Microbial Cell Factories

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

Wensi Meng[1], Yongjia Zhang[1], Menghao Cao[1], Wen Zhang[2], Chuanjuan Lü[1*], Chunyu Yang[1], Chao Gao[1], Ping Xu[3] and Cuiqing Ma[1*]

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

Background

Whey, a liquid by-product generated during cheese production, contains most of the water-soluble components in milk [1, 2]. Despite 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 (BOD, 30,000–50,000 mg/L) and chemical oxygen demand (COD, 60,000–80,000 mg/L) [3]. Economic disposal of whey has become a worldwide problem for the dairy industry. Lactose, a utilizable disaccharide for many microbial strains, is the major contributor to BOD and COD of whey [4, 5]. Using the lactose in whey as a substrate for industrial microbial fermentation may transform a potential pollutant into a value-added product and this prospect deserves an intensive study.

2,3-Butanediol (BDO) is an important platform chemical that can be applied in many industrial fields [6–8]. Derivatives of BDO are estimated to have a potential global market of around 32 million tons per year. One common method for BDO synthesis is performed under harsh conditions (160–220 °C, 50 bar) with a C_4 hydrocarbon fraction of cracked gases as the substrate [9, 10]. However, due to shortage of fossil fuels and increasing global environmental concerns, green production of BDO through microbial fermentation is desirable [11–16]. Renewable resources such as rice waste biomass,

*Correspondence: chuanjuanlv@mail.sdu.edu.cn; macq@sdu.edu.cn
[1] State Key Laboratory of Microbial Technology, Shandong University, Qingdao 266237, People's Republic of China
Full list of author information is available at the end of the article

© The Author(s) 2020. This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

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).

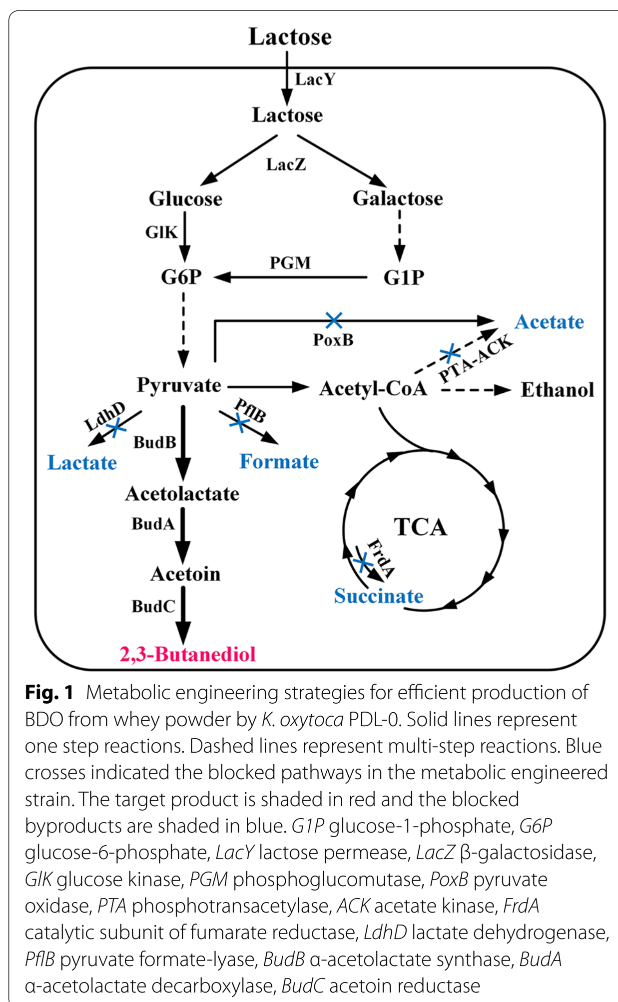

Fig. 1 Metabolic engineering strategies for efficient production of BDO from whey powder by *K. oxytoca* PDL-0. Solid lines represent one step reactions. Dashed lines represent multi-step reactions. Blue crosses indicated the blocked pathways in the metabolic engineered strain. The target product is shaded in red and the blocked byproducts are shaded in blue. *G1P* glucose-1-phosphate, *G6P* glucose-6-phosphate, *LacY* lactose permease, *LacZ* β-galactosidase, *GlK* glucose kinase, *PGM* phosphoglucomutase, *PoxB* pyruvate oxidase, *PTA* phosphotransacetylase, *ACK* acetate kinase, *FrdA* catalytic subunit of fumarate reductase, *LdhD* lactate dehydrogenase, *PflB* pyruvate formate-lyase, *BudB* α-acetolactate synthase, *BudA* α-acetolactate decarboxylase, *BudC* acetoin reductase

