Engineering of phosphoenolpyruvate: carbohydrate phosphotransferase system increased acetate assimilation in *Escherichia coli*

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Abstract

Acetate is a potential low-cost carbon source and can be used for microbial production of valuable chemicals in *Escherichia coli*. In this study, separate and simultaneous inactivation of the *ptsG*, *ptsI*, and *ptsP* genes involved in the phosphoenolpyruvate:carbohydrate phosphotransferase system was performed in *E. coli* and the effects on cell growth and acetate assimilation were evaluated. The mutant strain with double deletion of *ptsG* and *ptsP* exhibited faster acetate use than the other mutants. Inactivation of *ptsI* seriously reduced acetate consumption. This work provides a novel engineering target for improving acetate use rates.

Keywords Acetate · Phosphoenolpyruvate:carbohydrate phosphotransferase system · Metabolic engineering · *Escherichia coli*

Introduction

With the advantages of a clear genetic background, convenient metabolic engineering tools and fast growth in cheap media, *Escherichia coli* has been widely used to produce various valuable chemicals. Sugars such as glucose are often selected as the carbon source. However, with concerns about global food shortages, non-food substrates have attracted increasing attention. Acetate can be generated by chemical processes, bacterial fermentation, and anaerobic digestion of cellulosic biomass hydrolysate (Bhatia and Yang 2017; Wu et al. 2016). Common industrial microbes, including *E. coli*, *Corynebacterium glutamicum* and *Saccharomyces cerevisiae*, can use acetate as a carbon source. Accordingly, many valuable chemical compounds, such as polyhydroxyalkanoates, mevalonate, and fatty acids, have been successfully produced from acetate (Xiao et al. 2013; Chen et al.

2018; Xu et al. 2018). In *E. coli*, acetate is mainly used via the AMP-forming acetyl CoA synthetase (encoded by *acs*) or the phosphotransacetylase/acetate kinase pathways (encoded by *pta* and *ackA*) (Fig. 1). Acetate is first converted into acetyl-CoA, and then acetyl-CoA enters the TCA cycle and glyoxylate cycle, providing energy and precursors for cell growth (Noh et al. 2018).

In *E. coli*, the phosphoenolpyruvate:carbohydrate phosphotransferase system (C-PTS) is the main system involved in the transport and phosphorylation of glucose and other sugars (Gosset 2005). This system is composed of non-sugar specific protein components enzyme I (EI), the phosphohistidine carrier protein, and sugar-specific enzymes IIA^{Glc} and IICB^{Glc}, which are encoded by *ptsI*, *ptsH*, *ptsG*, and *ptsP*, respectively (Fig. 2). Apart from glucose transport, the PTS system also takes part in carbon catabolite repression, which is mainly modulated by the phosphorylation state of EI^{Glc}. The *ptsP* gene encodes an EI paralogue designated enzyme I nitrogen (EI^{Ntr}) involved in the nitrogen-PTS (N-PTS), which also functions in carbon and nitrogen metabolism (Ninfa 2011; Rabus et al. 1999). Like in the C-PTS, a phosphate group is transferred from phosphoenolpyruvate to EI^{Ntr}, NPr (encoded by *ptsO/npr*) and EI^{Ntr} (encoded by *ptsN*). However, the N-PTS was evaluated to have only regulatory functions (Jahn et al. 2013). It was reported that the N-PTS could take part in the regulation of K⁺ uptake (Lee

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Materials and methods

Bacterial strains

All strains, plasmids and oligonucleotides used in this study are listed in Tables 1 and 2. *E. coli* MG1655 was selected as the base (parental) strain.

Gene deletion

The genes *ptsI*, *ptsG*, and *ptsP* were knocked out in MG1655 by a one-step inactivation method (Datsenko and Wanner 2000). Primers ptsI-QF/ptsI-QR, ptsG-QF/ptsG-QR, ptsP-QF/ptsP-QR and template plasmids pKD3 and pKD4 were used to obtain linearized DNA flanked by FLP recognition target sites and homologous sequences. The PCR products were purified and electroporated into electrocompetent strains containing plasmid pTKRED. Transformant cells were selected on solid SOB medium containing chloramphenicol (17 mg/L) or kanamycin (25 mg/L). Candidate clones were screened by PCR employing ptsI-JF/ptsI-JR, ptsG-JF/ptsG-JR, and ptsP-JF/ptsP-JR, respectively. The chloramphenicol or kanamycin cassette was removed with the helper plasmid pCP20. All primers were synthesized by TSINGKE Biological Technology (Qingdao, China).

qPCR

Samples for mRNA preparation were taken in the exponential growth phase. Total mRNA of samples was extracted with an RNA Simple Total RNA kit (Tiangen, Beijing, China). Reverse transcription was performed using random 6-mer and oligo dT primers with a PrimeScript RT reagent kit (Takara, Dalian, China). qPCR was carried out with SYBR Premix Ex

Taq II (Takara) following the LightCycler 480 RT-PCR System protocol (Roche, Basel, Switzerland).

Growth conditions

Starter cultures were grown in Luria–Bertani medium (1% tryptone, 0.5% yeast extract and 1% NaCl). For batch fermentation, SMAC medium was used, and 50 mM sodium acetate and 2 g/L of yeast extract were added (Li et al. 2016). A single clone was precultured overnight in 5 mL LB medium at 37 °C with shaking at 250 rpm; 1 mL overnight cells were inoculated into 50 mL SMAC medium for batch fermentation, and flasks were incubated at 37 °C with shaking at 250 rpm.

Analytical methods

Cell growth was monitored by OD₆₀₀ with a spectrophotometer (Metash, China). For analyzing acetate, high-performance liquid chromatography (Thermo Fisher Scientific, USA) was employed with a column of Aminex HPX-87H ion exclusion particles (300 mm × 7.8 mm; Bio-Rad, Hercules, CA, USA). Samples were centrifuged at 12,000 rpm for 5 min and then filtered with a 0.22-μm aqueous membrane. The mobile phase was 5 mM sulfuric acid (in Milli-Q water) with a flow rate of 0.6 mL/min, and the column was maintained at 65 °C.

Results and discussion

As reported by Hara et al., inactivation of *ptsG*, *ptsI*, and *ptsP* involved in the PTS resulted in obviously increased ATP synthesis activity (Hara et al. 2009). As ATP is important for the assimilation of acetate in *E. coli*, the effect of inactivation of *ptsG*, *ptsI*, and *ptsP* was explored. First, these

Table 1 Strains and plasmids used in this study

Strains and plasmids	Genotype	References
Strains		
MG1655	F ⁻ , λ ⁻ , <i>rph-1</i>	Lab stock
MG1655P	MG1655 (Δ <i>ptsP</i>)	This study
MG1655I	MG1655 (Δ <i>ptsI</i>)	This study
MG1655G	MG1655 (Δ <i>ptsG</i>)	This study
MG1655PI	MG1655 (Δ <i>ptsP</i> Δ <i>ptsI</i>)	This study
MG1655IG	MG1655 (Δ <i>ptsI</i> Δ <i>ptsG</i>)	This study
MG1655GP	MG1655 (Δ <i>ptsG</i> Δ <i>ptsP</i>)	This study
MG1655PGI	MG1655 (Δ <i>ptsP</i> Δ <i>ptsG</i> Δ <i>ptsI</i>)	This study
Plasmids		
pKD3	<i>bla</i> , FRT- <i>cat</i> -FRT	Datsenko and Wanner (2000)
pKD4	<i>bla</i> , FRT- <i>kan</i> -FRT	Datsenko and Wanner (2000)
pTKRED	<i>spc</i> , helper plasmid	Kuhlman and Cox (2010)
pCP20	<i>bla</i> and <i>cat</i> , helper plasmid	Cherepanov and Wackernagel (1995)

Table 2 Primers used in this study

Name	Sequence (5'–3')
ptsI-QF	GCGGAACCTCGAGTAATTTCCCGGGTTCTTTTAAAAATCAGTCACAAGTAAGGTAGGGTTGTGTAGGCTGGAGCTGCTTC
ptsI-QR	GTTTATCGAACAAACCCATGATCTTCTCCTAAGCAGTAAATTGGGCCGCATCTCGTGGAATGGGAATTAGCCATGGTCC
ptsI-JF	GATTCAGCCTGTCGGAACCTGGTATTT
ptsI-JR	AGCCTTCGCCTTTTCAGTTCAACGGTGTCG
ptsG-QF	ACGTAAAAAAGCACCCATACTCAGGAGCACTCTCAATTGTGTAGGCTGGAGCTGCTTC
ptsG-QR	AGCCATCTGGCTGCCCTAGTCTCCCCAACGCTTACGGAATGGGAATTAGCCATGGTCC
ptsG-JF	CCTGTACACGGCGAGGCTCT
ptsG-JR	AATAACACCTGTAAAAAAGGCAGCC
ptsP-QF	ATGCTCACTCGCCTGCGCGAAATAGTCGAAAAGGTAGCCAGCGCACCCAGCCTGAATGAGTGTAGGCTGGAGCTGCTTC
ptsP-QR	CTATAACCTCCGCGAATCAGCCCGCCCATGCCGCGACGCTCCATAAAGGCTGCAACCTATGGGAATTAGCCATGGTCC
ptsP-JF	GTTTGACGGCTGGCGATGGGTAAGT
ptsP-JR	GATAGCTACTGGTCATCTGTAC
qRT-PCR	
rrsG-QF	GGTGTAGCGGTGAAATGCGTAG
rrsG-QR	TCAAGGGCACAACCTCCAAGTC
ackA-QF	GATGAGTCTGTATTTCAGGGTA
ackA-QR	GCATAGTCTGGTGGAACG
pta-QF	CTGAACCGCTGAAAATGAG
pta-QR	CCCTGAGACATAACGAAGACGA
actP-QF	GATTATTATGTTCTGATTTC
actP-QR	CGCCTGCGGTGTAGTAGTC
acs-QF	TACGAGGACGGCACGCTGAATCT
acs-QR	AGCAGGGTATTGGCGAAG

three genes were knocked out individually in *E. coli* strain MG1655, and then batch fermentation was performed. As Fig. 3a and Table S1 shows, the growth of all three mutants was similar and a littler poorer than that of the parental strain, indicated by the OD₆₀₀ values (4.98, 4.80, and 4.85, respectively, vs 5.50). In 2015, Liang et al. carried out a comparison between mutant strains with individual component deletions in the PTS and wild-type strain W3110 (Liang et al. 2015). When glucose was used as the carbon source, the mutant strains showed various lag periods. In particular, the lag time of W3110I was about 38 h, while the final biomass was 45.42% higher than that of the wild-type strain W3110. However, as the PTS is not directly involved in acetate consumption, deletion of *ptsG*, *ptsI*, or *ptsP* may not result in an obvious lag time when acetate is used as the sole carbon source. Nevertheless, the final OD₆₀₀ values of MG1655G, MG1655I, MG1655P were all less than that of the wild-type strain MG1655. In 2008, Sigala et al. found that PTS mutant strains had lower transcription levels of important gluconeogenic genes when acetate was selected as the sole carbon source (Sigala et al. 2009), which may lead to poorer growth of PTS[−] derivatives than wild-type *E. coli*.

The acetate consumption rate of the PTS knockout strains was then detected (Fig. 3b and Table S1). MG1655G showed slightly slower acetate use than MG1655. However, the acetate consumption of MG1655I was obviously lower than that of wild-type MG1655 and the other two mutant strains. During the first 6 h, a lag time was observed and only a little acetate was consumed for MG1655. Surprisingly, MG1655P exhibited faster acetate assimilation than MG1655, indicating the positive outcome of deleting *ptsP* (encoding EI^{Ntr}) on the use of acetate. When EI^{Ntr} is inactivated, the phosphorylation state of EIIA^{Glc}, encoded by *crr*, will be changed (Jahn et al. 2013). It was reported that the phosphorylation level of EIIA^{Glc} regulates various signal transduction process and central metabolism by indirectly controlling the cAMP:CRP ratio (Gosset 2005). Accordingly, deletion of *ptsP* may increase the cAMP/CRP level, thus increasing transcription of acetyl CoA synthetase Acs. In contrast, it was reported that the deletion of *ptsG* and *ptsI* in *E. coli* leads to low cAMP production (Sigala et al. 2009). As a result, the transcription of the *acs* gene may be decreased, and, in turn, the acetate assimilation rate decreased.

Fig. 3 **a** Growth curves of *E. coli* strains MG1655, MG1655P, MG1655G and MG1655I; **b** acetate consumption by MG1655, MG1655P, MG1655G and MG1655I; **c** growth curves of strains MG1655PI, MG1655IG, MG1655GP and MG1655PGI; **d** ace-

tate consumption by MG1655PI, MG1655IG, MG1655GP and MG1655PGI. Error bars represent standard deviations from three replicate fermentations

Another possible explanation for the different acetate use rates of PTS mutant strains could be different transcription levels of acetate assimilation genes. To test this hypothesis, qPCR was carried out for our mutant strains, and four genes related to acetate use—*acs*, *ackA*, *pta* and *actP*—were selected for analysis. As Fig. 4 shows, transcription of all four genes was downregulated in MG1655I compared with MG1655, which was consistent with the acetate assimilation of this strain being the lowest we observed. The acetate use rate of MG1655G was a little lower than that of MG1655 but higher than that of MG1655I. This was also consistent with the levels of transcription of the four genes. Interestingly, the transcription of *actP*, encoding an acetate permease, was increased to 1.91-fold in MG1655P compared with MG1655, and the transcription of *ackA*, *pta* and *acs* encoding the phosphotransacetylase/acetate kinase pathway were also

increased (1.36, 1.42 and 1.56-fold to wild MG1655). The enhanced transcription of *actP*, *ackA*, *pta* and *acs* may be the reason for improved acetate assimilation in MG1655P.

Mutants with double deletions of *ptsG*, *ptsI*, and *ptsP* were then constructed to further elucidate the effect on acetate assimilation. In batch cultivation, these three mutants, MG1655PI, MG1655GP, and MG1655IG, showed similar growth compared to the mutants with deletions of a single gene (Fig. 3c and Table S1). The maximum OD₆₀₀ values of the three double mutants were 4.82, 4.86, and 4.91, respectively, and these results further indicated there was little effect on cell growth when these three genes were deleted. Then, the acetate consumption was analyzed for the three double mutant strains. As shown in Fig. 3d and Table S1, MG1655PG exhibited a faster acetate assimilation rate than any of the other mutant strains or wild-type MG1655. *ptsG*

Fig. 4 Gene expression in strains MG1655P, MG1655I and MG1655G relative to the parental strain MG1655. *rrsG* transcripts were selected as the standard and three replicates were performed for each sample. The error bars indicate standard deviations

encodes the sugar-specific enzyme $\text{EIICB}^{\text{Glc}}$, which is responsible for transferring a phosphoryl group from $\text{EIICB}^{\text{Glc}}$ to glucose. It may be that the deletion of *ptsG* from MG1655P further changed the proportion of phosphorylated $\text{EIICB}^{\text{Glc}}$ and accelerated acetate use. Notably, for MG1655PI and MG1655IG, a lag phase was observed for acetate use but not for cell growth. It was previously reported that a basal activity of adenylate cyclase, responsible for the generation of cAMP from ATP, was retained even if its regulator-PTS component was inactivated (Bettenbrock et al. 2007). However, that basal level of cAMP was not enough to activate the maximum expression of acetate assimilation-related genes (Sigala et al. 2009). As a result, the generation of cAMP and transcription of *acs*, *ackA*, *pta* and *actP* must be activated by other factor(s). The induction time may lead to the lag time for acetate use by strains MG1655PI and MG1655IG. In the medium used in the present study, 2 g/L yeast extract was supplemented, and the relatively abundant nutrients may be the reason why no lag time was observed for the growth of these two strains.

Finally, MG1655PGI with triple gene deletion was constructed, and batch fermentation was performed (Fig. 4c, d). Consistent with expectations, it exhibited similar behavior to other mutant strains with *ptsI* deletion. In particular, the acetate assimilation was even lower than those of MG1655I, MG1655PI, and MG1655IG.

In summary, the effects of knocking out *ptsG*, *ptsI*, and *ptsP* individually and simultaneously were explored. Mutant

strains with *ptsG* and *ptsP* inactivation exhibited rapid acetate use, while the acetate use of mutants with *ptsI* deletion was slower than that of the parental strain MG1655. Further experiments should be performed to investigate the detailed regulatory mechanism of the PTS system on acetate use. Nevertheless, this work provides a novel strategy for engineering recombinant strains when acetate is selected as the carbon source.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

- Bettenbrock K, Sauter T, Jahreis K, Kremling A, Lengeler JW, Gilles ED (2007) Correlation between growth rates, $\text{EIICB}^{\text{Glc}}$ phosphorylation, and intracellular cyclic AMP levels in *Escherichia coli* K-12. *J Bacteriol* 189(19):6891–6900
- Bhatia SK, Yang YH (2017) Microbial production of volatile fatty acids: current status and future perspectives. *Rev Environ Sci Biotechnol* 16(6):1–19

- Chen J, Li W, Zhang ZZ, Tan TW, Li ZJ (2018) Metabolic engineering of *Escherichia coli* for the synthesis of polyhydroxyalkanoates using acetate as a main carbon source. *Microb Cell Fact* 17(1):102
- Cherepanov PP, Wackernagel W (1995) Gene disruption in *Escherichia coli*: TcR and KmR cassettes with the option of Flp-catalyzed excision of the antibiotic-resistance determinant. *Gene* 158(1):9–14
- Datsenko KA, Wanner BL (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci USA* 97(12):6640–6645
- Gosset G (2005) Improvement of *Escherichia coli* production strains by modification of the phosphoenolpyruvate:sugar phosphotransferase system. *Microb Cell Fact* 4(1):14
- Hara KY, Shimodate N, Ito M, Baba T, Mori H, Mori H (2009) Systematic genome-wide scanning for genes involved in ATP generation in *Escherichia coli*. *Metab Eng* 11(1):1–7
- Hayden JD, Ades SE (2008) The extracytoplasmic stress factor, sigmaE, is required to maintain cell envelope integrity in *Escherichia coli*. *PLoS One* 3(2):e1573
- Jahn S, Haverkorn van Rijsewijk BR, Sauer U, Bettenbrock K (2013) A role for EIIA(Ntr) in controlling fluxes in the central metabolism of *E. coli* K12. *Biochim Biophys Acta* 1833(12):2879–2889
- Kuhlman TE, Cox EC (2010) Site-specific chromosomal integration of large synthetic constructs. *Nucleic Acids Res* 38(6):e92
- Lee CR, Cho SH, Yoon MJ, Peterkofsky A, Seok YJ (2007) *Escherichia coli* enzyme IIA^{Ntr} regulates the K⁺ transporter TrkA. *Proc Natl Acad Sci USA* 104(10):4124–4129
- Li Y, Huang B, Wu H, Li Z, Ye Q, Zhang YP (2016) Production of succinate from acetate by metabolically engineered *Escherichia coli*. *ACS Synth Biol* 5(11):1299–1307
- Liang Q, Zhang F, Li Y, Zhang X, Li J, Yang P, Qi Q (2015) Comparison of individual component deletions in a glucose-specific phosphotransferase system revealed their different applications. *Sci Rep* 5:13200
- Luttmann D, Gopel Y, Gorke B (2012) The phosphotransferase protein EIIA(Ntr) modulates the phosphate starvation response through interaction with histidine kinase PhoR in *Escherichia coli*. *Mol Microbiol* 86(1):96–110
- Ninfa AJ (2011) Unnecessary signaling: poorly named? *J Bacteriol* 193(18):4571–4573
- Noh MH, Lim HG, Woo SH, Song J, Jung GY (2018) Production of itaconic acid from acetate by engineering acid-tolerant *Escherichia coli* W. *Biotechnol Bioeng* 115(3):729–738
- Rabus R, Reizer J, Paulsen I, Saier MH Jr (1999) Enzyme I^{Ntr} from *Escherichia coli*. A novel enzyme of the phosphoenolpyruvate-dependent phosphotransferase system exhibiting strict specificity for its phosphoryl acceptor, NPr. *J Biol Chem* 274(37):26185–26191
- Sigala JC, Flores S, Flores N, Aguilar C, de Anda R, Gosset G, Bolivar F (2009) Acetate metabolism in *Escherichia coli* strains lacking phosphoenolpyruvate: carbohydrate phosphotransferase system; evidence of carbon recycling strategies and futile cycles. *J Mol Microbiol Biotechnol* 16(3–4):224–235
- Wu QL, Guo WQ, Zheng HS, Luo HC, Feng XC, Yin RL, Ren NQ (2016) Enhancement of volatile fatty acid production by co-fermentation of food waste and excess sludge without pH control: the mechanism and microbial community analyses. *Bioresour Technol* 216:653–660
- Xiao Y, Ruan Z, Liu Z, Wu SG, Varman AM, Liu Y, Tang YJ (2013) Engineering *Escherichia coli* to convert acetic acid to free fatty acids. *Biochem Eng J* 76(28):60–69
- Xu X, Xie M, Zhao Q, Xian M, Liu H (2018) Microbial production of mevalonate by recombinant *Escherichia coli* using acetic acid as a carbon source. *Bioengineered* 9(1):116–123