Different performance of *Escherichia coli* mutants with defects in the phosphoenolpyruvate: carbohydrate phosphotransferase system under low glucose condition

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Abstract

In *Escherichia coli*, the transport and phosphorylation of glucose is mainly accomplished by the phosphoenolpyruvate-dependent glucose-specific phosphotransferase system (PTS^{Glc}), which is, therefore, frequently selected as a target for engineering to increase the intracellular level of phosphoenolpyruvate. Here we characterized the effects of a low glucose concentration on the growth, glucose consumption, and acetate secretion of individual strains with a single PTS^{Glc} mutation. We found that most mutants accumulated similar amounts of biomass, consumed glucose at lower rates, and secreted less acetate compared with the wild-type parental strain. The exception was the growth-impaired strain MG1655I harboring a *ptsI* deletion. In summary, the fermentation performance of mutant strains under 5 g/L glucose was obviously different with those strains under 20 g/L glucose. This study is a good complement to the knowledge of PTS^{Glc} in *E. coli* and indicates that engineering the components of PTS^{Glc} should be carefully optimized, particularly during fermentation in the presence of low concentrations of glucose.

Keywords *E. coli* · PTS^{Glc} · Glucose · Acetate

Introduction

With the advantage of clear genetic background, simple gene manipulation and fast growth in cheap media, various valuable compounds have been produced by engineered *Escherichia coli* (Ding et al. 2017; Gu et al. 2012; Zhang et al. 2016). Generally, glucose, xylose, or fatty acids serve as sole carbon and energy sources for fermentations employing *E. coli* (Chen et al. 2018; Guo et al. 2018; Li et al. 2018). Among these compounds, glucose affords the highest growth rate and is widely employed as raw material in industrial fermentations.

Escherichia coli employs the phosphoenolpyruvate: carbohydrate phosphotransferase system (PTS) as its

main system to transport and phosphorylate of glucose. This system comprises non-sugar specific protein components enzyme I (EI), the phosphohistidine carrier protein (HPr), and sugar-specific enzymes, such as glucose-specific EIIABC^{Glc} group and mannose-specific EIIABCD^{Man} group (Fig. 1). When glucose is present, a phosphoryl group is transferred from phosphoenolpyruvate (PEP) to the sugar-specific enzymes EIIA^{Glc} and EIIB^{Glc} via HPr and EI components (Gosset 2005). Glucose, which can be transported by the integral membrane protein permease EIIC^{Glc}, is then phosphorylated by EIIB^{Glc} (Fig. 1). When one glucose molecule was assimilated into *E. coli* cells by PTS^{Glc}, one molecule of PEP will be consumed. Accordingly, PTS components are often selected as targets to improve the intracellular level of PEP in *E. coli* (Liu et al. 2017; Wei et al. 2016). In addition, the mannose-specific EIIABCD^{Man} complex encoded by *manXYZ* operon can also transport glucose when the *ptsG* gene encoding EIIBC^{Glc} is inactivated (Curtis and Epstein 1975).

Apart from PTS, glucose can also be transported into the cytoplasm by galactose internalization systems, such as *galP* encoding a low-affinity galactose: H⁺ symporter. When PTS components are deleted, GalP can function as a glucose

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Fig. 1 The phosphoenolpyruvate-dependent glucose-specific phosphotransferase system and other glucose transport systems of *Escherichia coli*

transporter even at relatively high glucose concentrations. In addition, the Mgl system encoded by *mglABC* operon is also involved in glucose import only when glucose is present at a very low concentration (Death and Ferenci 1994). Notably, glucose internalized by GalP or the Mgl systems must be phosphorylated by glucokinase Glk to enter into the glycolytic pathway.

When a sufficient amount of glucose is supplied under aerobic conditions, wild-type *E. coli* consumes substrates at a high rate, which contributes to its rapid growth rate. Accordingly, a metabolic imbalance between glycolysis and the tricarboxylic acid (TCA) cycle usually occurs, generating excess acetyl-coenzyme A that is directed into the biosynthetic pathways of byproducts such as acetate, ethanol, and lactate (Han et al. 1992; Majewski and Domach 1990). Among them, acetate is the main byproduct for *E. coli* fermentation. Acetate is a toxic organic acid whose accumulation in culture media inhibits the growth of *E. coli* and reduces the yields of target compounds. In addition, the secretion of acetate is a waste of glucose (Shiloach et al.

1996). To solve this problem, a relatively low concentration of glucose is often maintained in fed-batch fermentation.

We previously constructed a recombinant *E. coli* strain, designated GPT1002, which accumulates 1.70 g/L L-tryptophan in a 36-h batch fermentation (Gu et al. 2012). To further reduce the secretion of acetate, a lower concentration of glucose (5 g/L) was employed, but with no significant beneficial effect. In GPT1002, *ptsG* encoding the IICB^{Glc} was deleted to save PEP, which possibly led to poor strain performance. To explore the influence of PTS^{Glc} defects on growth, glucose consumption, and acetate secretion by *E. coli* in batch fermentations under low concentrations of glucose, here we generated and tested a collection of mutants with single defects in genes encoding the components of the PTS^{Glc} system.