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 (Additional file 1: Fig. S1, Fig. 2a–c). *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

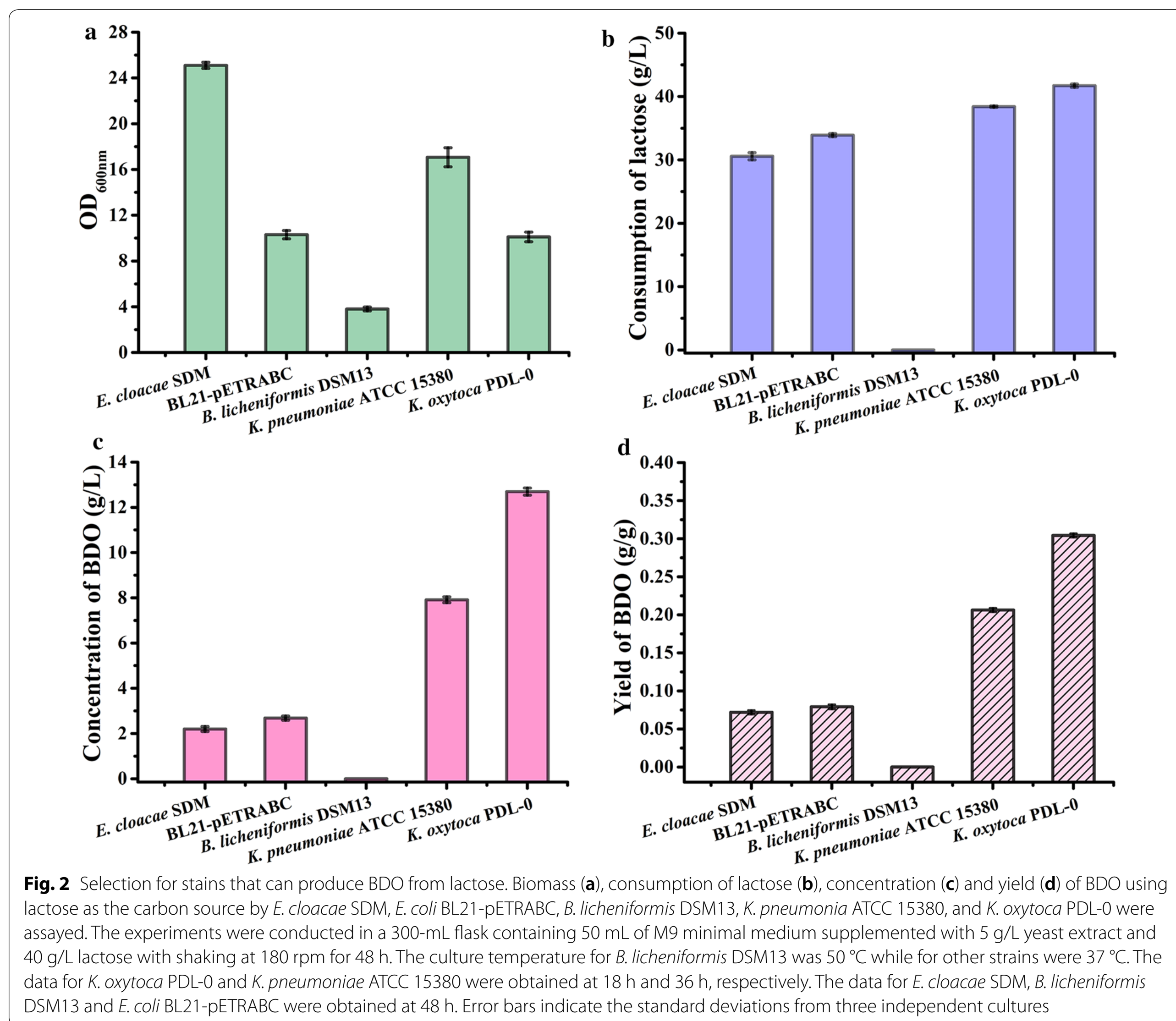

Fig. 2 Selection for stains that can produce BDO from lactose. Biomass (**a**), consumption of lactose (**b**), concentration (**c**) and yield (**d**) of BDO using lactose as the carbon source by *E. cloacae* SDM, *E. coli* BL21-pETRABC, *B. licheniformis* DSM13, *K. pneumonia* ATCC 15380, and *K. oxytoca* 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. The data for *K. oxytoca* PDL-0 and *K. pneumoniae* ATCC 15380 were obtained at 18 h and 36 h, respectively. The data for *E. cloacae* SDM, *B. licheniformis* DSM13 and *E. coli* BL21-pETRABC were obtained at 48 h. Error bars indicate the standard deviations from three independent cultures

