J Ind Microbiol Biotechnol (2014) 41:1249–1258
DOI 10.1007/s10295-014-1455-y

GENETICS AND MOLECULAR BIOLOGY OF INDUSTRIAL ORGANISMS

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Investigation of *ptsG* gene in response to xylose utilization in *Corynebacterium glutamicum*

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Received: 15 January 2014 / Accepted: 28 April 2014 / Published online: 25 May 2014
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Abstract *Corynebacterium glutamicum* strains NC-2 were able to grow on xylose as sole carbon sources in our previous work. Nevertheless, it exhibited the major shortcoming that the xylose consumption was repressed in the presence of glucose. So far, regarding *C. glutamicum,* there are a number of reports on *ptsG* gene, the glucose-specific transporter, involved in glucose metabolism. Recently, we found *ptsG* had influence on xylose utilization and investigated the *ptsG* gene in response to xylose utilization in *C. glutamicum* with the aim to improve xylose consumption and simultaneously utilized glucose and xylose. The *ptsG*-deficient mutant could grow on xylose, while exhibiting noticeably reduced growth on xylose as sole carbon source. A mutant deficient in *ptsH*, a general PTS gene, exhibited a similar phenomenon. When complementing *ptsG* gene, the mutant Δ*ptsG*-*ptsG* restored the ability to grow on xylose similarly to NC-2. These indicate that *ptsG* gene is not only essential for metabolism on glucose but also important in xylose utilization. A *ptsG*-overexpressing recombinant strain could not accelerate glucose or xylose metabolism. When strains were aerobically cultured in a sugar mixture of glucose and xylose, glucose and xylose could not be utilized simultaneously. Interestingly, the Δ*ptsG* strain could co-utilize glucose and xylose under oxygen-deprived conditions, though the consumption rate of glucose and xylose dramatically declined. It was the first report of *ptsG* gene in response to xylose utilization in *C. glutamicum*.

Keywords *Corynebacterium glutamicum* · *ptsG* · Xylose utilization · Biomass hydrolysate · Simultaneous utilization

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Introduction

Lignocellulosic biomass from agricultural residues is an abundant, cost-effective and attractive renewable feedstock for production of biofuel and commodity chemicals. Lignocellulose is mainly composed of cellulose, hemicellulose and lignin [41]. Whereas cellulose is a homopolymer of D-glucose, hemicellulose contained various hexose sugar and pentose sugar, such as glucose and xylose [2]. On the one hand, relatively few native strains of industrial microorganisms can metabolize pentose sugars as substrates, even though the recombinant strains with improved pentose sugar metabolic pathways [14]. On the other hand, a classic pattern of diauxic growth occurs when cells are exposed to mixture sugars in the presence of glucose, with a lag period occurring between growth phases [1]. Microorganisms that consume mixture sugars such as glucose and xylose sequentially must have lower productivities for the generation of a product than that if the organism were able to utilize the sugars simultaneously [42]. For industrialization of chemical production from lignocellulosic biomass, developing microorganism which simultaneously consumes hexose and pentose sugars becomes a major technological bottleneck. Xylose is widely contained in lignocellulosic biomass. Xylose utilization is an important trait for an economically feasible production of commodity chemicals from lignocellulosic biomass by microbial [15]. In such hydrolysates, rapid and efficient conversion of xylose in the

presence of glucose can immensely impact the commercial viability of an entire process of bulk chemicals production.

Xylose metabolism has been researched in *Escherichia coli* [3, 37]. Moreover, *E. coli* PTS mutants have been observed to simultaneously use glucose and xylose [11, 22]. It plays a central role in CCR of *E. coli*. Therefore, the effects of mutation in *ptsG* encoding EIIglu have been investigated on sugar mixture utilization. A recombinant ethanologenic *E. coli* with *ptsG* mutation was constructed and its utilization of sugar mixtures was investigated [22]. The wild-type strain showed sequential glucose–pentose utilization, while the corresponding *ptsG* mutant consumed these sugars simultaneously and produced ethanol at comparable yields with the parental strain. A similar study was performed with a PTS-glucose+ mutant strain [11]. The authors showed that the uptake of ^{14}C-labeled xylose by the PTS-glucose+ mutant was inhibited by glucose. In *E. coli*, glucose inhibiting uptake of xylose is a regulatory phenomenon called inducer exclusion, which is mediated by the glucose-specific enzyme IIA ($EIIA^{glc}$) of the PTS [30]. When glucose is present, the phosphate group of PTS proteins is drained to the incoming sugar. $EIIA^{glc}$ exists mainly in its unphosphorylated form. This form of $EIIA^{glc}$ binds to non-PTS sugar permeases. Finally, transport of non-PTS sugars is inhibited and formation of inducers is prevented [4].

C. glutamicum is used for the industrial production of various amino acids [10]. Moreover, due to arrested cell growth under oxygen deprivation, it has great potential capacity for efficient production ethanol [13] or organic acid: D-lactate [26], succinate [24]. It was known that *C. glutamicum* usually did not use D-xylose as a substrate owing to lack of a gene encoding the xylose isomerase *xylA*, which was capable to catalyze D-xylose to D-xylulose, even though *C. glutamicum* possesses a functional *xylB* gene xylulokinase, which is capable to catalyze phosphorylation of D-xylulose to the PPP intermediate D-xylulose-5-phosphate, the last step of D-xylose catabolism. To break through the bottleneck of xylose transport and accelerate xylose utilization, the heterologous expression of xylose transport systems, e.g. *araE*, was used for research. The low-affinity L-arabinose H^+ symporter (*araE*) was identified as xylose transporter though a protein-only exits in *C. glutamicum* ATCC31831. Moreover, *araE* introduction enhanced xylose consumption threefold at low xylose concentrations in a recombinant *C. glutamicum* with heterologous xylose-catabolizing pathway [33]. On the other hand, Meiswinkel and co-workers [20] demonstrated that introducing higher *xylA* (from *Xanthomonas campestris*) and *xylB* (from *C. glutamicum*) activities could improve xylose utilization. Productivity of the glutamate, lysine, ornithine as well as diamine putrescine was increased.