Materials and methods

Bacterial strains

Escherichia coli strains, plasmids, and primers are listed in Tables 1 and 2, respectively. *E. coli* MG1655 was used for constructing PTS mutant strains.

Gene deletion

We used a one-step inactivation method to individually delete the *ptsH*, *ptsG*, *ptsI*, and *crr* genes, which encode components of the PTS glucose transport system, from MG1655 to generate MG1655H, MG1655G, MG1655I, and MG1655C (Datsenko and Wanner 2000). The primers ptsG-QF/ptsG-QR, ptsH-QF/ptsH-QR, ptsI-QF/ptsI-QR, and crr-QF/crr-QR and template plasmids pKD3 and pKD4 were used to obtain linearized DNA flanked by FLP recombinase recognition target sites and homologous sequences, respectively. Primers were synthesized by TSINGKE Biological Technology (Qingdao, China). PCR

Table 1 Strains and plasmids used in this study

Strains and plasmids	Genotype	References
Strains		
MG1655	F ⁻ , λ ⁻ , <i>rph-1</i>	Lab stock
MG1655G	MG1655 ($\Delta ptsG$)	This study
MG1655I	MG1655 ($\Delta ptsI$)	This study
MG1655H	MG1655 ($\Delta ptsH$)	This study
MG1655C	MG1655 (Δcrr)	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	GCGGAAC TCGAGTAATTTCCCGGGTCTTTTAAAAATCAGTCACAAGTAAGGTAGGG TTGTGTAGGCTGGAGCTGCTTC
ptsI-QR	GTTTATCGAACAAACCCATGATCTTCTCTAAGCAGTAAATTGGGCCGCATCTCGTG GAATGGGAATTAGCCATGGTCC
ptsI-JF	GATTCAGCCTGTCGGAAC TGGTATT
ptsI-JR	AGCCTTCGCCTTTCAGTTCAACGGTGTCG
ptsG-QF	ACGTAAAAAAGCACCCATACTCAGGAGCACTCTCAATTGTGTAGGCTGGAGCTGCTTC
ptsG-QR	AGCCATCTGGCTGCCTTAGTCTCCCCAACGTCTTACGGAATGGGAATTAGCCATGGTCC
ptsG-JF	CCTGTACACGGCGAGGCTCT
ptsG-JR	AATAACACCTGTAAAAAAGGCAGCC
ptsH-QF	GGCTTCACTTCTGAAATTACTGTGACTTCCAACGGCAAAGTGTAGGCTGGAGCTGCTTC
ptsH-QR	GGTACCTTGAGTCAGGCCAGAGTCTGCAGTTTAAACAGATGGGAATTAGCCATGGTCC
ptsH-JF	ATGTTCCAGCAAGAAGTTAC
ptsH-JR	TTACTCGAGTTCCGCCATCA
crr-QF	TTTTTGCGGAAAAAATCGTTGGTGATGGTATTGCTATCAGTGTAGGCTGGAGCTGCTTC
crr-QR	GCGGCAGATCAAATTCAATGACAGTATCGCCAACTTTCAATGGGAATTAGCCATGGTCC
crr-JF	ATGGGTTTGTTTCGATAAACT
crr-JR	TTACTTCTTGATGCGGATAA

products were purified and used to electroporate MG1655 strains harboring the plasmid pTKRED. Candidate clones were screened using PCR with ptsG-JF/ptsG-JR, ptsH-JF/ptsH-JR, ptsI-JF/ptsI-JR, or crr-JF/crr-JR. The chloramphenicol or kanamycin cassette was removed using the helper plasmid pCP20.

Fermentation conditions

Batch fermentations were performed using mineral AM1 medium (Majewski and Domach 1990) supplemented with 1 g/L yeast extract and 5 g/L glucose. A single clone was cultured overnight in 5 mL of LB medium at 37 °C with shaking (250 rpm), 1 mL of the culture was used to inoculate 50 mL of AM1 medium, and flasks were incubated overnight at 37 °C with shaking (250 rpm).

Analytical methods

Cell growth (OD_{600}) was measured using a spectrophotometer (Metash, China). Glucose and acetate concentrations were measured using high-performance liquid chromatography (Thermo Fisher Scientific, USA) with a column containing 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 through a 0.22-μm aqueous membrane.