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 *pox* and *pta*, *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 *pox*, *pta*, *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

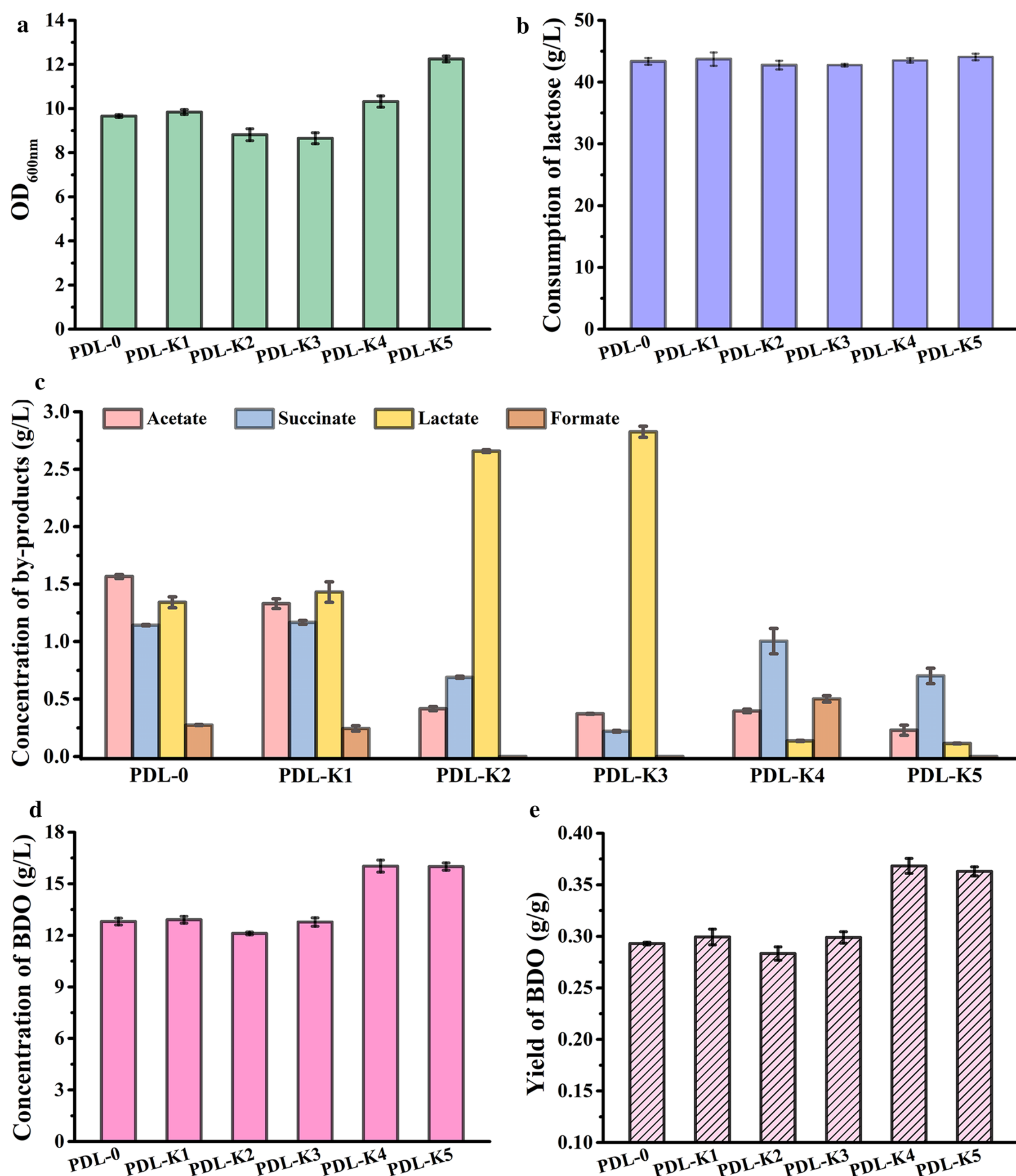

Fig. 3 Effects of by-product pathway genes knockout when using lactose as the carbon source. Biomass (**a**), consumption of lactose (**b**), by-products (**c**), concentration (**d**) and yield (**e**) of BDO by *K. oxytoca* PDL-0 and its derivatives were assayed. The experiments were conducted in a 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

