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Acetate production from corn stover hydrolysate using recombinant *Escherichia coli* BL21 (DE3) with an EP-bifido pathway

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Abstract

Background Acetate is an important chemical feedstock widely applied in the food, chemical and textile industries. It is now mainly produced from petrochemical materials through chemical processes. Conversion of lignocellulose biomass to acetate by biotechnological pathways is both environmentally beneficial and cost-effective. However, acetate production from carbohydrate in lignocellulose hydrolysate via glycolytic pathways involving pyruvate decarboxylation often suffers from the carbon loss and results in low acetate yield.

Results *Escherichia coli* BL21 (DE3) was confirmed to have high tolerance to acetate in this work. Thus, it was selected from seven laboratory *E. coli* strains for acetate production from lignocellulose hydrolysate. The byproduct-producing genes *frdA*, *ldhA*, and *adhE* in *E. coli* BL21 (DE3) were firstly knocked out to decrease the generation of succinate, lactate, and ethanol. Then, the genes *pfkA* and *edd* were also deleted and bifunctional phosphoketolase and fructose-1,6-bisphosphatase were overexpressed to construct an EP-bifido pathway in *E. coli* BL21 (DE3) to increase the generation of acetate from glucose. The obtained strain *E. coli* 5K/pFF can produce 22.89 g/L acetate from 37.5 g/L glucose with a yield of 0.61 g/g glucose. Finally, the *ptsG* gene in *E. coli* 5K/pFF was also deleted to make the engineered strain *E. coli* 6K/pFF to simultaneously utilize glucose and xylose in lignocellulosic hydrolysates. *E. coli* 6K/pFF can produce 20.09 g/L acetate from corn stover hydrolysate with a yield of 0.52 g/g sugar.

Conclusion The results presented here provide a promising alternative for acetate production with low cost substrate. Besides acetate production, other biotechnological processes might also be developed for other acetyl-CoA derivatives production with lignocellulose hydrolysate through further metabolic engineering of *E. coli* 6K/pFF.

Keywords Acetate, Corn stover hydrolysate, EP-bifido pathway, Carbon catabolite repression, *Escherichia coli*

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Introduction

Acetate is an important platform feedstock expansively applied in the production of bulk chemicals like vinyl acetate, monochloroacetic acid, acetate esters, and acetic anhydride [1–8]. It also has potential applications in the food preservation, adhesives and textile treatments [9–12]. Acetate is now mainly produced via petrochemical pathways including methanol carbonylation, ethylene oxidation, and acetaldehyde oxidation [13–15]. These chemical processes rely on non-renewable feedstocks under harsh reaction conditions, and thus are costly and environmentally unfriendly. Some biochemical processes have also been developed for acetate production from regenerable resources. However, only 10% of the world acetate demand is fulfilled by microbial fermentation up to now [16].

Currently, acetic acid bacteria are regarded as the most efficient acetate producers which mainly produce acetate from ethanol through two oxidization reactions for making vinegar [17–21]. Autotrophic acetogenic bacteria can also utilize syngas to produce acetate using the Wood–Ljungdahl pathway [22, 23]. Besides ethanol and syngas, lignocellulose is termed as another promising renewable resource for biological acetate production. Hydrolysis of lignocellulose results in a hydrolysate with glucose and xylose as the major utilizable sugars [24–26]. Most of the studies toward the conversion of lignocellulose biomass to acetate used heterotrophic acetogens species. Pyruvate produced from glucose and xylose is oxidatively decarboxylated to generate acetyl-CoA, which will be channeled into TCA cycle for energy generation, utilized for different chemicals production or transformed into acetate through phosphate acetyltransferase (Pta) and acetate kinase (AckA), with acetyl-phosphate (AcP) as the key intermediate. However, the pyruvate decarboxylation results in the carbon loss and decreases the theoretical yield of acetate from lignocellulose hydrolysate.

Non-oxidative glycolytic (NOG) pathway was created by Bogorad et al. to prevent CO_2 release during C2 metabolites production from glucose [27]. The key enzyme in the NOG pathway was bifunctional phosphoketolase (Fxpk) [28], which splits fructose-6-phosphate (F6P) into AcP and erythrose-4-phosphate (E4P), as well as xylulose-5-phosphate (X5P) into glyceraldehyde-3-phosphate (G3P) and AcP in "bifid shunt" of bifidobacteria. E4P and G3P can also be transformed into AcP via carbon atom rearrangements, which was pushed by fructose-1,6-bisphosphatase (Fbp), another key enzyme in NOG pathway. Recently, Wang et al. deleted 6-phosphofructokinase (PfkA) and 6-phosphogluconate dehydratase (Edd), overexpressed Fxpk and Fbp, and successfully constructed an EP-bifido pathway combining Embden-Meyerhof-Parnas pathway, Pentose Phosphate pathway and bifid shunt in *Escherichia coli* [29]. The utilization of EP-bifido pathway can result in theoretical maximum production of C2 metabolites from glucose and generate enough NADPH to support growth of *E. coli*. Xylose can also be converted into X5P with endogenous xylose isomerase (XylA) and xylulokinase (XylB) [30–32]. Thus, C2 metabolites production from xylose in *E. coli* may also be improved via the EP-bifido pathway.

In this work, *E. coli* BL21 (DE3) was metabolically engineered to produce acetate from lignocellulose hydrolysate (Fig. 1). Byproduct-producing genes in *E. coli* BL21 (DE3), including *frdA*, *ldhA*, and *adhE*, were knocked out to decrease the generation of succinate, lactate, and ethanol. The EP-bifido pathway was introduced to increase the generation of acetate with glucose. The obtained strain *E. coli* 5K/pFF produced 22.89 g/L acetate from 37.5 g/L glucose with a yield of 0.61 g/g glucose. Then, the glucose-specific transporter $EIICB^{Glc}$ coding gene *ptsG* was also knockout and the strain *E. coli* 6K/pFF produced 20.09 g/L acetate from corn stover hydrolysate.