Results and discussion

Deletion of genes encoding components of the PTS^{Glc} system alters cell growth and glucose consumption

The assimilation of glucose by *E. coli* via the PTS^{Glc} system is mainly accomplished by EI, HPr, IIA^{Glc} , IIB^{Glc} - IIC^{Glc} . To investigate the effect of each on substrate consumption and cell growth in the presence of 5 g/L glucose, we deleted the corresponding genes from wild-type *E. coli* MG1655 to generate the mutants designated MG1655I, MG1655H, MG1655C, and MG1655G (Table 1). In aerobic batch fermentation with 5 g/L glucose, these mutants grew at rates similar to that of the wild-type, except for MG1655I. MG1655I exhibited weak growth (maximum OD_{600} = 3.24), which was 59.1% lower compared with that of the wild-type strain (Fig. 2). However, no obvious lag phase was exhibited for MG1655I in 5 g/L glucose. In contrast, when 20 g/L glucose was employed, MG1655I showed a 9-h lag phase and only a litter higher final biomass accumulation (Fig. S1).

Consistent with the growth curves, MG1655I assimilated glucose more slowly compared with the wild-type strain and the other mutants (Fig. 3). After 24 h of culture, 0.84 g/L glucose remained. In contrast, MG1655 completely consumed 5 g/L glucose within 9 h. In addition, inactivation of *ptsH*, *ptsG*, and *crr* reduced glucose consumption rates by 16.1%, 36.9%, and 29.2%, respectively.

Fig. 2 Growth of mutant and wild-type strains. 5 g/L glucose was selected as carbon source. The error bars represent the standard deviations from three replicate fermentations

Fig. 3 Glucose consumption of the mutant and wild-type strains. 5 g/L glucose was selected as carbon source. The error bars represent the standard deviations of three replicate fermentations

The differences in performance of the mutant strains may be explained by different induction times and the activity of an alternative glucose transport system after deleting the genes encoding PTS^{Glc} components. *E. coli* can utilize the MglABC, GalP, and PTS^{Man} systems to transport glucose (Fig. 1). It is noteworthy that all the PTS system share EI and HPr transporter proteins. Therefore, strains MG1655I and MG1655H may employ a non-PTS transport system with a relatively long induction time. However, MG1655H exhibited similar growth and glucose consumption rates compared with those of wild-type MG1655. In contrast, when 20 g/L glucose was supplied, MG1655H exhibited an obviously long lag time than wild-type strain (Fig. S2).

Maybe recruiting of few other glucose transport proteins was enough for MG1655H to assimilate 5 g/L glucose. This phenomenon was also reported for W3110 mutant strain with deletion of *ptsH* (Liang et al. 2015).

Otherwise, the glucose consumption rate of MG1655I was significantly lower compared with those of the other four strains, irrespective of whether the concentration of glucose is 5 g/L or 20 g/L (Fig. 3 and Fig. S2). This phenomenon indicated that the protein EI plays a crucial role in physiological signaling (Cases et al. 2007) and is more sensitive to glucose induction. In PTS^{Glc} mediated glucose transport, EI autophosphorylation and the subsequent phosphor transfer reaction are the limiting steps, which further exacerbated the glucose assimilation defect of MG1655I (Gabor et al. 2011). In contrast, M1655C and M1655G could recruit both PTS system and non-PTS system for glucose internalization. Therefore, the glucose consumption and growth rates of these strains and wild-type MG1655 were similar.

Deletions of individual PTS^{Glc} components exhibited different acetate secretion

Acetate, which is an undesirable byproduct of metabolic engineering, is usually accumulated by *E. coli* because of metabolic overflow (Peebo et al. 2014). As shown in Fig. 4, all the four mutants exhibited an obvious reduction of acetate secretion. The maximum acetate accumulation of MG1655, MG1655H, MG1655C, and MG1655G were 1.24, 0.75, 0.79, and 0.75 g/L, respectively, and acetate was undetectable in cultures of MG1655I. These findings indicate diminished metabolic overflow in these mutants. Compared with the performance of all five strains at 20 g/L glucose (Fig. S3), the absolute value of acetate secretion was only a litter lower at 5 g/L. This suggests only decreasing the supplement

Fig. 4 Acetate secretion of the mutant and wild-type strains. 5 g/L glucose was selected as carbon source. The error bars represent the standard deviations of three replicate fermentations

concentration of glucose may be not effective for decreasing the secretion of acetate, when 5–20 g/L glucose was utilized.

In conclusion, this paper described the effect of single PTS^{Glc} mutation on cell growth, glucose consumption, and acetate secretion under low concentrations of glucose. When 5 g/L glucose was used, we found MG1655I with *ptsI* deletion did not show an obvious lag phase, and four mutant strains MG1655H, MG1655C, MG1655G, and MG1655I exhibited different glucose assimilation profile compared with the wild-type strain. These fermentation performances of mutant strains were obviously different compared with the same strains under 20 g/L. Accordingly, this study is a good complement to the knowledge of PTS^{Glc} in *E. coli*.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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