A successful demonstration of the heterologous expression of xylose utilization genes made them interesting biocatalysts for xylose fermentation, which are the main components in lignocellulosic hydrolysates. Genetically engineered xylose utilizing *C. glutamicum* strains are constructed by introducing *E. coli xylA-xylB* to 1,5-diaminopentane producer DAP-3c strain [5, 6] and lysine producer DM1729 [20]. The recombinant of *C. glutamicum* DAP-3c with xylose as the sole carbon source produced significant quantities of 1,5-diaminopentane [5]. Similarly, heterologous expression of the *araE* in *C. glutamicum* R resulted in a mutant strain for production xyllitol [32].

We have previously constructed a xylose utilization metabolic engineering strain for production of succinate under the oxygen-deprived conditions which arrest the cellular growth [40]. In the present study, we investigated the *ptsG* gene involved in xylose utilization in *C. glutamicum*. The results would lead us to simultaneously utilize glucose and xylose. Moreover, it was the first report of *ptsG* gene in response to xylose utilization in *C. glutamicum*.

Materials and methods

Bacterial strains, media, plasmids and cultivation conditions

All bacterial strains and plasmids used in this study are listed in Table 1. For genetic manipulations, *E. coli* strains were grown at 37 °C in LB medium [31]. *C. glutamicum* strains were routinely cultivated at 30 °C. The nutrient-rich medium (A medium) was used for aerobic growth: 7 g l^{-1} casamino acids, 7 g l^{-1} $(NH_4)_2SO_4$, 2 g l^{-1} urea, 2 g l^{-1} yeast extract, 0.5 g l^{-1} KH_2PO_4, 0.5 g l^{-1} K_2HPO_4, 0.5 g l^{-1} $MgSO_4{\cdot}7H_2O$, 6 mg l^{-1} $FeSO_4{\cdot}7H_2O$, 4.2 mg l^{-1} $MnSO_4{\cdot}H_2O$, 0.2 mg l^{-1} biotin and 0.2 mg l^{-1} thiamine. The minimal medium is A medium without yeast extract and casamino acids. The mineral salts medium including 0.5 g l^{-1} KH_2PO_4, 0.5 g l^{-1} K_2HPO_4, 0.5 g l^{-1} $MgSO_4{\cdot}7H_2O$, 6 mg l^{-1} $FeSO_4{\cdot}7H_2O$, 4.2 mg l^{-1} $MnSO_4{\cdot}H_2O$, 100 μg l^{-1} biotin and 100 μg l^{-1} thiamine was used for anaerobic fermentation. If appropriate, the final antibiotic concentrations were as follows: for *E. coli* 50 μg ml^{-1} chloramphenicol and 50 μg ml^{-1} kanamycin, and for *C. glutamicum* 10 μg ml^{-1} chloramphenicol and 10 μg ml^{-1} kanamycin.

Fermentation under oxygen deprivation

C. glutamicum cells precultured under aerobic conditions described above were harvested by centrifugation (5,000×*g*, 4 °C; 10 min) and were subsequently washed twice with mineral salts medium. Following the second

Table 1 Strains and plasmids used in this study

Strain, plasmid or primer	Relevant characteristics	Source or reference
E. coli		
JM109	*recA1 endA1 gyrA96 thi hsdR17 supE44 relA1* Δ(*lac-proAB*)/F′ [*traD36 proAB* + *lacI*^{q}*lacZ*ΔM15]	Takara
C. glutamicum		
13032	Wild-type strain, biotin auxotroph	ATCC13032
NC-2	chromosomal integration into the *pta-ackA* locus of *xylA* and *xylB* gene from *E. coli* K-12 under the control of the *trc* promoter	[40]
NC-2a	pXMJ19 were introduced into NC-2	This work
NC-2b	pXMJ19-*ptsG* were introduced into NC-2	This work
Δ*ptsG*	NC-2 derivative with an in-frame deletion of the *ptsG*	This work
Δ*ptsH*	NC-2 derivative with an in-frame deletion of the *ptsH*	This work
Δ*ptsG-ptsG*	pXMJ19-*ptsG* were introduced into Δ*ptsG*	This work
Δ*ptsH-ptsH*	pXMJ19-*ptsH* were introduced into Δ*ptsH*	This work
Plasmids		
pK18mobsacB	Kan^r; vector for allelic exchange in *C. glutamicum*	[35]
pK18mobsacB-Δ*ptsG*	Kan^r; pK18mobsacB with a 1.3 kb *Eco*RI–*Xba*I DNA fragment containing Δ*ptsG* gene	This work
pK18mobsacB-Δ*ptsH*	Kan^r; pK18mobsacB with a 1.6 kb *Eco*RI–*Bam*HI DNA fragment containing Δ*ptsH* gene	This work
pXMJ19	Cm^r; *E. coli–C. glutamicum* shuttle vector, source of tac promoter	[14]
pXMJ19-*ptsG*	Cm^r; pXMJ19 with a 2 kb *Xba*I–*Kpn*I *ptsG* fragment	This work

wash, cells were resuspended in 40 ml of mineral salts medium and were incubated at 30 °C with constant agitation in a lidded 100 ml medium bottle. Under oxygen condition, medium were supplemented with 300 mM Na_2CO_3.

DNA techniques

Plasmid DNA was isolated using a Plasmid Miniprep Kit (Biomiga, USA) according to the manufacturer's instructions. DNA was extracted by using 4 mg ml^{-1} lysozyme at 37 °C for 30 min. Genomic DNA of *C. glutamicum* was isolated using a TIANamp Bacteria DNA Kit (TIANGEN, China). Oligonucleotides were synthesized by Genscript Corporation. Routine methods like PCR, restriction, or ligation were carried out according to standard protocols [8]. PCR products were generated with Prime STAR HS DNA Polymerase (Takara, Japan). DNA fragment was purified by Gel/PCR Extraction Kit (Biomiga, USA). Transformation of *E. coli* cells was performed by the $CaCl_2$ procedure [31]. Transformation of *C. glutamicum* was performed as described previously [39].