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

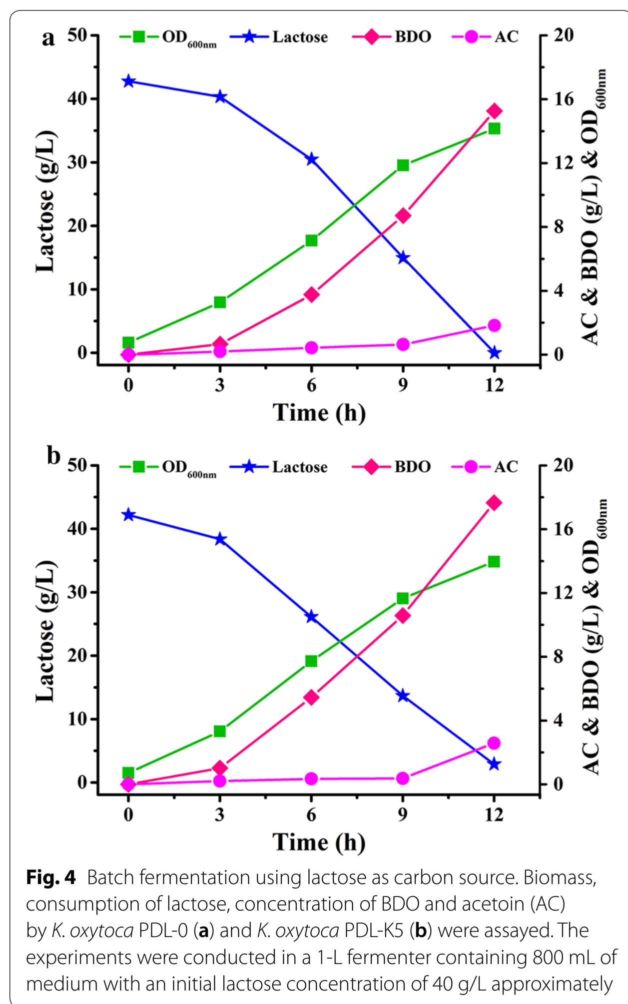

Fig. 4 Batch fermentation using lactose as carbon source. Biomass, consumption of lactose, concentration of BDO and acetoin (AC) by *K. oxytoca* PDL-0 (**a**) and *K. oxytoca* PDL-K5 (**b**) were assayed. The experiments were conducted in a 1-L fermenter containing 800 mL of medium with an initial lactose concentration of 40 g/L approximately