Materials and methods

Chemicals and reagents

Glucose was supplied by Sinopharm Chemical Reagent Co. (Shanghai, China). DNA Restriction enzymes and T4 DNA ligase were purchased from Thermo Fisher Scientific (Waltham, USA). Polymerase chain reaction (PCR) primers were synthesized by Tsingke Biotechnology Co., Ltd. (Qingdao, China). DNA oligonucleotides and DNA polymerase were obtained from Vazyme Biotechnology Co., Ltd. (Nanjing, China). Corn stover hydrolysate containing glucose (411.0 g/L), xylose (140.8 g/L), and arabinose (5.0 g/L) was a kindly gift from Dacheng Group Co., Ltd. (Changchun, China). All other chemicals are commercially available.

Bacterial strains, plasmids and growth conditions

The bacterial strains and plasmids used in this work are listed in Table 1. *E. coli* BL21 (DE3) and its derivatives were generally cultivated in Lysogeny Broth (LB) medium at 37℃ and 180 rpm. Defined medium with 5 g/L yeast extract (DYM) containing 13.5 g/L KH_2PO_4, 4 g/L $(NH_4)_2HPO_4$, 1.7 g/L citric acid, 1.4 g/L $MgSO_4{\cdot}7H_2O$, 4.5 mg/L vitamin B1, 10% (v/v) trace metals solution and different carbon sources (glucose or corn stover hydrolysate) was used for acetate synthesis [33].

DNA manipulation in *E. coli* BL21 (DE3)

Primers used in this work are listed in Additional file 1: Table S1. Gene knockout of *E. coli* BL21 (DE3) was conducted through CRISPR/Cas9 system containing the pEcCas and pEcgRNA plasmids as described previously [34, 35]. Herein, the knockout of *frdA* was taken as an example. Single-strand oligonucleotides (gRNA-*frdA*-F/gRNA-*frdA*-R) targeting *frdA* were annealed and inserted