Construction of deletion mutants, chromosomal gene replacements and plasmids

Plasmid pK18mobsacB-Δ*ptsG* was constructed for deleting the chromosomal *ptsG* gene. First, the regions upstream and downstream (0.6 and 0.7 kb, respectively) of the Δ*ptsG* deletion region were amplified with the oligonucleotide pairs ptsGF1/ptsGF2 and ptsGR1/ptsGR2, respectively. The two PCR products severed as the templates for an overlap extension PCR with oligonucleotide pair with oligonucleotide pair ptsGF1/ptsGR2. The PCR product of about 1.3 kb, which carried cloning sites, was digested with *Eco*RI and *Xba*I and cloned into pK18mobsacB cut with the same enzymes (Table 2).

Plasmid pK18mobsacB-Δ*ptsH* was constructed for deleting the chromosomal *ptsH* gene. First, the regions upstream and downstream (0.9 and 0.7 kb, respectively) of the Δ*ptsH* deletion region were amplified with the oligonucleotide pairs ptsHF1/ptsHF2 and ptsHR1/ptsHR2, respectively. The two PCR products severed as the templates for an overlap extension PCR with oligonucleotide pair with oligonucleotide pair ptsHF1/ptsHR2. The PCR product of about 1.6 kb, which carried cloning sites, was digested with *Eco*RI and *Bam*HI and cloned into pK18mobsacB cut with the same enzymes.

Plasmid pXMJ19-*ptsG* was constructed for overexpressing the chromosomal *ptsG* gene. The *C. glutamicum* ATCC13032 chromosome served as the template for a PCR with oligonucleotide pair ptsG1/ptsG2. The PCR product of about 2 kb, which carried cloning sites, was digested with *Xba*I and *Kpn*I and cloned into pXMJ19 cut with the same enzymes.

Plasmid pXMJ19-*ptsH* was constructed for overexpressing the chromosomal *ptsH* gene. The *C. glutamicum* ATCC13032 chromosome served as the template for a PCR with oligonucleotide pair ptsH1/ptsH2. The PCR product

Table 2 Oligonucleotides used in this study

Primer	Target gene	Sequence (5′–3′)	Overhanged restriction site
ptsGF1	*ptsG* upstream	GATGAATTCATGGCGTCCAAACTG	*Eco*RI
ptsGF2	*ptsG* upstream	TGTTTAAGTTTAGTGGATGGGTGGCAGGAAGTAGAA	
ptsGR1	*ptsG* downstream	CCCATCCACTAAACTTAAACATTCCAACGAAGAGCG	
ptsGR2	*ptsG* downstream	GATTCTAGATACTCGTTCTTGCCG	*Xba*I
ptsHF1	*ptsH* upstream	GATGAATTCGGTTGTTGGTCTCGT	*Eco*RI
ptsHF2	*ptsH* upstream	CCGGTACCCTAAACTTAAACAACAGTCTTGGAAGCC	
ptsHR1	*ptsH* downstream	TGTTTAAGTTTAGGGTACCGGGCTGCGCTTATCGCA	
ptsHR2	*ptsH* downstream	GATGGATCCTTTCCCCTATCCCTA	*Bam*HI
ptsG1	*ptsG*	GATTCTAGAAAAGGAGGACAACCATGGCGTCCAAACTG	*Xba*I
ptsG2	*ptsG*	GGGGTACCTTACTCGTTCTTGCCG	*Kpn*I
ptsH1	*ptsH*	GATCTGCAGAAAGGAGGACAACCCGTTCGGATTAACGG	*Pst*I
ptsH2	*ptsH*	GATGAATTCAACAAGCTTCTAACG	*Eco*RI

of about 0.4 kb, which carried cloning sites, was digested with *Pst*I and *Eco*RI and cloned into pXMJ19 cut with the same enzymes.

Resultant plasmids were introduced into each cell by electroporation. *C. glutamicum* NC-2 was transformed by electroporation with plasmids pK18mobsacB-Δ*ptsG*. The transfer of the resulting deletion plasmids into *C. glutamicum* and selection for the first and second recombination events were performed as described previously [13]. The first recombinant colonies that grew were kanamycin-sensitive. Southern hybridization and PCR analyses of the *ptsG* gene region on the chromosome of such colonies with the oligonucleotide pairs ptsGF1/ptsGR2, exhibited mutant *ptsG* gene in which the middle fragment of the *ptsG* gene was deleted, whereas the remaining 50 % exhibited the wild-type *ptsG* gene. Thus, due to the lethality of its expression when cells are grown in LB plate with 10 % sucrose as a sole carbon source, the sacB gene inserted in the vicinity of the *ptsG* gene in the chromosome of *C. glutamicum* could serve as an efficient selection mechanism for the positive selection. After verification of the deletions through PCR analyses with the oligonucleotide pairs ptsGF1/ptsGR2. The recombinant strain was used to investigate corynebacteria for xylose utilization. Similarly, *C. glutamicum* NC-2 was transformed by electroporation with plasmids pK18mobsacB-Δ*ptsH*. The markerless *ptsH* mutant was constructed.

Enzyme assay

PTS activity of *C. glutamicum* was assayed following phosphoenolpyruvate (PEP)-dependent phosphorylation of methyl α-[^{14}C]glucoside ([^{14}C]αMG). The assay was carried out for 30 min at 30 °C in a reaction volume of 0.1 ml containing 12 μM [^{14}C]αMG (40 mCi mmol^{-1}) 50 mM Tris/HCl pH 7.5, 5 mM $MgCl_2$ and 3 mM DTT [27].

Analytical methods

The cell growth was monitored by the optical density at 600 nm (OD_{600}) with a spectrophotometer (UV-2800, UNICO, USA) and was transformed into dry cell weight (DCW) using the following equation: $DCW\left(gl^{-1}\right) = 0.4 \times OD_{600}$.

The culture samples were centrifuged (12,000 rpm, 4 °C, 1 min) and the resulting supernatants were analyzed for the presence of sugars. Xylose and glucose were quantified in hydrolysates with a Agilent HPLC on an Aminex HPX-87H column (Bio-Rad) operating at 45 °C with 5 mM H_2SO_4 as the mobile phase at a flow rate of 0.6 ml min^{-1} and detected with an RI-detector.