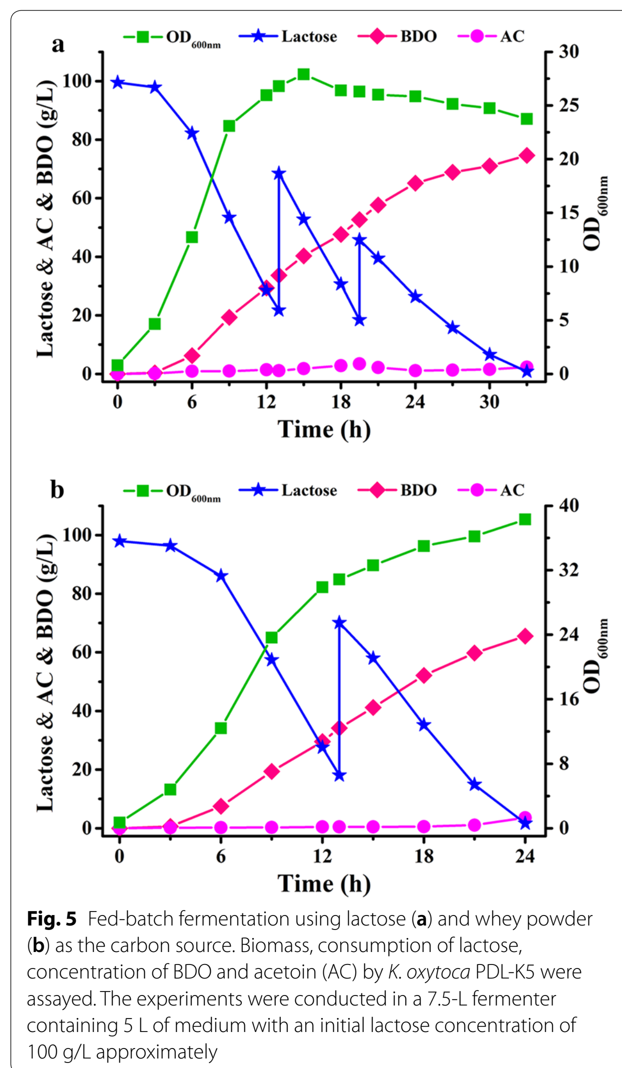

Fig. 5 Fed-batch fermentation using lactose (**a**) and whey powder (**b**) as the carbon source. Biomass, consumption of lactose, concentration of BDO and acetoin (AC) by *K. oxytoca* PDL-K5 were assayed. The experiments were conducted in a 7.5-L fermenter containing 5 L of medium with an initial lactose concentration of 100 g/L approximately

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 1 vvm, 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

Table 1 Comparison of BDO production using whey/lactose as substrate by different microorganisms

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*, *butBA*, 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

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-0 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

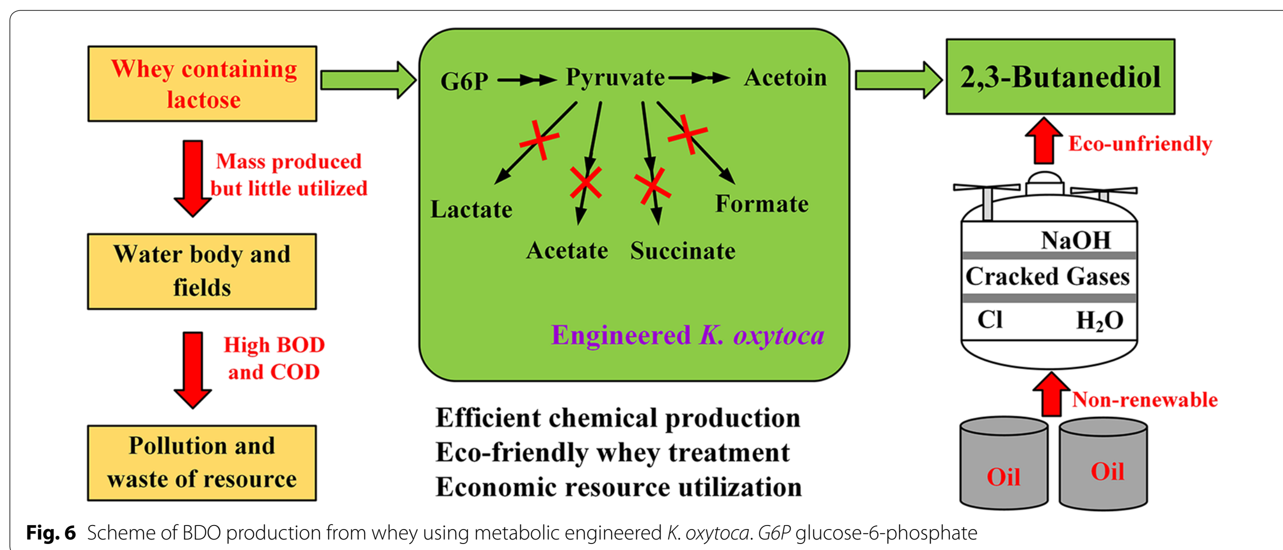

Fig. 6 Scheme of BDO production from whey using metabolic engineered *K. oxytoca*. *G6P* glucose-6-phosphate

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, by-product 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 $pKR6K_{Cm}$ 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 $pKR6K_{Cm}$ [27]. The left and right flanking sequences were amplified from *K. oxytoca* PDL-0 and then ligated through

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		
$pKR6K_{Cm}$	Cm^r, gene replacement vector derived from plasmid pK18*mobsacB*, R6K origin, Mob^+ *sac*B, and the Km^r resistance was replaced by Cm^r	[27]
pKDΔ*pox*	$pKR6K_{Cm}$ derivative, carries a 580 bp deletion of *pox*	This study
pKDΔ*pta*	$pKR6K_{Cm}$ derivative, carries a 1152 bp deletion of *pta*	This study
pKDΔ*frdA*	$pKR6K_{Cm}$ derivative, carries a 720 bp deletion of *frdA*	This study
pKDΔ*ldhD*	$pKR6K_{Cm}$ derivative, carries a 386 bp deletion of *ldhD*	This study
pKDΔ*pflB*	$pKR6K_{Cm}$ derivative, carries a 1150 bp deletion of *pflB*	This study