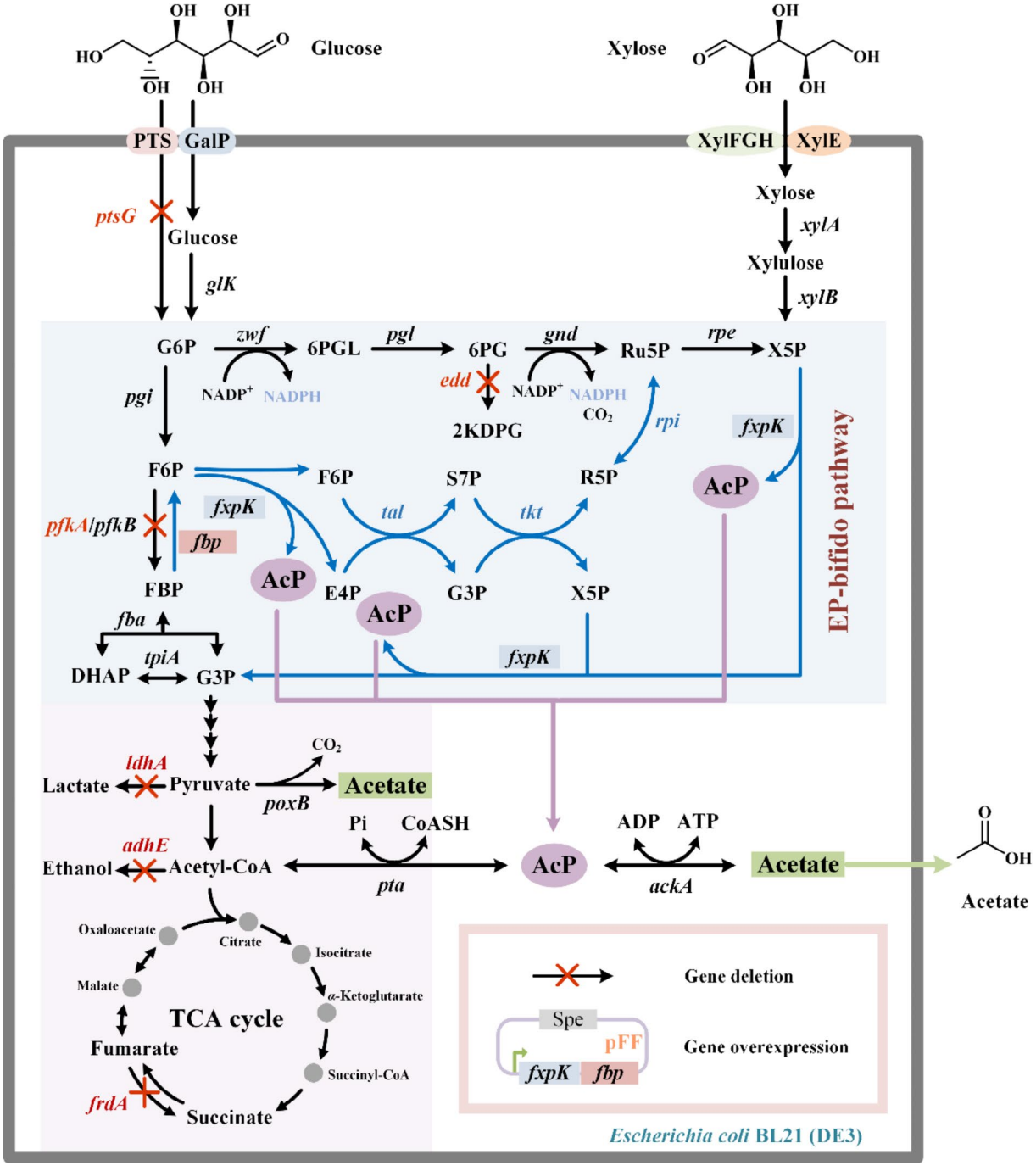

Fig. 1 Metabolic engineering strategies for acetate production from corn stover hydrolysate by *E. coli* BL21 (DE3). PTS, phosphotransferase system; GalP, galactose permease; XylE, xylose transporter; XylFGH, xylose ABC transporter; *ptsG*, glucose-specific transporter $EIICB^{Glc}$ coding gene; *glK*, glucose kinase coding gene; *xylA*, xylose isomerase coding gene; *xylB*, xylulose kinase coding gene; *zwf*, glucose-6-phosphate dehydrogenase coding gene; *pgl*, 6-phosphogluconolactonase coding gene; *gnd*, 6-phosphogluconate dehydrogenase coding gene; *rpe*, ribulose-5-phosphate 3-epimerase coding gene; *pgi*, glucose-6-phosphate isomerase coding gene; *edd*, 6-phosphogluconate dehydratase coding gene; *fxpk*, phosphoketolase coding gene; *fbp*, fructose-1,6-bisphosphatase coding gene; *tal*, transaldolase coding gene; *tkt*, transketolase coding gene; *rpi*, ribulose-5-phosphate epimerase coding gene; *pfkA*/*pfkB*, 6-phosphofructokinase coding gene; *fba*, fructose-1,6-bisphosphate aldolase coding gene; *tpiA*, triosephosphate isomerase coding gene; *ldhA*, lactate dehydrogenase coding gene; *adhE*, acetaldehyde/alcohol dehydrogenase coding gene; *frdA*, fumarate reductase subunit A coding gene; *pta*, phosphate acetyltransferase coding gene; *ackA*, acetate kinase coding gene; *poxB*, pyruvate oxidase coding gene. Abbreviations for metabolites: G6P, glucose-6-phosphate; 6PGL, gluconolactone-6-phosphate; 6PG, 6-phosphogluconate; Ru5P, ribulose-5-phosphate; X5P, xylulose-5-phosphate; 2KDPG, 2-keto-3-deoxy-6-phosphogluconate; F6P, fructose-6-phosphate; FBP, fructose-1,6-bisphosphate; AcP, acetyl-phosphate; E4P, erythrose-4-phosphate; S7P, sedoheptulose-7-phosphate; G3P, glyceraldehyde-3-phosphate; R5P, ribose-5-phosphate; DHAP, dihydroxyacetone phosphate

Table 1 Strains and plasmids used in this study

Strain or plasmid	Description	Source
Strain		
E. coli MG1655	K-12 $F^{-}\lambda^{-}ilvG^{-}rfb$-50 rph^{-1}	Laboratory stock
E. coli BL21 (DE3)	$F^{-}ompT\ hsdSB\ (rB^{-}mB^{-})\ gal\ (\lambda\ c\ I\ 857\ ind1\ sam7\ nin5\ lacUV5\text{-}T7gene1)\ dcm$ (DE3)	Laboratory stock
E. coli W3110	$F^{-}\ \lambda^{-}$ IN (*rrnD-rrnE*)1 rph^{-1}	Laboratory stock
E. coli TOP10	$F^{-}mcrA\ \Delta$(*mrr-hsdRMS-mcrBC*) Φ80*lacZ*ΔM15 Δ*lacX*74 *recA*1 *araD*139 Δ(*araleu*)7697 *galU galK rpsL* (StrR) *endA*1 *nupG*	Laboratory stock
E. coli JM109	*endA*1, *recA*1, *gyrA*96, *thi*, *hsdR*17 (rk^{-}, mk^{+}), *relA*1, *supE*44, λ^{-}, Δ(*lac-proAB*), [F' *traD*36, *proAB*, *laqI*^{q}ZΔM15]	Laboratory stock
E. coli HB101	$F^{-}mcrB\ mrr\ hsdS$20($r_B^{-}m_B^{-}$) *recA*13 *supE*44 *ara*14 *proA*2 *lacY1 galK*2 *xy*15 $\lambda^{-}mtl$1 *rpsL*20(Sm^{r}) *glnV*44 λ^{-}	Laboratory stock
E. coli S17-1	*TpR SmR recA*, *thi*, *pro*, *hsdR-M* + *RP4:2-Tc: Mu: Km Tn*7 *λpir*	Laboratory stock
E. coli DH5*α*	F^{-} φ80*lacZ*ΔM15 Δ(*lacZYA-argF*)U169 *deoR recA*1 *endA*1 *hsdR*17(r_K^{-}, m_K^{+}) *phoA supE*44 $\lambda^{-}thi$-1 *gyrA*96 *relA*1	Invitrogen
E. coli 0K	*E. coli* BL21 (DE3)	Laboratory stock
E. coli 1K	*E. coli* BL21 (DE3) (Δ*frdA*)	This study
E. coli 2K	*E. coli* BL21 (DE3) (Δ*frdA*Δ*ldhA*)	This study
E. coli 3K	*E. coli* BL21 (DE3) (Δ*frdA*Δ*ldhA*Δ*adhE*)	This study
E. coli 5K	*E. coli* BL21 (DE3) (Δ*frdA*Δ*ldhA*Δ*adhE*Δ*pfkA*Δ*edd*)	This study
E. coli 5K/pFF	*E. coli* 5K harboring the plasmid pFF	This study
E. coli 6K	*E. coli* BL21 (DE3) (Δ*frdA*Δ*ldhA*Δ*adhE*Δ*pfkA*Δ*edd*Δ*ptsG*)	This study
E. coli 6K/pFF	*E. coli* 6K harboring the plasmid pFF	This study
Plasmid		
pFF	Inducible expression of the *fxpk* gene from *Bifidobacterium adolescentis* and the *fbp* gene from *E. coli*, Spe^{r}	Wang et al.
pEcCas	Constitutive expression of Cas9 and inducible expression of λ-Red recombination systems, Kan^{r}	Addgene
pEcgRNA	Expression the targeting sgRNA, Spe^{r}	Addgene
pEcgRNA-Δ*frdA*	Targeting *frdA* in *E. coli* BL21 (DE3), Spe^{r}	This study
pEcgRNA-Δ*ldhA*	Targeting *ldhA* in *E. coli* BL21 (DE3), Spe^{r}	This study
pEcgRNA-Δ*adhE*	Targeting *adhE* in *E. coli* BL21 (DE3), Spe^{r}	This study
pEcgRNA-Δ*pfkA*	Targeting *pfkA* in *E. coli* BL21 (DE3), Spe^{r}	This study
pEcgRNA-Δ*edd*	Targeting *edd* in *E. coli* BL21 (DE3), Spe^{r}	This study
pEcgRNA-Δ*ptsG*	Targeting *ptsG* in *E. coli* BL21 (DE3), Spe^{r}	This study