Results

Disruption of *ptsG* gene and complementation analysis in xylose utilization in *C. glutamicum*

To identify the physiological function and confirm the essential involvement of *ptsG* in the catabolism of glucose and xylose in *C. glutamicum*, a mutant strain deficient in *ptsG*, Δ*ptsG*, was constructed. Protein extracts of strains were used for PEP-dependent phosphorylation of methyl α-[^{14}C] glucoside (αMG) to assay the glucose PTS. Enzymatic assays indicated that the mutant Δ*ptsG* lost the PTS activity (Table 3). This mutant was tested for the ability to grow on glucose or xylose as the sole carbon source under aerobic conditions. Although the strain NC-2 was able to grow in minimal medium containing xylose as the sole carbon source, the growth on xylose was considerably slower than on glucose (Fig. 1a, b). The mutant Δ*ptsG* exhibited noticeably reduced growth on glucose or xylose compared with the strain NC-2. When the complementary plasmid

Table 3 PEP-dependent phosphorylation of [^{14}C]αMG in extracts of *C. glutamicum*

Strain	Phosphorylation activity [nmol sugar-P 30 min^{-1} (mg protein^{-1})]
NC-2	4.36 ± 0.08
NC-2a	4.24 ± 0.02
NC-2b	7.21 ± 0.12
Δ*ptsG*	<0.01
Δ*ptsG-ptsG*	4.13 ± 0.03
Δ*ptsH*	<0.01
Δ*ptsH-ptsH*	3.78 ± 0.10

Extracts were dialyzed and used at 80 μg protein

Cells of *C. glutamicum* were grown in LB medium

Average values and standard deviations of three independent experiments are shown

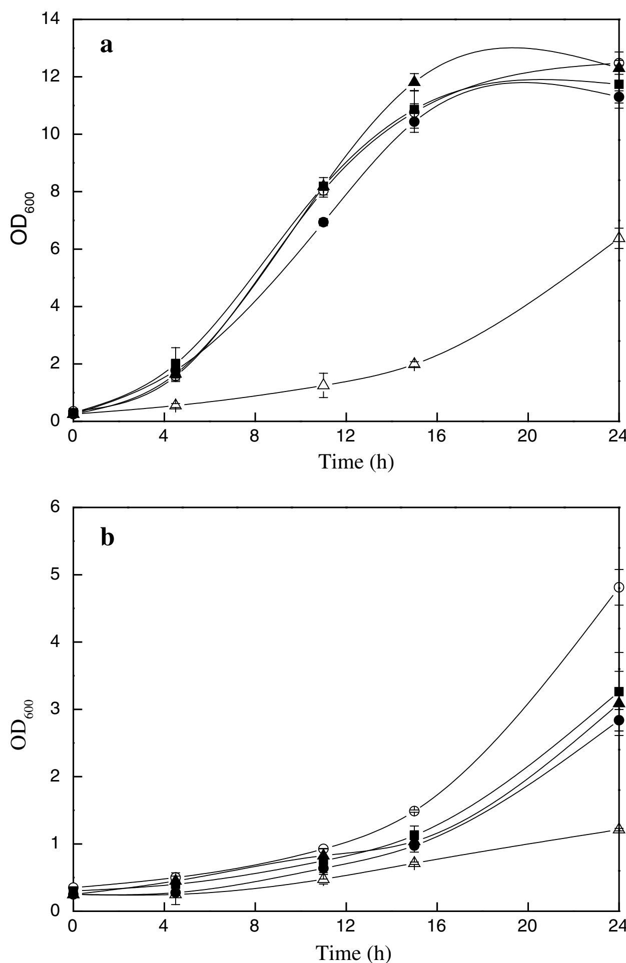

Fig. 1 Aerobic growth of *Corynebacterium glutamicum* strain NC-2 (*empty circles*), *ptsG*-deficient strain Δ*ptsG* (*empty triangles*), *ptsG*-overexpressing strain NC-2b (*filled squares*), Δ*ptsG-ptsG* (*filled triangles*) and NC-2a (*filled circles*). Strains were cultured in BT mineral medium containing 110 mM glucose (**a**) or 110 mM xylose (**b**). Initial OD_{600} was 0.3. Average values and standard deviations of three independent experiments are shown

pXMJ19-*ptsG* was introduced into the mutant Δ*ptsG* and induced with IPTG, the mutant Δ*ptsG-ptsG* restored the ability to grow on glucose or xylose, as we expected. A *ptsG*-overexpressing strain, NC-2b, was constructed. When cultured on glucose or xylose, NC-2b grew as well as the parent strain NC-2 (Fig. 1a, b). These results indicated that, in *C. glutamicum*, *ptsG* was essential for the growth not only on glucose but also on xylose.

Under conditions of oxygen deprivation, *C. glutamicum* had been observed to produce organic acids from glucose or xylose at high yields in mineral medium [15, 25]. To evaluate the capacity of glucose or xylose consumption under conditions of oxygen deprivation, the cells of the *C. glutamicum* parent strain and mutant strains were aerobically grown with either glucose or xylose and subsequently used to inoculate mineral medium containing glucose or xylose as the sole carbon source for fermentation under oxygen deprivation (Fig. 2a, b). The glucose consumption rate of strain Δ*ptsG* (1.3 mmol h^{-1} l^{-1}) was much slower than strain NC-2 (7.5 mmol h^{-1} l^{-1}). Similarly, the *ptsG*-deficient strain slightly consumed xylose at a lower rate (0.8 mmol h^{-1} l^{-1}) than the parent strain (5.48 mmol h^{-1} l^{-1}) (Fig. 2b). When complementing the *ptsG* gene, the capacity of consume xylose was restored. The xylose consumption rate of the *ptsG*-overexpressing strain NC-2b (5.09 mmol h^{-1} l^{-1}) was close to the parent strain (Fig. 2b).

