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REVIEW

The glucose uptake systems in *Corynebacterium glutamicum*: a review

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Abstract
The phosphoenolpyruvate-dependent glucose phosphotransferase system (PTS^{Glc}) is the major uptake system responsible for transporting glucose, and is involved in glucose translocation and phosphorylation in *Corynebacterium glutamicum*. For the longest time, the PTS^{Glc} was considered as the only uptake system for glucose. However, some PTS-independent glucose uptake systems (non-PTS^{Glc}) were discovered in recent years, such as the coupling system of inositol permeases and glucokinases (IPGS) and the coupling system of β-glucoside-PTS permease and glucokinases (GPGS). The products (e.g. lysine, phenylalanine and leucine) will be increased because of the increasing intracellular level of phosphoenolpyruvate (PEP), while some by-products (e.g. lactic acid, alanine and acetic acid) will be reduced when this system become the main uptake pathway for glucose. In this review, we survey the uptake systems for glucose in *C. glutamicum* and their composition. Furthermore, we summarize the latest research of the regulatory mechanisms among these glucose uptake systems. Detailed strategies to manipulate glucose uptake system are addressed based on this knowledge.

Keywords *Corynebacterium glutamicum* · Glucose uptake system · Regulatory mechanism · Manipulation strategy

Introduction

Corynebacterium glutamicum is a non-pathogenic Gram-positive bacterium, which is widely used as workhorse for producing various amino acids in industry (Wang et al. 2019). Since 1984, the research on genetics and metabolism of *C. glutamicum* has accelerated because of the successful application of tools for genetically modifying *C. glutamicum* and the DNA sequencing of entire genome of two model strains (i.e., *C. glutamicum* ATCC13032, *Brevibacterium flavum* ATCC14067) (Xu et al. 2020). The metabolic engineering strategy for *C. glutamicum* has been extended from core biosynthetic pathways to central metabolism route, cofactor regeneration systems, energy metabolism, global regulation and material transmembrane transports (Ikeda 2012; Xu et al. 2018). On the other hand, Michiko and Shiio (1987) firstly discovered a system for glucose uptake and phosphorylation in *C. glutamicum*, and it was named as phosphoenolpyruvate-dependent sugar phosphotransferase system (i.e., PTS). At present, the physiological significance and mechanism of glucose uptake systems have been a hot topic in breeding high-productive strain of target products.

Glucose has been widely used as raw materials in fermentation industry because of the low price and the ubiquitous effect. The uptake and phosphorylation of glucose in *C. glutamicum* is mainly through PTS^{Glc} (Blombach and Seibold 2010; Xu et al. 2019). PTS is composed of a membrane-bound carbohydrate-specific EIIABC component (EII) and two cytoplasmic components [i.e., enzyme I (EI) and histidine protein (HPr)]. PTS is widely found in bacteria but not in archaea and fungi (Ikeda 2012), which can efficiently transport and phosphorylate a variety of sugars at the same time. For example, glucose is transported and phosphorylated by PTS^{Glc} to generate glucose-6-phosphate (Xu et al. 2019). However, the assimilation of glucose by PTS^{Glc} requires phosphoenolpyruvate (PEP) as phosphoryl group donor, resulting in a reduction in intracellular PEP level and in production of PEP-derived products, such as L-lysine, succinic acid, aromatic compounds (Becker et al. 2011; Xu et al. 2019). In addition, the high efficiency of glucose utilization by PTS^{Glc} causes the unbalance between glycolytic pathway and tricarboxylic acid cycle, resulting in overflow metabolism and deficient cell growth (Becker et al. 2011; Lara et al. 2008; Xu et al. 2020). Therefore, researchers have been

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looking for PTS-independent glucose uptake systems (i.e., non-PTS^{Glc}) to replace PTS^{Glc} to increase glucose utilization and intracellular PEP levels.

It has long been thought that PTS^{Glc} is the only way to transport and phosphorylate glucose, but in fact there are a series of non-PTS^{Glc} in *C. glutamicum*. For example, Kumar et al. (2019) reported that glucose can be transported into cells by glucose permease. In addition, Ikeda et al. (2011) pointed out that HPr-deficient strain also uses glucose as the sole carbon source and resists to 2-deoxyglucose, indicating that there is non-PTS^{Glc} in *C. glutamicum*. Further studies confirmed that the coupling system of inositol permeases and glucokinases (designed as IPGS) participates in glucose uptake and phosphorylation in *C. glutamicum*. Moreover, Ikeda et al. (2015) found that the recombinant strain with double inactivation of PTS^{Glc} and IPGS also grows on a medium with glucose as the sole carbon source, indicating that there is a third glucose uptake system in *C. glutamicum*. It is now established that the third glucose uptake system is the coupling system of β-glucoside-PTS permease and glucokinases (designed as GPGS).