Kan^{r}, kanamycin resistant; Spe^{r}, spectinomycin resistant

into pEcgRNA to obtain plasmid pEcgRNA-Δ*frdA*. To obtain donor DNA, the upstream and downstream homologous arms of *frdA* were amplified from the genome of *E. coli* BL21 (DE3) by uf-*frdA*/ur-*frdA* and df-*frdA*/dr-*frdA*, and connected through recombinant PCR. Then, a mixture containing 250 ng of pEcgRNA-Δ*frdA* and 1000 ng of donor DNA was co-transferred into *E. coli* BL21 (DE3) harboring pEcCas plasmid by electroporation. The correct colonies were screened out from LB plate containing 50 μg/mL spectinomycin and 50 μg/mL kanamycin, and then grown in LB supplemented with 10 mM rhamnose and 10% (w/v) sucrose to induce the curing of the pEcCas and pEcgRNA-Δ*frdA* plasmids. Other *E. coli* BL21 (DE3) mutants were obtained using the same approach as described above.

Batch fermentation in shake flask for acetate production

E. coli BL21 (DE3) and its derivatives were cultured in 300 mL shake flask with 50 mL DYM containing 20 g/L glucose. Spectinomycin at a concentration of 50 μg/mL and isopropyl-*β*-D-1-thiogalactopyranoside (IPTG) at a concentration of 0.1 mM were supplemented into the medium when the engineered *E. coli* strains carrying plasmid pFF. Cultivations were conducted at 37℃ and 180 rpm. The pH of fermentation broth was adjusted to 6.8 before fermentation. Samples were taken periodically and the cell density, concentrations of glucose, acetate, and by-products were determined after an appropriate dilution.

Batch fermentation in a 1-L bioreactor for acetate production

Batch fermentation for acetate production was also conducted in a 1-L bioreactor (Infors AG, Bottmingen, Switzerland) with a working volume of 0.8 L. The seed culture (10%, v/v) was injected into DYM containing different carbon sources and 0.1 mM IPTG. For acetate production by *E. coli* 5K/pFF, DYM containing 40 g/L glucose was used. For acetate production by *E. coli* 6K/pFF, corn stover hydrolysate was added to DYM to make glucose at a concentration of about 30 g/L and xylose at a concentration of about 10 g/L. Batch fermentation was carried out at 37℃, with an airflow rate of 1.2 vvm and a stirrer speed of 400 rpm. The pH of the fermentation broth

was automatically adjusted to 6.8 by the addition of 5 M NaOH via a peristaltic pump.

Analytical methods

Cell growth was measured through detecting the optical density at 600 nm by visible spectrophotometer (V5100H, METASH, China). Concentrations of acetate, lactate, succinate and ethanol in the fermentation broth were detected by high-performance liquid chromatography (HPLC) (Shimadzu LC-20AT) as described previously [29]. The glucose concentration was determined by SBA-40D bioanalyzer (Shandong Academy of Sciences, China).

Results

Tolerance to acetate of *E. coli* BL21 (DE3)

E. coli has fast growth rate, powerful genetic manipulation tools and mature fermentation techniques, and is generally considered as a suitable host for bulk chemicals production [36]. Acetate is an overflow metabolite secreted by *E. coli* to support fast cell growth. However, excessive accumulation of acetate may inhibit the growth of *E. coli* and conversely decreased its final titer and productivity [37–39]. Thus, an ideal recombinant strain for acetate production should be acetate-tolerant. In this study, the tolerance of different laboratory *E. coli* strains including *E. coli* BL21 (DE3), *E. coli* MG1655, *E. coli* W3110, *E. coli* TOP10, *E. coli* JM109, *E. coli* HB101, and *E. coli* S17-1 to acetate was firstly evaluated. These strains were cultured in DYM with 20 g/L glucose and various concentrations (0, 5, 10 g/L) of acetate for 12 h. As shown in Fig. 2A, *E. coli* BL21 (DE3) exhibited higher biomass in DYM with either 5 g/L or 10 g/L acetate than other *E. coli* strains. In addition, this strain can still slightly grow in the presence of 30 g/L acetate (Fig. 2B).

As shown in Fig. 2C, *E. coli* BL21 (DE3) (referred to as *E. coli* 0K) consumed 17.33 g/L glucose within 15 h and accumulated 1.25 g/L succinate, 6.91 g/L lactate, 1.84 g/L ethanol and 5.25 g/L acetate (Fig. 2D). The yield of acetate was 0.30 g/g glucose and it accounted for 34.43%