PTS-dependent xylose uptake in *C. glutamicum*

To evaluate the role of PTS in utilization of xylose, a mutant strain deficient in a general PTS component HPr, Δ*ptsH*, was constructed. The strain Δ*ptsH* exhibited a phenotype of little growth on glucose, while the mutant Δ*ptsG* still exhibited some growth, albeit weak growth (Fig. 3a). When cultured on xylose, Δ*ptsH* showed similarly weak growth on xylose as Δ*ptsG* (Fig. 3b), implying that the PTS played a significant role in xylose transport. Moreover, Δ*ptsH* strain was complemented by *ptsH* expressed from a plasmid. The complemental strain was designated strain Δ*ptsH-ptsH*. The complemental plasmid conferred the ability to grow in minimal medium with glucose or xylose as the sole carbon source on the PTS-negative strain, proving that each PTS component played a role in glucose and xylose uptake.

Under oxygen deprivation, glucose or xylose consumption of the mutant strains were investigated (Fig. 4a, b). The glucose consumption rates dramatically decreased in mutants compared with the parent strain NC-2. Moreover, the consumption rate of strain Δ*ptsH* (1.3 mmol h^{-1} l^{-1}) was much slower than that of strain NC-2 (7.5 mmol h^{-1} l^{-1}). When cultured on xylose, the strains Δ*ptsG* and Δ*ptsH* almost did not consume xylose under

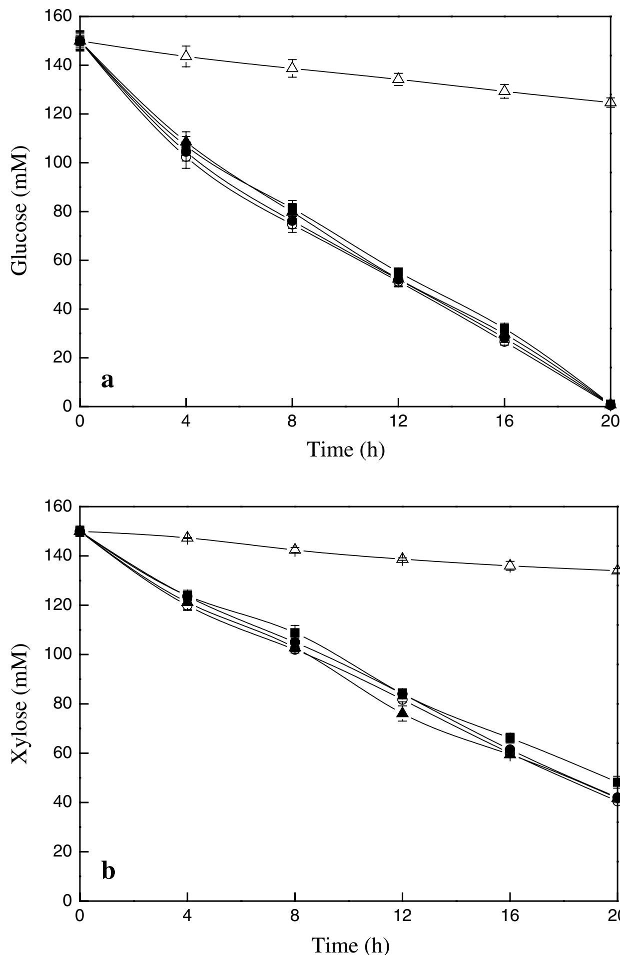

Fig. 2 Glucose (**a**) or xylose (**b**) utilization of *Corynebacterium glutamicum* NC-2 strain (*empty circles*), *ptsG*-deficient strain Δ*ptsG* (*empty triangles*), *ptsG*-overexpressing strain NC-2b (*filled squares*), Δ*ptsG-ptsG* (*filled triangles*) and NC-2a (*filled circles*) under oxygen deprivation. Initial glucose, xylose and Na_2CO_3 were 150, 150 and 300, respectively. **a** Biomass concentration was 8.5 g-dry cell l^{-1}; **b** biomass was 6.0 g-dry cell l^{-1}. Average values and standard deviations of three independent experiments are shown

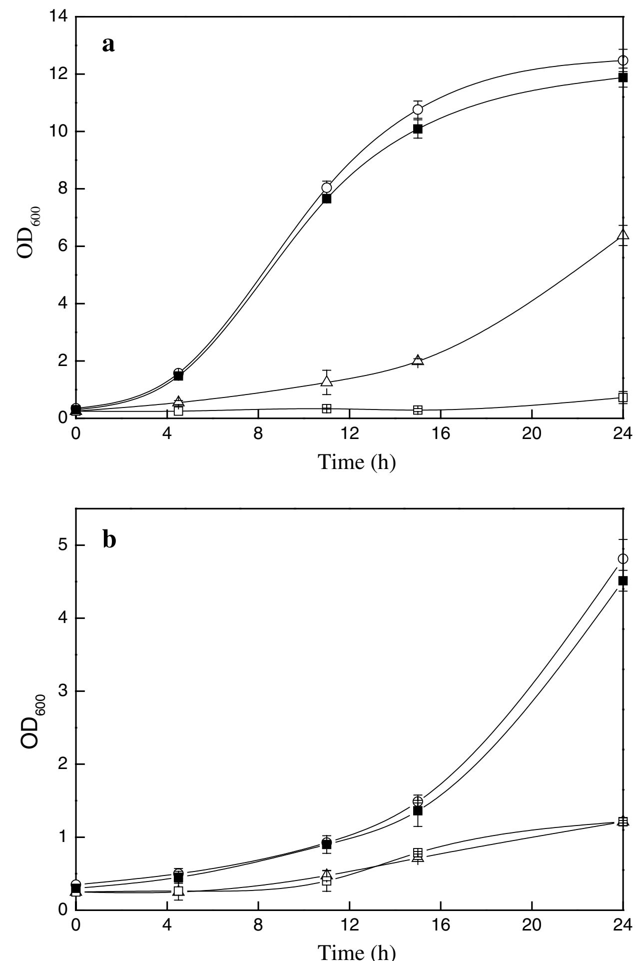

Fig. 3 Aerobic growth of *Corynebacterium glutamicum* NC-2 strain (*empty circles*), *ptsG*-deficient strain Δ*ptsG* (*empty triangles*), *ptsH*-deficient strain Δ*ptsH* (*empty squares*) and Δ*ptsH-ptsH* (*filled squares*). Strains were cultured in BT mineral medium containing 110 mM glucose (**a**) or 110 mM xylose (**b**). Initial OD_{600} was 0.3. Average values and standard deviations of three independent experiments are shown

these conditions (Fig. 4b), whereas that of the Δ*ptsH-ptsH* strain was almost consistent with the strain NC-2. These data promoted the view that the PTS system affected xylose metabolism, although the xylose was metabolized through *xylA* and *xylB*. The deficient PTS system mutants caused decreasing xylose consumption.

