Efficient Production of 2,3-Butanediol from whey Powder by Metabolic Engineered Klebsiella Oxytoca

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Abstract

Backgrounds: Whey is the major pollution source from the dairy industry. Exploring new outlets for whey utilization is urgently needed to decline its environmental pollution. In this study, we explored the possibility of using whey powder to produce 2,3-butanediol (2,3-BD), an important platform chemical.

Results: A Klebsiella oxytoca strain PDL-0 was selected from five 2,3-BD producing strains based on its ability to efficiently produce 2,3-BD from lactose, the major fermentable sugar in whey. Five genes including pox, pta, frdA, ldhD, and pflB were knocked out in K. oxytoca PDL-0 to decrease the production of byproducts like acetate, succinate, lactate, and formate. Using fed-batch fermentation of K. oxytoca PDL-0 ΔpoxΔptaΔfrdAΔldhDΔpflB, 74.9 g/L 2,3-BD was produced 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 2,3-BD 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 proved the efficiency of K. oxytoca PDL-0 to metabolize whey for 2,3-BD production. Due to its characteristics of 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.

Background

Whey is a liquid byproduct in cheese making process which contains most of the water-soluble components in milk [1, 2]. Despite its annual production of 145 million tons worldwide, only a little over one-half of the whey produced is utilized [3]. Whey is regarded as a serious pollutant because of its high biochemical oxygen demand (30,000–50,000 mg/L) and chemical oxygen demand (60,000–80,000 mg/L) [3]. Economic disposal of whey becomes a world-wide problem of the dairy industry. Lactose, a utilisable disaccharide of many microbial strains, is the major contributor to biochemical oxygen demand and chemical oxygen demand in whey [4, 5]. Using the lactose in whey as a substrate for microbial fermentation may transform a potential pollutant into a value-added product and deserves an intensive study.

2,3-Butanediol (2,3-BD) is an important platform chemical which can be applied in many industrial fields [6 – 8]. It is estimated that the derivatives of 2,3-BD have a potential global market of around 32 million tons per year. Nowdays, the common method for 2,3-BD synthesis is a chemical route conducted under a harsh condition (160–220 °C, 50 bar) with a C₄ hydrocarbon fraction of crack gases as the substrate [9, 10]. Due to the shortage of fossil fuels and increasing global environmental concerns, the green production of 2,3-BD through microbial fermentation using renewable resources has received great attentions [11 – 16].

Several 2,3-BD producing microorganisms can use fermentable sugars including glucose, xylose, fructose, and lactose as the sole carbon source for growth [17 – 20]. However, these strains exhibited unsatisfactory fermentative performances of 2,3-BD production when using lactose as the carbon source. For example, Klebsiella oxytoca NRRL-B199 can use the mixture of glucose and galactose as substrate for growth and produce 2,3-BD as its main product. Nevertheless, 2,3-BD was present in a low concentration and the strain produced acetate as the major product in the fermentation broth with lactose [21, 22]. Thus, it is of vital necessity to select suitable microbial strains with potential of efficient 2,3-BD production from lactose.

In this study, five strains including Klebsiella pneumonia ATCC 15380, Enterobacter cloacae SDM, Bacillus licheniformis DSM13, K. oxytoca PDL-0 and Escherichia coli BL21-pETRABC were cultured in fermentation broth with lactose as the carbon source. K. oxytoca PDL-0 was found to possess the best performance in lactose utilization and 2,3-BD production. Then, byproduct-producing genes including pox, pta, frdA, ldhD, and pflB in K. oxytoca PDL-0 were knocked out to improve the efficiency of 2,3-BD production from lactose. Finally, high production of 2,3-BD from whey powder was achieved through fed-batch fermentation using the recombinant strain K. oxytoca PDL-0 ΔpoxΔptaΔfrdAΔldhDΔpflB (Fig. 1).
Selection of *K. oxytoca* PDL-0 for 2,3-BD production from lactose

To select a strain for efficient 2,3-BD production from whey, we first looked for strains that can utilize lactose and produce 2,3-BD. *K. pneumonia*, *E. cloacae*, *B. licheniformis*, and *K. oxytoca* can produce 2,3-BD from glucose [16]. *E. coli* BL21-pETRABC carrying the 2,3-BD pathway gene cluster from *E. cloacae* can also efficiently biotransform glucose into 2,3-BD [23]. In the present study, we first compared the abilities of *K. pneumonia* ATCC 15380, *E. cloacae* SDM, *B. licheniformis* DSM13, *K. oxytoca* PDL-0, and *E. coli* BL21-pETRABC to produce 2,3-BD from lactose, and the results are shown in Fig. 2.