As mentioned above, there are three glucose uptake systems in *C. glutamicum* (Fig. 1). Therefore, many strategies can be used to improve the effective use of glucose. This review summarizes the current status and application prospects of glucose uptake systems in *C. glutamicum*. Moreover, regulatory mechanisms of different uptake systems were highlighted, which will provide theoretical bases for genetically modifying strains to improve glucose uptake rate. Finally, this review covers strategies for manipulating glucose uptake systems to construct high-productive strain of target products.

The glucose uptake systems in *C. glutamicum*

The phosphoenolpyruvate-dependent glucose phosphotransferase system (i.e., PTS^{Glc})

Glucose is an important carbon source for *C. glutamicum*, which is mainly transported and phosphorylated by PTS^{Glc}. PTS^{Glc} is a glucose-specific uptake system consisting of two cytoplasmic protein components (i.e., EI and HPr; encoded by *ptsI* and *ptsH*, respectively) and one membrane-bound glucose-specifc EIIBCA component (i.e., EII^{Glc}; encoded by *ptsG*). EI is a highly conserved protein with a molecular weight of about 64 kDa, and it contains two functional domains, i.e., phosphopyruvate kinase and PEP synthetase (Kotrba et al. 2001b). The conserved sequence of EI contains a histidine active site to ensure that PEP or ATP can be used as a phosphate donor for phosphorylation (Kotrba et al. 2001b). HPr has a molecular weight of about 9 kDa and contains a single domain. The histidine located at position 15 of HPr is the highly conserved site, in which HPr

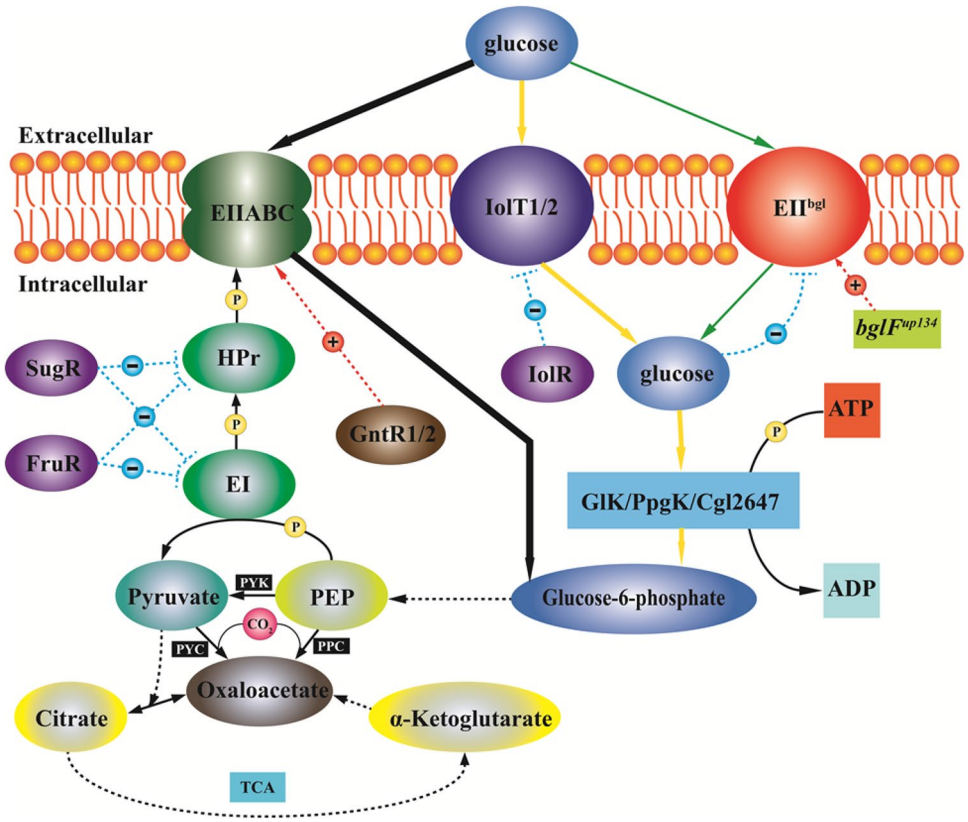

Fig. 1 Three variant glucose uptake systems in *Corynebacterium glutamicum*. Black, yellow and green lines indicate three variant pathways for glucose transfer and transformation in *C. glutamicum*, i.e., PTS^{Glc}, IPGS and GPGS. The green route (i.e., GPGS) is only found in *C. glutamicum* ATCC31833. PTS^{Glc} is negatively affected by DeoR-type transcriptional regulators, e.g., SugR and FruR. IPGS is negatively affected by RpiR-Like transcriptional regulators, e.g., IolR. GPGS is negatively affected by glucose, but it is activated during introducing mutation G134T at the upstream of *bglF*, i.e., *bglF*^{up134}. *PYK* Pyruvate kinase, *PYC* Pyruvate car boxylase, *PPC* Phosphoenolpyruvate carboxylase