Effect of *ptsG* on simultaneous utilization of xylose and glucose

To evaluate whether xylose was utilized in the presence of glucose during aerobic culture, Δ*ptsG* mutant, NC-2b and NC-2 were incubated in a sugar mixture of glucose and xylose under both aerobic and oxygen-deprived conditions. 2 ml precultures in LB medium were used to inoculate 50 ml of minimal medium containing 30 mM of glucose and 30 mM of xylose to a final OD_{600} of 0.1. All the strains could not utilize simultaneously glucose and xylose (Fig. 5). During an initial phase, glucose was uptaken, while all of xylose remained in the medium. Xylose consumption started once glucose pool was completely exhausted. The strains demonstrated a typical diauxic behavior, with glucose as preferred substrate.

Under conditions of oxygen deprivation, NC-2 and the NC-2b *ptsG*-overexpressing strain were unable to consume xylose in the presence of glucose. Interestingly, the Δ*ptsG* strain could consume glucose and xylose simultaneously under oxygen deprivation, although its total sugar consumption rate (4.36 mmol h^{-1} l^{-1}) was markedly lower than that of NC-2 (7.65 mmol h^{-1} l^{-1}). NC-2b

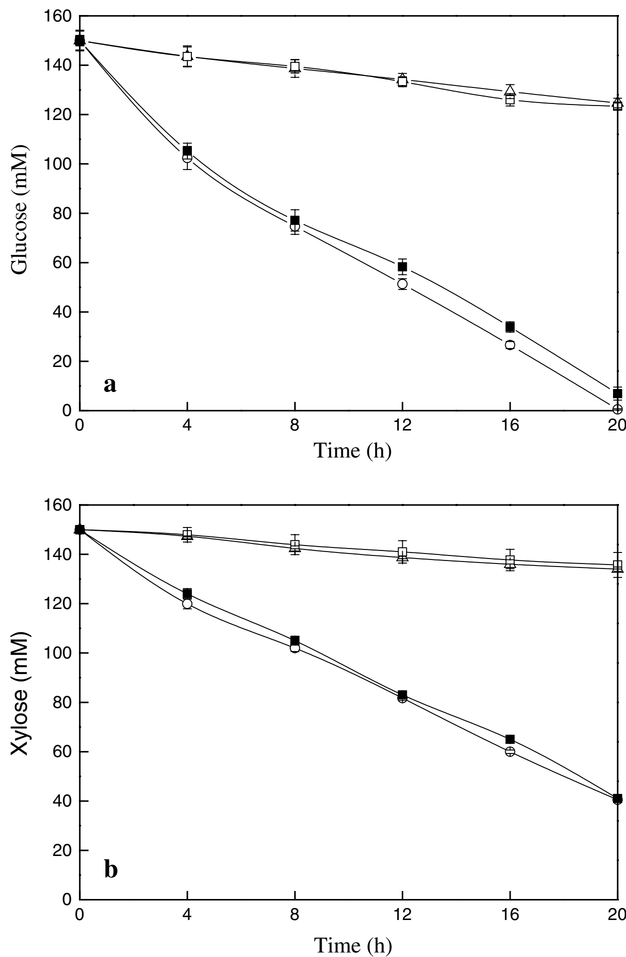

Fig. 4 Glucose (**a**) or xylose (**b**) utilization of *Corynebacterium glutamicum* NC-2 strain (*empty circles*), *ptsG*-deficient strain Δ*ptsG* (*empty triangles*), *ptsH*-deficient strain Δ*ptsH* (*empty squares*) and Δ*ptsH-ptsH* (*filled squares*) under oxygen deprivation. Initial glucose, xylose and Na_2CO_3 were 150, 150 and 300, respectively. **a** Biomass concentration was 8.5 g-dry cell l^{-1}; **b** biomass was 6.0 g-dry cell l^{-1}. Average values and standard deviations of three independent experiments are shown

subsequently consumed glucose and xylose with a total sugar consumption rate (7.68 mmol h^{-1} l^{-1}) equivalent to that of NC-2 (Fig. 6).

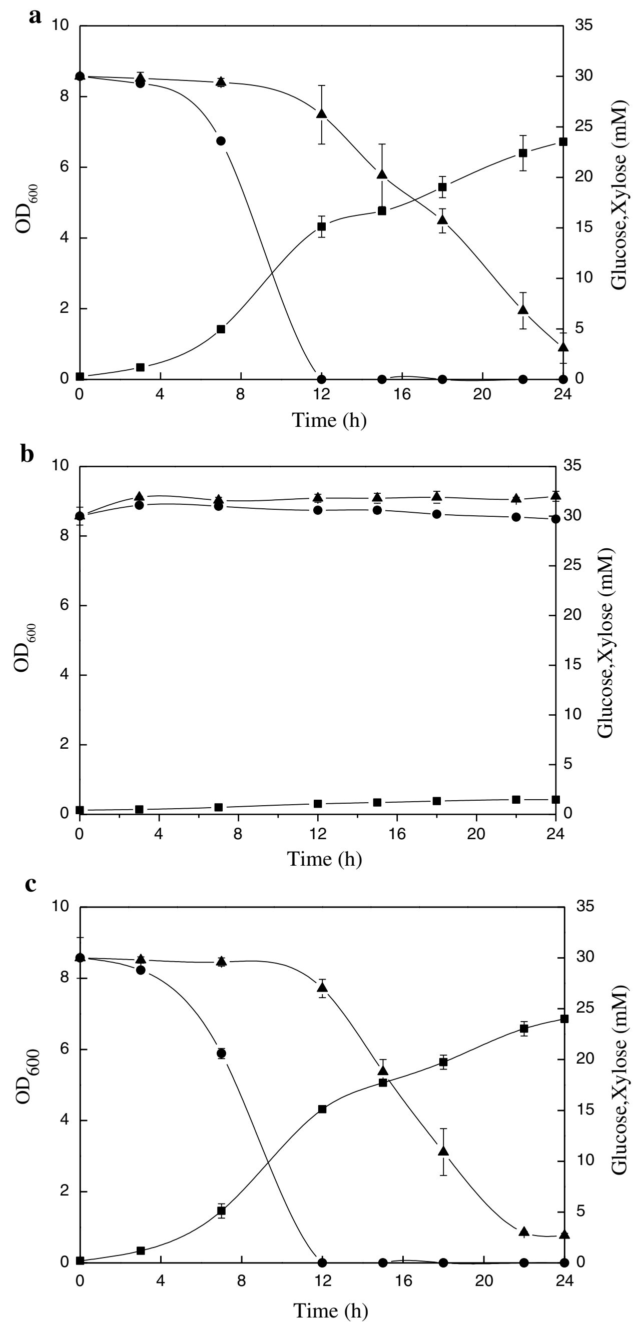

Fig. 5 Sugar consumption and aerobic growth (*filled squares*) of *Corynebacterium glutamicum* strain NC-2 (**a**), *ptsG*-deficient strain Δ*ptsG* (**b**) and *ptsG*-overexpressing strain NC-2b (**c**) in minimal medium containing glucose and xylose mixture. Cell growth was monitored by OD_{600} (*filled squares*). The time course of changes in the concentrations of glucose (*filled circles*) and xylose (*filled triangles*) in the medium. Initial OD_{600}, glucose and xylose was 0.1, 30 and 30 mM, respectively. Average values and standard deviations of three independent experiments are shown

Discussion

This study revealed that *ptsG*, the glucose transporter, is essential for growth on glucose or xylose as the sole carbon source in *C. glutamicum*. The *ptsG*-deficient mutant exhibited the slow growth phenotype in minimal medium containing glucose as the sole carbon source (Fig. 1a). Of particular interest was the fact that not only the growth of Δ*ptsG* on glucose was impacted, but also the utilization of xylose was subject to the PTS-deficient (Figs. 1b, 2b).

The growth of Δ*ptsG* on xylose was slower compared with NC-2 (Fig. 1b). Furthermore, growth-independent xylose consumption had negative influence (Fig. 2b). These results

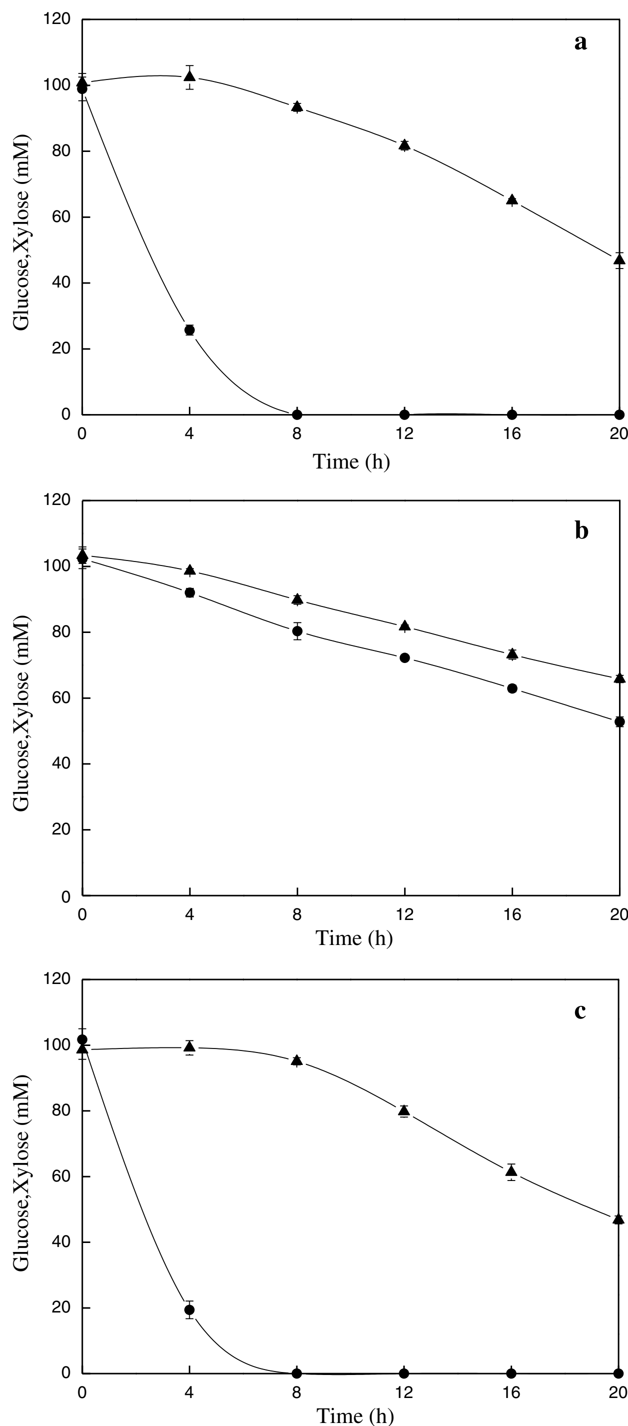

Fig. 6 Sugar consumption by *Corynebacterium glutamicum* strain NC-2 (**a**), *ptsG*-deficient strain Δ*ptsG* (**b**) and *ptsG*-overexpressing strain NC-2b (**c**) incubated in mineral medium containing a mixture of glucose (*filled circles*) and xylose (*filled triangles*) under oxygen deprivation. Initial glucose, xylose, Na_2CO_3 and biomass were 105, 105, 300 mM and 17.8 g-dry cell l^{-1}, respectively. Average values and standard deviations of three independent experiments are shown

suggest *ptsG* may be essential for xylose catabolism. It was likely that *C. glutamicum* PTS played a key role in the flux control of the xylose catabolic pathway. A similar result appeared in mannose utilization, the growth of Δ*ptsG* on agar plate containing mannose as the sole carbon source was repressed compared with the wild-type strain [34]. When complementing *ptsG* gene, the mutant Δ*ptsG*-*ptsG* restored the ability to grow on xylose as same as NC-2. The results support the assumption that *ptsG* gene is important for the closely linked xylose transport in *C. glutamicum*. The phenotype of *ptsG*-overexpressing recombinant strain exhibited the PTS transport xylose was not a rate-limiting step. In *C. glutamicum*, *ptsG* was involved in the transport of other sugars that are structurally related to glucose, i.e. methyl α-glucoside, 2-deoxyglucose, L-sorbose and 5-thio-glucose [23]. The EII^{Glc} transporter of *C. glutamicum* also accepts glucosamine as substrate [38]. This was not to be expected since the well-studied PTS system in *E. coli* does not accept glucosamine as substrate. Glucosamine is taken up mainly by the PTS^{Man} system, which has rather broad substrate specificity [28].