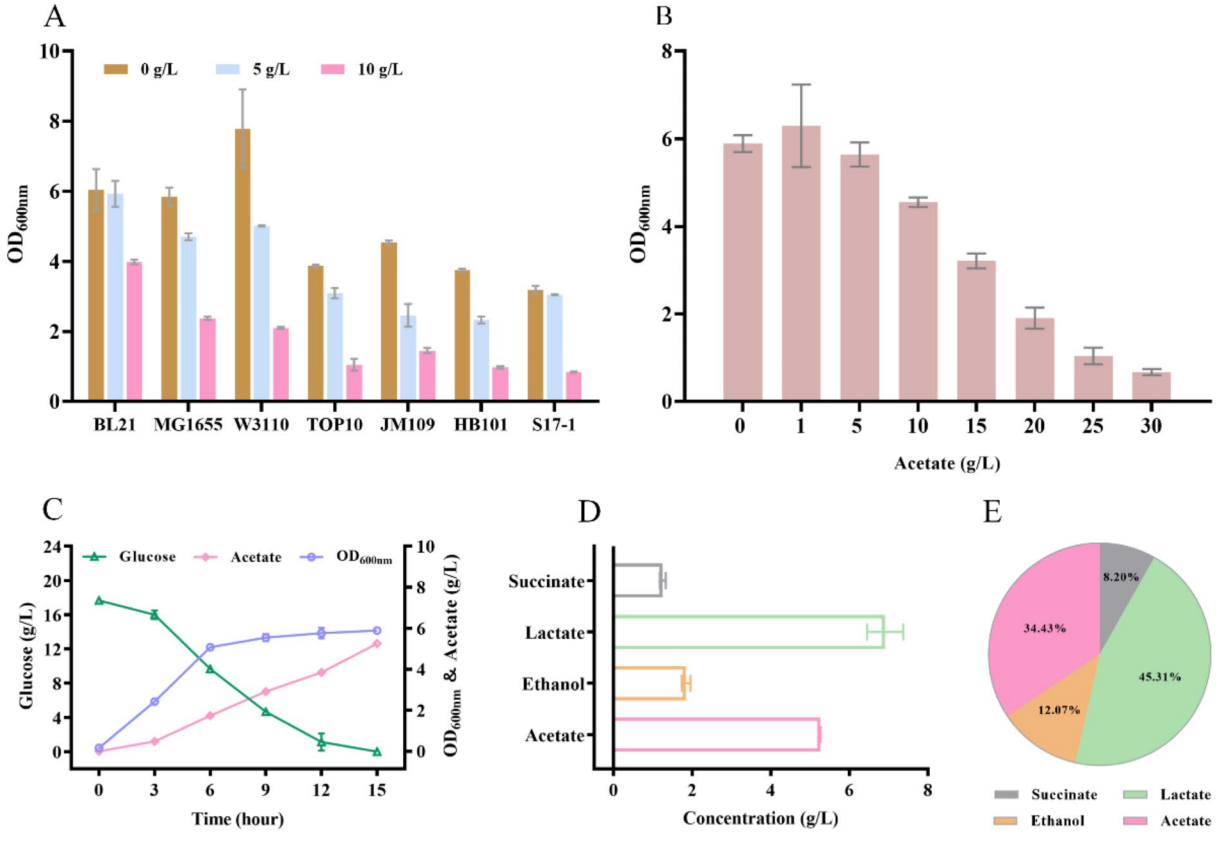

Fig. 2 *E. coli* BL21 (DE3) has the potential for high acetate production. (**A**) The biomass of different laboratory *E. coli* strains after cultivation in DYM with 20 g/L glucose and 0 g/L, 5 g/L, or 10 g/L acetate for 12 h. (**B**) The biomass of *E. coli* BL21 (DE3) after cultivation in DYM with 20 g/L glucose and different concentrations of acetate. *E. coli* BL21 (DE3) was inoculated into DYM with 20 g/L glucose and different concentrations of acetate to reach an initial OD_{600nm} of 0.08. (**C**) Time profiles of biomass, glucose consumption, and acetate production of *E. coli* BL21 (DE3) during shake flask fermentation. (**D**) The concentrations of accumulated metabolites in the fermentation broth by *E. coli* BL21 (DE3). (**E**) The proportion of different metabolites during shake flask fermentation of *E. coli* BL21 (DE3). The data are shown as averages of three independent experiments with standard deviations

(concentration ratio) of the total fermentation products (Fig. 2E). These results indicated that *E. coli* BL21 (DE3) had the potential for high acetate production and was metabolically engineered in subsequent experiments.

Inactivating by-products generation pathways in *E. coli* 0K to enhance acetate production

Besides acetate, succinate (8.20%), lactate (45.31%), and ethanol (12.07%) were also produced by *E. coli* 0K through the mixed acid fermentation pathway [40]. The generation of these by-products would decrease the metabolic flux toward acetate production and restrict the yield of acetate from glucose. Thus, the genes *frdA*, *ldhA*, and *adhE* in *E. coli* 0K responsible for succinate, lactate and ethanol production, were knocked out sequentially to construct the recombinant strains *E. coli* 1K, *E. coli* 2K, and *E. coli* 3K. The cell growth, glucose consumption, and acetate accumulation of *E. coli* 1K, *E. coli* 2K, and *E. coli* 3K in DYM supplemented with glucose were studied (Fig. 3A-C). As expected, deletion of *frdA*, *ldhA*, and *adhE* obviously decreased the generation of succinate, lactate and ethanol, respectively (Fig. 3D-F). The strain *E. coli* 3K produced 7.93 g/L acetate from 18 g/L glucose within 24 h. The proportion of acetate in the total