All of the five strains can grow in M9 medium supply with 5 g/L yeast extract and about 40 g/L lactose. *B. licheniformis* DSM13 is the only strain that can not consume lactose (Fig. 2a and Fig. 2b). *E. cloacae* SDM and *E. coli* BL21-pETRABC could efficiently utilize lactose (about 30 g/L) but these two strains only accumulated about 2 g/L 2,3-BD (Fig. 2b and Fig. 2c). *K. pneumonia* ATCC 15380 and *K. oxytoca* PDL-0 can produce 2,3-BD from lactose, with a yield of 0.21 g/g and 0.30 g/g lactose, respectively. Considering the fact that *K. oxytoca* PDL-0 belongs to the Risk Group 1 [15] and produces 2,3-BD with a higher yield from lactose, this strain was selected for further study in successive experiments.

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

*K. oxytoca* PDL-0 produced 2,3-BD as its major fermentative product during lactose fermentation in a shake flask culture. However, only 58% of theoretical yield (0.526 g/g) was observed (Fig. 2). 2,3-BD is produced by a fermentative pathway known as the mixed acid-2,3-BD pathway in *K. oxytoca*. Succinate, lactate, formate, and acetate were also detected as by-products in the fermentation broth [7, 15].

In *K. oxytoca* PDL-0, the formation of acetate, succinate, lactate, and formate is catalyzed by *pox* and *pta*, *frdA*, *ldhD*, and *pflB*, respectively [24]. To achieve higher yield of 2,3-BD, these genes were successively deleted in strain *K. oxytoca* PDL-0 (Additional file 1: Fig. S1). Effects of these genes deletion on growth, lactose consumption, by-product accumulation, and 2,3-BD production were studied in M9 medium supply with 5 g/L yeast extract and about 40 g/L lactose. As shown in Fig. 3a and Fig. 3b, deletion of all these by-product pathways in *K. oxytoca* PDL-0 had no effect on lactose consumption but slightly increased its growth. Accumulation of by-products including acetate, succinate, lactate, and formate reduced remarkably due to deletion of *pox*, *pta*, *frdA*, *ldhD*, and *pflB* (Fig. 3c). The final strain *K. oxytoca* PDL-0 Δ*pox*Δ*pta*Δ*frdA*Δ*ldhD*Δ*pflB* exhibited higher concentration and yield of 2,3-BD (Fig. 3d and Fig. 3e) and lower byproducts production (Fig. 3c) than other recombinant strains.

Performance of recombinant strain in 1-L batch fermentation

Then, the effects of inactivation of by-product pathways on 2,3-BD production were further studied through batch fermentation in a 1-L fermenter. The strains *K. oxytoca* PDL-0 and *K. oxytoca* PDL-0 Δ*pox*Δ*pta*Δ*frdA*Δ*ldhD*Δ*pflB* were cultured in a fermentation medium containing corn steep liquor powder as a cheap nitrogen source and about 40 g/L lactose as carbon source. As shown in Fig. 4a and 4b, *K. oxytoca* PDL-0 consumed 42.75 g/L lactose and produced 15.26 g/L 2,3-BD with a yield of 0.36 g/g at 12 h, while *K. oxytoca* PDL-0 Δ*pox*Δ*pta*Δ*frdA*Δ*ldhD*Δ*pflB* consumed 39.29 g/L lactose and produced 17.65 g/L 2,3-BD with a yield of 0.45 g/g. Thus, the recombinant strain *K. oxytoca* PDL-0 Δ*pox*Δ*pta*Δ*frdA*Δ*ldhD*Δ*pflB* possesses advantages over wild type in both concentration and yield of 2,3-BD.

Utilization of lactose for 2,3-BD production in fed-batch fermentation

To achieve a higher product concentration, fed-batch fermentation using strain *K. oxytoca* PDL-0
ΔpoxΔptaΔfrdAΔldhDΔpflB with an initial lactose concentration of 100 g/L was conducted. 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 2,3-BD was produced within 33 h. The productivity was 2.27 g/L/h, and the yield was 0.43 g/g lactose. The concentration of acetate, which was included in the medium, was 0.59 g/L at the end of the fermentation. The concentration of lactate, which was also included in the medium, decreased to 0.13 g/L at 33 h. The final concentration of 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 2,3-BD production in fed-batch fermentation

Fed-batch fermentation using whey powder as the carbon source by strain K. oxytoca PDL-0 ΔpoxΔptaΔfrdAΔldhDΔpflB was also carried out. After 24 h of fermentation, 65.5 g/L 2,3-BD was obtained from 148.3 g/L lactose (Fig. 5b). The productivity and yield of 2,3-BD were 2.73 g/L/h and 0.44 g/g, respectively. The major by-products in 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).

Several microbial strains have been screened to produce 2,3-BD from whey or lactose. However, as shown in Table 1, the final concentration and yield of 2,3-BD produced by wild type isolates were relatively low. For example, Vishwakarma tried to use strain K. oxytoca NRRL-13-199 for 2,3-BD production from whey. After the addition of 50 mM acetate, a concentration of 8.4 g/L 2,3-BD was acquired with a yield of 0.365 g/g lactose [25]. Barrett et al studied production of 2,3-BD from whey by K. pneumoniae ATCC 13882 [20]. After 60 h of fermentation, 19.3 g/L 2,3-BD was produced from whey with a productivity of 0.32 g/L/h. Ramachandran et al got a concentration of 32.49 g/L 2,3-BD from lactose by using K. oxytoca (formerly known as Aerobacter aerogenes or K. pneumoniae ATCC 8724), however, the yield (0.207 g/g lactose) and productivity (0.861 g/L/h) of 2,3-BD were still unsatisfactory [26]. In a previous work, Lactococcus lactis MG1363 was metabolic engineered to produce 2,3-BD from residual whey permeate and the final titer of 51 g/L was acquired [27]. Exogenous antibiotics was needed for the maintenance of two plasmids pJM001 and pLP712, which respectively carries the genes needed for 2,3-BD production and metabolism of lactose. To make bio-based 2,3-BD production from whey more economically efficient and environment-friendly, 2,3-BD production without antibiotic addition in the fermentation system for the maintenance of plasmid should be initiated. In this work, K. oxytoca PDL-0 was metabolic engineered to efficiently produce 2,3-BD from lactose through deleting pox, pta, frdA, ldhD, and pflB. Using whey powder as the carbon source, the recombinant strain can produce 65.5 g/L 2,3-BD (Table 1). Compared with other strains used for 2,3-BD 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 unnecessary exogenous antibiotics.
Table 1
Comparison of 2,3-BD production using whey/lactose as substrate by different microorganisms.

| Strain                      | Substrate                  | Method                                                                 | Concentration (g/L) | Yield (g/g) | Productivity (g/L/h) | Reference |
|-----------------------------|-----------------------------|------------------------------------------------------------------------|---------------------|-------------|-----------------------|-----------|
| *Bacillus polymyxa* ATCC 1232 | Cheese whey                | Wild-type                                                              | 5.5                 | 0.25        | 0.03                  | [19]      |
| *K. pneumoniae* NCIB 8017   | Rennet whey permeate        | Wild-type                                                              | 7.5                 | 0.46        | 0.08                  | [36]      |
| *K. oxytoca* NRRL-13-199    | Whey                       | Wild-type                                                              | 8.4                 | 0.37        | –                     | [25]      |
| *Enterobacter aerogenes* 3889 | Whey                       | Wild-type, using neutralized acid whey with 50 mM acetate              | 15.1                | –           | 0.24                  | [20]      |
| *K. pneumoniae* ATCC 13882  | Whey                       | Wild-type, using unsterilized acid whey and adjusting pH to 6.5         | 19.3                | –           | 0.32                  | [20]      |
| *Lactococcus lactis* MG1363 | Residual whey permeate (lactose) | Deletion of *ldh*, *ldhB*, *ldhX*, *pta*, *adhE*, *butBA*, overexpression of *bdh* and lactose utilizing pathway | 51                  | 0.47        | 1.46                  | [27]      |
| *K. oxytoca* PDL-0          | Whey powder                | Deletion of *pox*, *pta*, *frdA*, *ldhD*, *pflB*                       | 65.5                | 0.44        | 2.73                  | This study|
| *K. pneumoniae* KG1         | Lactose                    | Wild-type                                                              | 4.4                 | 0.33        | 0.37                  | [18]      |
| *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                  | [22]      |
| *K. oxytoca* (K. pneumoniae ATCC 8724) | Lactose | Wild-type                                                              | 32.49               | 0.21        | 0.86                  | [26]      |
| *K. oxytoca* PDL-0          | Lactose                    | Deletion of *pox*, *pta*, *frdA*, *ldhD*, *pflB*                       | 74.9                | 0.43        | 2.27                  | This study|
Recently, lactose or whey have been used to produce various biochemicals, e.g., ethanol [28], butanol [29], lactic acid [30], citric acid [31], poly(3-hydroxybutyrate) (PHB) [32], and gluconic acid [33], through endogenous or exogenous biosynthetic pathways (Table 2). However, because of the low utilization efficiency of lactose in these chassis cell, it is difficult to produce the target chemicals with high productivity and high yield [29, 31].

Ahn et al constructed a fermentation strategy with cell recycle membrane system for the production of PHB from whey [32]. High consumption rate of lactose (7.67 g/L/h) was acquired using this complicated fermentation strategy. In this work, the engineered *K. oxytoca* PDL-0 was confirmed to have the ability to efficiently transform lactose into 2,3-BD with relatively high yield (0.44 g/g) and high consumption rate of lactose (6.18 g/L/h) (Table 1 and Table 2). Considering its excellent characteristics of non-pathogenicity (Risk Group 1) and efficient lactose utilization, *K. oxytoca* PDL-0 might be a promising chassis for production of various chemicals from whey through metabolic engineering.
Table 2
Other products using whey/lactose as substrate by different strains.

| Product   | Strain                                      | Substrate | Strategies                                                                 | Concentration (g/L) | Yield (%)<sup>a</sup> | Lactose consumption rate (g/L/h) | Reference |
|-----------|---------------------------------------------|-----------|---------------------------------------------------------------------------|----------------------|------------------------|-----------------------------------|-----------|
| Ethanol   | *Saccharomyces cerevisiae* STX 23-5B and *Kluyveromyces fragilis* 55-55 | Lactose   | Constructing hybrids between *S. cerevisiae* and *K. fragilis* through protoplast fusion | 105                  | -                      | -                                 | [28]      |
| Butanol   | *Clostridium saccharobutylicum* P262         | Lactose   | Using pervaporation membrane to recover and concentrate product           | 72.4                 | 79                     | 1.14                              | [29]      |
| Lactic acid | *Lactobacillus casei* SU No 22 and *L. lactis* WS 1042 | Deproteinized whey | Coimmobilization of *L. casei* and *L. lactis* cells | 47                   | 61                     | 3.04                              | [30]      |
| Citric acid | *Yarrowia lipolytica* B9                  | Partly deproteinized whey | Using immobilized cells of *Y. lipolytica* | 33.3                 | 47                     | 0.53                              | [31]      |
| PHB       | *E. coli* CGSC 4401                        | Whey      | Using cell recycle membrane system by *E. coli* expressing pha genes    | 168                  | -                      | 7.67                              | [32]      |
| Gluconic acid | *Aspergillus niger* NCIM 548           | Lactose and glucose | Using *A. niger* immobilized in polyurethane foam | 92                   | 80                     | 1.98                              | [33]      |
| 2,3-BD    | *K. oxytoca* PDL-0                        | Lactose   | Deletion of *pox, pta, frdA, ldhD, pflB*                                | 74.9                 | 82                     | 5.25                              | This study |
| 2,3-BD    | *K. oxytoca* PDL-0                        | Whey powder | Deletion of *pox, pta, frdA, ldhD, pflB*                          | 65.5                 | 84                     | 6.18                              | This study |

<sup>a</sup>Ratio between actual yield and theoretical yield of each product.

Conclusions
In this study, the ability of K. oxytoca PDL-0 to metabolize lactose and produce 2,3-BD was firstly identified. Then, by-product pathways encoding genes in K. oxytoca PDL-0 was knockout to improve the yield of 2,3-BD. The engineered strain K. oxytoca PDL-0 ΔpoxΔptaΔfrdAΔldhDΔpflB could utilize whey powder as the substrate for high production of 2,3-BD. The process developed here may be a promising alternative for both biotechnological production of 2,3-BD and whey 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 (AC) and 2,3-BD was purchased from Apple Flavor & Fragrance Group (Shanghai, China) and ACROS (The Kingdom of Belgium), respectively. Whey powder 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 3. 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 pKR6K_{Cm} was used for gene knockout in K. oxytoca [24].

| Strain or plasmid | Characteristic(s) | Reference or source |
|-------------------|-------------------|---------------------|
| Strain            |                   |                     |
| *Escherichia coli* S17-1 | *recA, pro, thi*, conjugative strain able to host λ-pir-dependent plasmids | [37] |
| *Enterobacter cloacae* SDM | Wild-type | [12] |
| E.coli BL21-pETRABC | *E. coli* BL21 (DE3) harboring pET-RABC | [23] |
| *Klebsiella pneumonia* ATCC 15380 | Wild-type | ATCC |
| *Bacillus licheniformis* DSM13 | Wild-type | DSMZ |
| *Klebsiella oxytoca* PDL-0 | Wild-type | [24] |
| K. *oxytoca* PDL-0 Δpox | K. *oxytoca* PDL-0 with deletion of *pox* | This study |
| K. *oxytoca* PDL-0 ΔpoxΔpta | K. *oxytoca* PDL-0 with deletion of *pox* and *pta* | This study |
| K. *oxytoca* PDL-0 ΔpoxΔptaΔfrdA | K. *oxytoca* PDL-0 with deletion of *pox*, *pta*, and *frdA* | This study |
| K. oxytoca PDL-0 ΔpoxΔptaΔfrdAΔldhD | K. oxytoca PDL-0 with deletion of pox, pta, frdA, and ldhD | This study |
|---------------------------------------|-------------------------------------------------------|-----------|
| K. oxytoca PDL-0ΔpoxΔptaΔfrdAΔldhDΔpflB | K. oxytoca PDL-0 with deletion of pox, pta, frdA, ldhD, and pflB | This study |

**Plasmid**

| pKR6K<sub>Cm</sub> | Cm<sup>r</sup>, gene replacement vector derived from plasmid pK18mobsacB, R6K origin, Mob<sup>+</sup> sacB, and the Km<sup>r</sup> resistance was replaced by Cm<sup>r</sup> [24] |
|---------------------|------------------------------------------------------------------|
| 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 |

Luria-Bertani (LB) medium was used for the cultivation of all the strains used. The M9 minimal medium [34] supplemented with 5 g/L yeast extract and 40 g/L lactose was used in shake flasks experiments for selection of the efficient 2,3-BD 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 [35]. Knockout mutants of *K. oxytoca* PDL-0 were generated via allele exchange using the suicide plasmid pKR6K<sub>Cm</sub> [24]. 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.<sub>f</sub> (EcoRI)/PΔpox.<sub>r</sub> (overlap) and PΔpox.<sub>f</sub> (overlap)/PΔpox.<sub>r</sub> (BamHI), respectively. The gel-purified Δpox fragments were ligated to the pKR6K<sub>Cm</sub>, 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 [24]. 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. Solid lactose or whey powder was added 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 [34]. The concentrations of AC and 2,3-BD were analyzed by gas chromatography (GC) (Shimadzu, GC2014c) using a capillary GC column as described previously [9].

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Availability of data and materials**

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

**Competing interests**

The authors declare that they have no competing interests.

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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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Metabolic engineering of *K. oxytoca* for production of platform chemical 2,3-butanediol from whey. G6P, glucose-6-phosphate.
Selection for stains that can produce 2,3-BD from lactose. Biomass (a), consumption of lactose (b), concentration (c) and yield (d) of 2,3-BD using lactose as the carbon source by E. cloacae SDM, E. coli BL21-pETRABC, B. licheniformis DSM13, K. pneumonia ATCC 15380, and K. oxytoca PDL-0 were assayed. The experiments were conducted in a 300-mL flask containing 50 mL of M9 minimal medium supplemented with 5 g/L yeast extract and 40 g/L lactose with shaking at 180 rpm for 48 h. The culture temperature for B. licheniformis DSM13 was 50 °C while for other strains were 37 °C. Error bars indicate the standard deviations from three independent cultures.
Figure 3

Effects of by-product pathway genes knockout when using lactose as the carbon source. Biomass (a), consumption of lactose (b), by-products (c), concentration (d) and yield (e) of 2,3-BD by K. oxytoca PDL-0 and its derivatives were assayed. The experiments were conducted in a 300-mL flask containing 50 mL of M9 minimal medium supplemented with 5 g/L yeast extract and 40 g/L lactose with shaking at 180 rpm for 24 h. The culture temperature was 37 °C. Error bars indicate the standard deviations from three independent cultures.
Figure 4
Batch fermentation using lactose as carbon source. Biomass, consumption of lactose, concentration of 2,3-BD and acetoin (AC) by K. oxytoca PDL-0 (a) and K. oxytoca PDL-0 ΔpoxΔptaΔfrdAΔIdhDΔpflB (b) were assayed. The experiments were conducted in a 1-L fermenter containing 800 mL of medium with an initial lactose concentration of 40 g/L approximately.
Fed-batch fermentation using lactose (a) and whey powder (b) as the carbon source. Biomass, consumption of lactose, concentration of 2,3-BD and acetoin (AC) by *K. oxytoca* PDL-0 ΔpoxΔptaΔfrdAΔldhDΔpfIB were assayed. The experiments were conducted in a 7.5-L fermenter containing 5 L of medium with an initial lactose concentration of 100 g/L approximately.
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