Genetic characterization of *ptsH* in this study confirmed that the PTS sugar transport system was essential for xylose utilization in *C. glutamicum* (Figs. 3, 4). Δ*ptsH*, a strain deficient in a general PTS component HPr, revealed little growth in minimal medium containing glucose as a sole source (Fig. 3a). However, these results are different from previous reports. Wendisch's group [19] reported that *C. glutamicum* strain Δ*hpr* (*ptsH*) showed a similar growth to *ptsG*-inactivated strain. Besides PTS, *C. glutamicum* possesses PTS-independent glucose uptake system, *iolT1*/*iolT2* (encoding glucose permeases), *glk*/*ppgk* (encoding glucokinases), which are not PTS. Glucose is a less preferred substrate of IolT1 and IolT2 from *C. glutamicum*, as the transport of inositol occurs with an almost 100-fold higher affinity than that of glucose [17]. PTS-independent glucose uptake via *iolT1*/*iolT2* and phosphorylation via glucokinases is relevant only at high glucose concentrations (more than 110 mM glucose) [18], while low glucose concentration (110 mM glucose) was contained in the media during the initial phase of cultivations. On the other hand, *C. glutamicum* might not possess EI paralogues which could supply the hexose-specific EI function [16], these may cause the ΔptsH hardly grew in glucose medium. In this study, the phenotype of mutant Δ*ptsG* or Δ*ptsH* suggested that PTS-deficiency resulted in a lack of xylose uptake. These results imply that PTS is responsible for xylose transport. EII^{glc} complex (*ptsG*) may have an important role in the process of facilitated diffusion of xylose in *C. glutamicum*

[36]. It had been previously demonstrated that the EII^{Man} gene had a role in the transport of xylose in *Lactobacillus pentosus*. Facilitated diffusion of a sugar may occur in the absence of phosphorylation [29]. Xylose was recognized by the $EIIC/D^{Man}$ domains and transported inside the cell by facilitated diffusion [7]. In *C. glutamicum*, EII^{glc} complex has the domain order IIB-IIC-IIA. The IIA domain is phosphorylated by Hpr and phosphorylates in turn the IIB domain [21]. The *C. glutamicum* $EIIC^{glc}$ might have a similar function as *L. pentosus* $EIIC/D^{Man}$, which is not phosphorylated on xylose transport. The Δ*ptsH* mutant phenotype implies that Hpr might control the active conformation of EII complex even if it is not functioning in phosphoryl transfer to xylose. These findings led us to predict the existence of an unidentified xylose transporter in this microbe.

In addition, to evaluate whether xylose was utilized in the presence of glucose in Δ*ptsG* mutant, high-density cells (17.8 g-dry cell l^{-1}) were used to converse substrate under oxygen-deprived conditions. As we expected, Δ*ptsG* mutant could co-utilize glucose and xylose under oxygen-deprived conditions (Fig. 6b). These results suggested that the inhibitory effects expected on xylose metabolism are attenuated under oxygen deprivation. On the other hand, when incubated under oxygen-deprived conditions, Δ*ptsG* mutant consumed each substrate at a similar rate when sugar mixtures were used (glucose-0.112 mmol h^{-1} g^{-1} CDW; xylose-0.084 mmol h^{-1} g^{-1} CDW) when either glucose (glucose-0.118 mmol h^{-1} g^{-1} CDW) or xylose (xylose-0.083 mmol h^{-1} g^{-1} CDW) alone was used (Fig. 4a, b). However, glucose and xylose could not be utilized simultaneously when aerobic culturing. In previous study, the *ptsG* gene was inactivated to deregulate the catabolite repression in *E. coli* [22]. It was recognized that the PTS took a significant role in glucose utilization. During glucose transport, the level of cyclic AMP (cAMP) is lowered by dephosphorylated EIIAGlc (encoded by crr), which in turn limits the availability of the cAMP and catabolite activator protein (cAMP–CAP) complex. The expression of genes that are involved in the catabolism of sugars generally requires the cAMP–CAP complex and, consequently, is repressed [9]. However, unlike *E. coli*, the inhibitory effects may attribute to a regulatory mechanism at the gene expression level in *C. glutamicum*. The PTS-dependent carbohydrate inhibited uptake of another carbon source. So far, the carrier protein for xylose uptake and the molecular mechanism of glucose repression were still unknown in *C. glutamicum*. It needs a more in-depth understanding of xylose transport and metabolism mechanism in *C. glutamicum*.

This study demonstrated that disruption of *ptsG* markedly decreased xylose utilization of *C. glutamicum*. Furthermore, we showed that the PTS system responds xylose utilization in *C. glutamicum*. Although we have not improved simultaneous utilization of glucose and xylose, we illustrated the possibility that a novel route involved in xylose transport. We have previously reported that genetic engineering of the lignocellulosic hydrolysates uptake and metabolism genes enable *C. glutamicum* to completely consume xylose and glucose. Moreover, xylose metabolism of the succinic acid production under oxygen deprivation was tolerant to the inhibitors contained in lignocellulose hydrolysates [40]. Further studies of efficient utilization of xylose simultaneously with glucose will be required to fully understand the role of xylose utilization in bacteria. These efforts will be integrated into the hydrolysates process to enhance the utilization of lignocellulosic materials and agricultural residues in *C. glutamicum*.

Acknowledgments This work was supported by the 973 Program of China (Grant No. 2011CB707405).

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