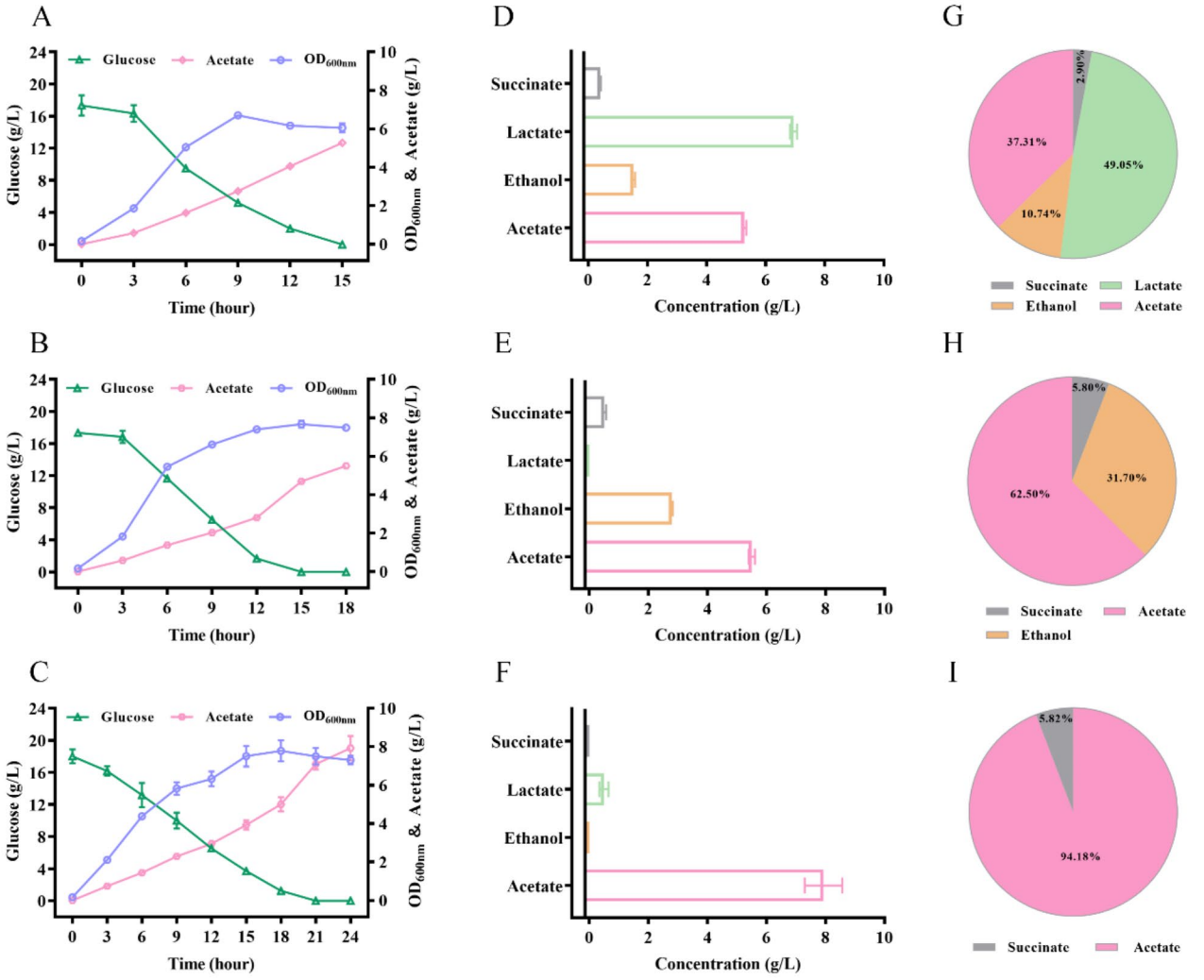

Fig. 3 Inactivating by-products generation pathways to increase acetate production by recombinant *E. coli* BL21 (DE3). (**A**) Time profiles of biomass, glucose consumption, and acetate production by *E. coli* 1K during shake flask fermentation. (**B**) Time profiles of biomass, glucose consumption, and acetate production by *E. coli* 2K during shake flask fermentation. (**C**) Time profiles of biomass, glucose consumption, and acetate production by *E. coli* 3K during shake flask fermentation. (**D**) The concentrations of accumulated metabolites in the fermentation broth by *E. coli* 1K. (**E**) The concentrations of accumulated metabolites in the fermentation broth by *E. coli* 2K. (**F**) The concentrations of accumulated metabolites in the fermentation broth by *E. coli* 3K. (**G**) The proportion of different metabolites during shake flask fermentation of *E. coli* 1K. (**H**) The proportion of different metabolites during shake flask fermentation of *E. coli* 2K. (**I**) The proportion of different metabolites during shake flask fermentation of *E. coli* 3K. The data are shown as averages of three independent experiments with standard deviations

fermentation products increased to 94.18% while the yield of acetate increased to 0.44 g/g glucose (Fig. 3G-I).

Introducing an EP-bifido pathway to enhance acetate production

Acetate is generated from acetyl-CoA by Pta and AckA with AcP as the key intermediate during the mixed acid fermentation of *E. coli*. However, the acetyl-CoA production from pyruvate with pyruvate dehydrogenase complex involves the inevitable decarboxylation reaction and may lead to low yield of acetate. Recently, Wang et al. designed an EP-bifido pathway in *E. coli* by overexpressing Fxpk from *B. adolescentis* and Fbp from *E. coli* and deleting PfkA and Edd [29]. Glucose can be converted into AcP with theoretical maximum yield via the EP-bifido pathway and then transformed into acetate through AckA. Thus, the *pfkA* and *edd* were also deleted in *E. coli* 3K to generate *E. coli* 5K. Then, the plasmid pFF with *fxpk* and *fbp* was introduced into *E. coli* 5K to obtain the recombinant strain *E. coli* 5K/pFF with the EP-bifido pathway.

The cell growth, glucose consumption, and acetate production of *E. coli* 5K and *E. coli* 5K/pFF were studied in DYM medium supplemented with glucose (Fig. 4A and B). As shown in Fig. 4C and D, *E. coli* 5K produced 6.23 g/L acetate from 17.33 g/L glucose, while *E. coli* 5K/pFF produced 10.52 g/L acetate from 14.97 g/L glucose (Additional file 1: Figure S1). The yield of acetate produced by *E. coli* 5K/pFF (0.70 g/g glucose) was slightly higher than theoretical value of acetate produced via pyruvate decarboxylation (0.67 g/g glucose) (Fig. 4F), which may be attributed to introduction of EP-bifido pathway and bioconversion of yeast extract added in the fermentation medium.

Acetate production by *E. coli* 5K/pFF from glucose through batch fermentation in a 1-L bioreactor

Then, the performance of *E. coli* 5K/pFF with the EP-bifido pathway in acetate production was evaluated in a 1-L bioreactor with DYM containing glucose as the substrate. IPTG at a concentration of 0.1 mM was added into the fermentation broth at 6 h to induce the expression of Fxpk and Fbp. As shown in Fig. 5A, the strain *E. coli* 5K/pFF finally consumed 37.5 g/L glucose and produced 22.89 g/L acetate with a yield of 0.61 g/g glucose. Acetate is the major product in the fermentation broth and accounted for 98.67% of the total products (Additional file 1: Figure S2A). Acetate can be transformed to acetyl-CoA and then channeled into TCA cycle under aerobic conditions. Microaerobic or even anaerobic conditions

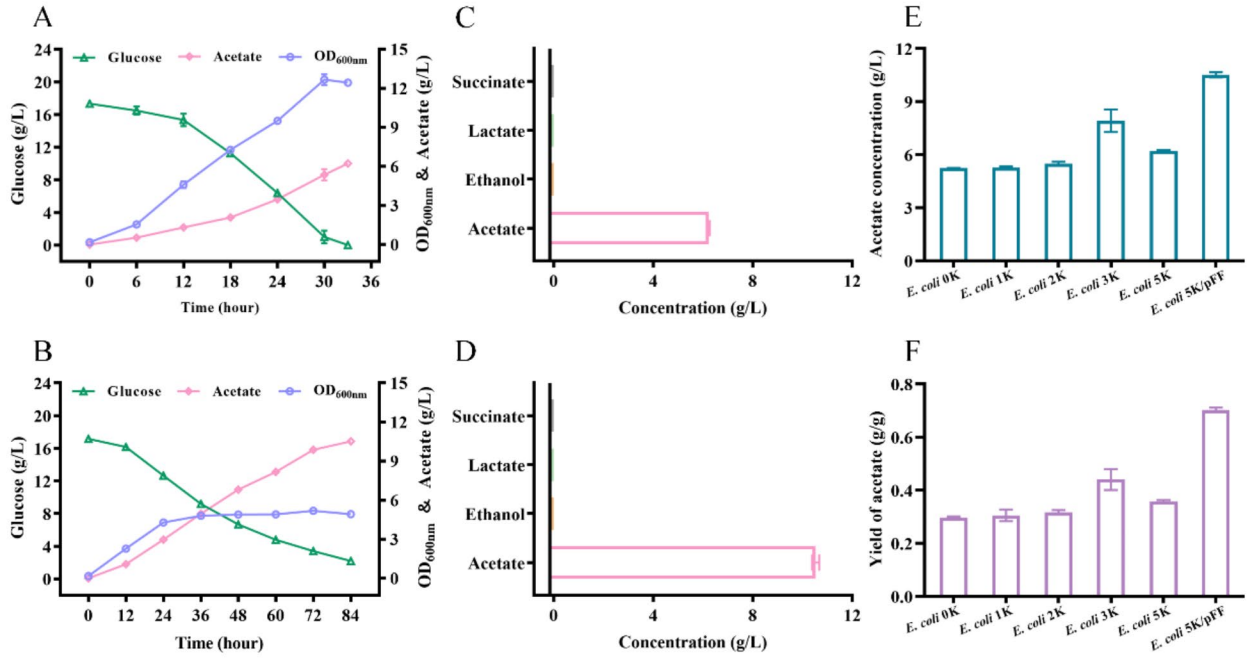

Fig. 4 Introduction of an EP-bifido pathway to increase the yield of acetate. (**A**) Time profiles of biomass, glucose consumption, and acetate production by *E. coli* 5K during shake flask fermentation. (**B**) Time profiles of biomass, glucose consumption, and acetate production by *E. coli* 5K/pFF during shake flask fermentation. (**C**) The concentrations of accumulated metabolites in the fermentation broth by *E. coli* 5K. (**D**) The concentrations of accumulated metabolites in the fermentation broth by *E. coli* 5K/pFF. (**E**) Comparison the concentrations of acetate produced by different recombinant *E. coli* strains from glucose during shake flask fermentation. (**F**) Comparison of the yields of acetate produced by different recombinant *E. coli* strains during shake flask fermentation. The data are shown as averages of three independent experiments with standard deviations

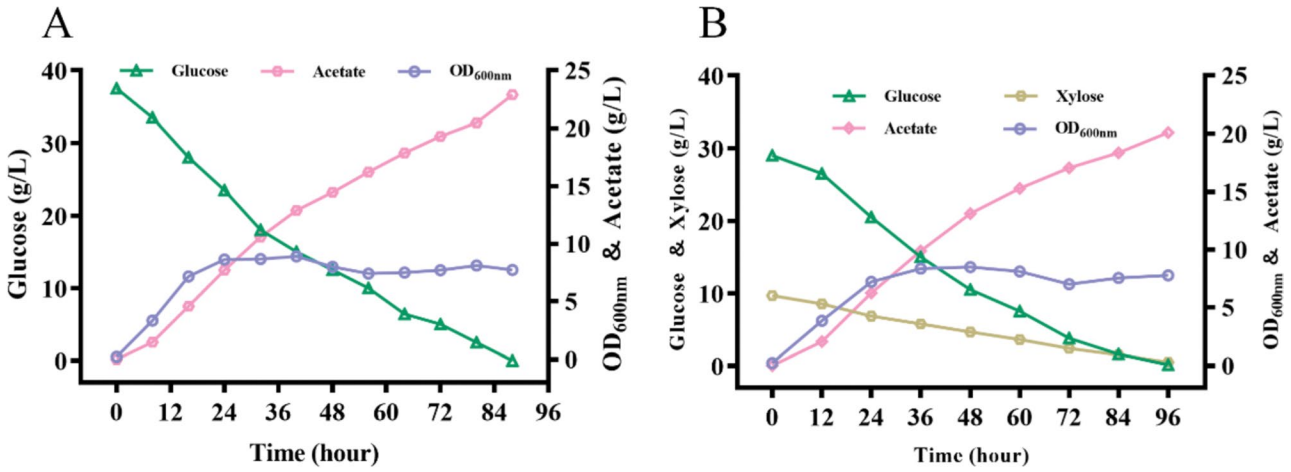

Fig. 5 Batch fermentation of recombinant *E. coli* 5K/pFF for acetate production in a 1-L bioreactor. (**A**) Time profiles of biomass, glucose consumption, and acetate production by *E. coli* 5K/pFF during batch fermentation in a 1-L bioreactor using glucose as the substrate. (**B**) Time profiles of biomass, glucose consumption, and acetate production by *E. coli* 6K/pFF during batch fermentation in a 1-L bioreactor using corn stover hydrolysate as the substrate

are beneficial for fermentative acetate production. The lower acetate yield of *E. coli* 5K/pFF in 1-L bioreactor might be due to high dissolved oxygen level (1.2 vvm and 400 rpm) induced higher biomass generation and TCA cycle flux.

Acetate production by *E. coli* 6K/pFF from corn stover hydrolysate through batch fermentation in a 1-L bioreactor

Corn stover hydrolysate is a promising sustainable feedstock for chemicals manufacture. In this work, the utilization of corn stover hydrolysate as feedstock for acetate production was also investigated. Glucose and xylose are two principal sugars in the corn stover hydrolysate. Due to the presence of carbon catabolite suppression, *E. coli* is theoretically unable to simultaneously metabolize both glucose and xylose [41, 42]. Thus, *ptsG* gene responsible for the carbon catabolite suppression was deleted in the strain *E. coli* 5K. Then, the plasmid pFF was transformed into the obtained strain *E. coli* 6K to generate *E. coli* 6K/pFF. The acetate production by *E. coli* 6K/pFF was also studied in a 1-L bioreactor. The corn stover hydrolysate was added into the DYM to make the glucose at a concentration of about 30 g/L and xylose at a concentration of about 10 g/L. As shown in Fig. 5B, *E. coli* 6K/pFF could simultaneously consume both glucose and xylose in corn stover hydrolysate to produce acetate. Acetate at a concentration of 20.09 g/L was produced from 29 g/L glucose and 9.66 g/L xylose with a yield of 0.52 g/g sugar, accounting for 98.34% of the total products (Additional file 1: Figure S2B).

Acetate is an important feedstock in chemicals production. Different biotechnological processes like ethanol oxidation by *Acetobacter* [18], syngas fermentation via the Wood–Ljungdahl pathway [43], and bioconversion of lignocellulosic substrates have been developed for acetate production (Additional file 1: Table S2). Among the reported biotechnological routes, the group of Ravinder obtained the highest acetate concentration of 30.98 g/L with *Clostridium lentocellum* SG6 from 100 g/L alkali-extracted paddy straw within 14 days [44]. Karekar et al. reported acetate production with corn stover hydrolysate by *Acetobacterium woodii* [45]. However, *A. woodii* can not use xylose for growth and 7.83 g/L acetate was produced within about 20 days from glucose in corn stover hydrolysate. In this study, the genes *frdA*, *ldhA*, and *adhE* in *E. coli* BL21 (DE3) were inactivated to reduce succinate, lactate, and ethanol generation. Then, the *ptsG* gene responsible for the carbon catabolite suppression was knocked out for co-utilization of glucose and xylose. Finally, an EP-bifido pathway was introduced to increase the yield of acetate. Acetate production from corn stover hydrolysate was achieved with high concentration (20.09 g/L), productivity (0.21 g/L/h), and yield (0.52 g/g sugar).

Acetyl-CoA is the precursor of many industrially relevant biochemicals like 3-hydroxypropionate [46], 1-butanol [47], isopropanol [48–52], 1,3-butanediol [53], poly-β-hydroxybutyrate [54], mevalonate [55] and fatty acids [56]. Wang et al. firstly constructed the EP-bifido pathway in *E. coli* [29] and achieved efficient production of mevalonate from glucose through dynamically regulation of glycolysis flux [55]. Recently, Shi et al. also established the EP-bifido pathway in *E. coli* and efficient poly-β-hydroxybutyrate production was realized using xylose as the substrate [57]. Corn is one of the main food crops in major agricultural countries such as China and the United States. Corn stover hydrolysate has been used for carbon sources to produce bioproducts like poly(3-hydroxybutyrate-co-lactate), glycolate, and ethanol [58–60]. In this study, *E. coli* 6K/pFF containing the EP-bifido

pathway was constructed to efficiently produce acetate with AcP as the key intermediate. Besides dephosphorylation to generate acetate by AckA, AcP can be catalyzed into acetyl-CoA by Pta and then utilized for acetyl-CoA derivatives synthesis. *E. coli* 6K/pFF thus may be used as a chassis strain for the production of derivatives of acetyl-CoA from corn stover hydrolysate through deleting AckA and further introducing suitable metabolic pathways.

Conclusion

Herein, the recombination strains *E. coli* 5K/pFF and *E. coli* 6K/pFF were constructed to produce acetate from glucose and corn stover hydrolysate, respectively. The recombination strain *E. coli* 5K/pFF can produce 22.89 g/L acetate from 29 g/L glucose with a yield of 0.61 g/g glucose and *E. coli* 6K/pFF can produce 20.09 g/L acetate from corn stover hydrolysate with a yield of 0.52 g/g sugar. This study provides a promising alternative for both fermentative acetate production and resource utilization of corn stover hydrolysate. In addition to acetate production, the recombinant strain *E. coli* 6K/pFF might also have the potential to be engineered for producing other acetyl-CoA derivatives with lignocellulose hydrolysate.

Abbreviations

AckA	Acetate kinase
Pta	Phosphate acetyltransferase
AcP	Acetyl-phosphate
NOG	Non-oxidative glycolytic
Fxpk	Bifunctional phosphoketolase
Fbp	Fructose-1,6-bisphosphatase
F6P	Fructose-6-phosphate
X5P	Xylulose-5-phosphate
E4P	Erythrose-4-phosphate
G3P	Glyceraldehyde-3-phosphate
PfkA	6-phosphofructokinase
Edd	6-phosphogluconate dehydratase
XylA	Xylose isomerase
XylB	Xylulokinase
IPTG	Isopropyl-β-D-1-thiogalactopyranoside

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12934-024-02575-y.

Supplementary Material 1: Additional file: Figure S1. Comparison of concentrations of the metabolites produced by different recombinant *E. coli* strains from glucose during shake flask fermentation. Figure S2. The proportion of different metabolites produced by *E. coli* 5K/pFF and *E. coli* 6K/pFF during batch fermentation in a 1-L bioreactor. Table S1. The primers used in this study. Table S2. Fermentative acetate production from different lignocellulose biomass.

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Author contributions

Jieni Zhu: Investigation, Writing original draft, Data curation. Wei Liu: Formal analysis, Software, Validation. Leilei Guo: Software, Validation. Xiaoxu Tan: Software, Investigation. Weikang Sun: Supervision, Formal analysis. Hongxu Zhang: Software, Validation. Hui Zhang: Software, Supervision. Wenjia Tian: Supervision. Tianyi Jiang: Supervision. Wensi Meng: Formal analysis. Yidong Liu: Supervision. Zhaoqi Kang: Formal analysis. Chuanjuan Lü: Writing, Supervision, Software, Formal analysis. Chao Gao: Supervision, Writing, Software, Funding acquisition. Ping Xu: Supervision. Cuiqing Ma: Conceptualization, Supervision, Writing, Funding acquisition. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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