PCR to get Δ*pox* fragment using primer pairs PΔ*pox*.f (EcoRI)/PΔ*pox*.r (overlap) and PΔ*pox*.f (overlap)/PΔ*pox*.r (BamHI), respectively. The gel-purified Δ*pox* fragments were ligated to the $pKR6K_{Cm}$ digested with EcoRI and BamHI. The resulting plasmid was designated pKDΔ*pox* and introduced into *E. coli* S17-1. Then, a three-step deletion procedure was applied to select the Δ*pox* mutant after conjugating the pKDΔ*pox* 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 $(NH_4)_2HPO_4$; 3 g/L sodium acetate; 0.4 g/L KCl; 0.1 g/L $MgSO_4$; 0.02 g/L $FeSO_4 \cdot 7H_2O$; 0.01 g/L $MnSO_4 \cdot 7H_2O$ and 40 g/L lactose. The cultivation was carried out at 37 °C, stirring at 400 rpm, airflow at 1.0 vvm and initial pH of 7.0. When pH dropped to 6.0, it was maintained at this level by automatic addition of 4 M H_3PO_4 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 H_2SO_4 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

3 min. Statistical analysis of the results was conducted using Origin 9.0 (OriginLab, USA). Unless otherwise specified, data are shown as the mean ± S.D. (standard deviations) from three independent experiments.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12934-020-01420-2.

Additional file 1. Experimental detail (Table S1, Figure S1) and Additioanl data (Figure S2).

Acknowledgements
We also thank Chengjia Zhang and Nannan Dong from Core Facilities for Life and Environmental Sciences (State Key Laboratory of Microbial Technology, Shandong University) for assistance in microbial fermentation.

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.

Funding
This work was supported by the National Natural Science Foundation of China (31670041), the Grant of National Key R&D Program of China (2019YFA0904900, 2019YFA0904803), Shandong Provincial Funds for Distinguished Young Scientists (JQ 201806), Natural Science Foundation of Shandong Provincial (ZR2018PC008), Key R&D Program of Shandong Provincial (2019GSF107034, 2019GSF107039) and Qilu Young Scholar of Shandong University. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

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.

Author details
[1] State Key Laboratory of Microbial Technology, Shandong University, Qingdao 266237, People's Republic of China. [2] Center for Gene and Immunotherapy, The Second Hospital of Shandong University, Jinan 250033, People's Republic of China. [3] State Key Laboratory of Microbial Metabolism, Joint International Research Laboratory of Metabolic & Developmental Sciences, and School of Life Sciences & Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, People's Republic of China.

Received: 5 May 2020 Accepted: 5 August 2020
Published online: 10 August 2020

References

1. Carvalho F, Prazeres AR, Rivas J. Cheese whey wastewater: characterization and treatment. Sci Total Environ. 2013;445–6:385–96.
2. Domingos JMB, Martinez GA, Scoma A, Fraraccio 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, Dabhi BK, Parmar SC, Aparnathi 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;110:48–68.
5. Asunis F, De Gioannis G, Isipato M, Muntoni A, Polettini A, Pomi R, Rossi A, Spiga D. Control of fermentation duration and pH to orient biochemicals and biofuels production from cheese whey. Bioresour 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, Polyiam 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 corncob 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. PLoS ONE. 2012;7: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 (2*R*,3*R*)-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. Saratale 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. Bioresour Technol. 2016;205:90–6.
18. Um J, Kim DG, Jung MY, Saratale GD, Oh MK. Metabolic engineering of *Enterobacter aerogenes* for 2,3-butanediol production from sugarcane bagasse hydrolysate. Bioresour Technol. 2017;245:1567–74.
19. Saratale RG, Shin HS, Ghodake GS, Kumar G, Oh MK, Saratale GD. Combined effect of inorganic salts with calcium peroxide pretreatment for kenaf core biomass and their utilization for 2,3-butanediol production. Bioresour 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 acetolactate synthase and acetoin reductase in *Klebsiella pneumoniae*. Biotechnol Appl Biochem. 2014;61:707–15.
22. Speckman RA, Collins EB. Microbial production of 2,3-butylene glycol from cheese whey. Appl Environ Microbiol. 1982;43:1216–8.
23. Barrett EL, Collins EB, Hall BJ, Matoi SH. Production of 2,3-butylene 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, Zanaroli 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;11:145.
30. Vishwakarma 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 (3*R*)-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;98:9859–67.
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-sterile 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 D-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 production of 2,3-butanediol from whey permeate. Biotechnol Lett. 1984;6:815–8.
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.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions