyl glycol from cheese whey. Appl Environ Microbiol. 1982;43:1216–8.
23. Barrett EL, Collins EB, Hall BJ, Matoi SH. Production of 2,3-butylenyl glycol from whey by Klebsiella pneumoniae and Enterobacter aerogenes. J Dairy Sci. 1983;66:2507–14.
24. Champluvier B, Decallonne J, Rouxhet PG. Influence of sugar source (lactose, glucose, galactose) on 2,3-butanediol production by Klebsiella oxytoca NRRL-B199. Arch Microbiol. 1989;152:411–4.
25. Champluvier B, Francart B, Rouxhet PG. Co-immobilization by adhesion of β-galactosidase in nonviable cells of Kluyveromyces lactis with Klebsiella oxytoca: conversion of lactose into 2,3-butanediol. Biotechnol Bioeng. 1989;34:844–53.
26. Xu Y, Chu H, Gao C, Tao F, Zhou Z, Li K, Li L, Ma C, Xu P. Systematic metabolic engineering of Escherichia coli for high-yield production of fuel bio-chemical 2,3-butanediol. Metab Eng. 2014;23:22–33.
27. Xin B, Tao F, Wang Y, Liu H, Ma C, Xu P. Coordination of metabolic pathways: enhanced carbon conservation in 1,3-propanediol production by coupling with optically pure lactate biosynthesis. Metab Eng. 2017;41:102–14.
28. Heyman B, Tulke H, Putri SP, Fukusaki E, Büchs J. Online monitoring of the respiratory quotient reveals metabolic phases during microaerobic 2,3-butanediol production with Bacillus licheniformis. Eng Life Sci. 2020;20:133–44.
29. Rebecchi S, Pinelli D, Zaranoli G, Fava F, Frascari D. Effect of oxygen mass transfer rate on the production of 2,3-butanediol from glucose and agro-industrial byproducts by Bacillus licheniformis ATCC9789. Biotechnol Biofuels. 2018;11:1145.
30. Vishvakarma S. Bioconversion of whey to 2,3-butanediol using Klebsiella oxytoca NRRL-13-199. Indian J Biotechnol. 2014;13:236–40.
31. Ramachandran KB, Hashim MA, Fernandez AA. Kinetic study of 2,3-butanediol production by Klebsiella oxytoca. J Ferment Bioeng. 1990;70:235–40.
32. Kandasamy V, Liu J, Dantoft SH, Solem C, Jensen PR. Synthesis of (3R)-acetoin and 2,3-butanediol isomers by metabolically engineered Lactococcus lactis. Sci Rep. 2016;6:36769.
33. Farahnak F, Seki T, Ryu DD, Ogrydziak D. Construction of lactose-assimilating and high-ethanol-producing yeasts by protoplast fusion. Appl Environ Microbiol. 1986;51:362–7.
34. Qureshi N, Friedl A, Maddox IS. Butanol production from concentrated lactose/whey permeate: use of pervaporation membrane to recover and concentrate product. Appl Microbiol Biotechnol. 2014;98:8559–67.
35. Roukas T, Kotzekidou P. Lactic acid production from deproteinized whey by mixed cultures of free and coimmobilized Lactobacillus casei and Lactococcus lactis cells using fedbatch culture. Enzyme Microb Technol. 1998;22:199–204.
36. Arslan NP, Aydogan MN, Taskin M. Citric acid production from partly deproteinized whey under non-sterele culture conditions using immobilized cells of lactose-positive and cold-adapted Yarrowia lipolytica B9. J Biotechnol. 2016;231:32–9.
37. Ahn WS, Park SJ, Lee SY. Production of poly(3-hydroxybutyrate) from whey by cell recycle fed-batch culture of recombinant Escherichia coli. Biotechnol Lett. 2001;23:235–40.
38. Mukhopadhyay R, Chatterjee S, Chatterjee BP, Banerjee PC, Guha AK. Production of gluconic acid from whey by free and immobilized Aspergillus niger. Int Dairy J. 2005;15:299–303.
39. Jang JW, Jung HM, Im DK, Jung MY, Oh MK. Pathway engineering of Enterobacter aerogenes to improve acetoin production by reducing by-products formation. Enzyme Microb Technol. 2017;106:114–8.
40. Zhang Y, Guo S, Wang Y, Liang X, Xu P, Gao C, Ma C. Production of α-xylonate from corn cob hydrolysate by a metabolically engineered Escherichia coli strain. ACS Sustain Chem Eng. 2019;7:2160–8.
41. Sambrook J, Russell DW. Molecular cloning: a laboratory manual. 3rd ed. Cold Spring Harbor Laboratory: Cold Spring Harbor; 2001.
42. Lee HK, Maddox IS. Microbial cloning: a laboratory manual. 3rd ed. Cold Spring Harbor Laboratory: Cold Spring Harbor, 2001.
43. Simon R, Priefer U, Pühler A. A broad host range mobilization system for in vivo genetic engineering: transposon mutagenesis in gram negative bacteria. Nat Biotechnol. 1983;1:784–91.

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