will be phosphorylated (Ikeda 2012). Unlike EI and HPr, EIIBCA components (i.e., EIIs) can specifically recognize one or more specific carbohydrates (Aboulwafa and Saier 2002). In general, EIIs are composed of two hydrophilic cytoplasmic domains (i.e., IIA and IIB) and a transmembrane domain (i.e., IIC)(Moon et al. 2005). IIA, containing 100 to 160 amino acid residues, adopts phosphate groups and then phosphorylates the active site to form IIA-P. IIA-P will promote the phosphorylation of IIB at active site to form IIB-P. IIC, consisting of 6 or 8 transmembrane helixes and containing about 350 amino acid residues, phosphorylates carbohydrates and then transports them into cytoplasm (Moon et al. 2007). However, the phosphorylation and transport of carbohydrates by IIC are regulated by IIB-P, resulting in rapid dissociation of phosphorylated carbohydrates from EIIs (Lee et al. 1994).

The tandem phosphorylation reaction by PTS is completed by five steps (Fig. 2). These five steps are catalysed by EI, HPr and EIIs, and reversibly transfer phosphate groups (Kotrba et al. 2001a). Firstly, EI adopts the high-energy phosphate groups from PEP to auto-phosphorylate to form EI-P. Then, the phosphate group from EI-P is transported to HPr and subsequently participates in the reactions catalyzed by EIIs (i.e., IIA, IIB, IIC). In these catalyzed reactions, IIA is phosphorylated by HPr, and then to phosphorylates IIB, thus opening the permease channel of IIC. Finally, the phosphate groups from PEP are transported to carbohydrates (Moon et al. 2007).

PTS-independent glucose uptake systems (i.e., non-PTS^{Glc})

The coupling system of inositol permeases and glucokinases (i.e., IPGS)

In 2011, Ikeda et al. (2011) found that HPr-deficient *C. glutamicum* strain SPH2 also uses glucose as the sole carbon source and resists to 2-deoxyglucose indicating that this recombinant strain does not rely on the PTS^{Glc} system to assimilate glucose. Further studies confirmed that the coupling system of inositol permeases and glucokinases (designed as IPGS, similarly hereinafter) participates in glucose uptake and phosphorylation, which belongs to PTS-independent glucose uptake systems (i.e., non-PTS^{Glc}). IPGS consists of *myo*-inositol permeases and glucokinases. In IPGS, carbohydrates are firstly transported into intracellular by *myo*-inositol permeases, and then phosphorylated by glucokinases (Lindner et al. 2013). IolT1 and IolT2 are two of *myo*-inositol permeases, which are responsible for transporting *myo*-inositol in *C. glutamicum* (Krings et al. 2006). Unlike *Bacillus subtilis* (Morinaga et al. 2010), IolT1 and IolT2 in *C. glutamicum* have sequence identity of up to 55%, and have similar gene expression levels and kinetic characteristics (Krings et al. 2006). Glk and PpgK are two of glucokinases in *C. glutamicum*. Glk and PpgK have sequence identity of 28% and have typical ATP binding sites (Lindner et al. 2010). However, IPGS will be activated only at the unavailability of PTS^{Glc} or at the excess glucose.

The coupling system of β-glucoside-PTS permease and glucokinases (i.e., GPGS)

The coupling system of β-glucoside-PTS permease and glucokinases (designed as GPGS, similarly hereinafter) was confirmed in 2015. Ikeda et al. (2015) found that the recombinant strain with double inactivation of PTS^{Glc} and IPGS also grow on a medium with glucose as the sole carbon source, indicating that GPGS is the third glucose uptake system in *C. glutamicum* except PTS^{Glc} and IPGS. GPGS consists of β-glucoside-PTS permease (i.e., EII^{Bgl}) and glucokinases. EII^{Bgl}, encoded by gene *bglF*, has been identified as *bglF*-specified EII component of β-glucoside-PTS (Kotrba et al. 2003). EII^{Bgl} has a molecular weight of about 65 kDa and contains three domains, i.e., IIA, IIB, and IIC (Chen and Amster-Choder 1998). In GPGS, carbohydrate is firstly transported into intracellular by EII^{Bgl}, and then

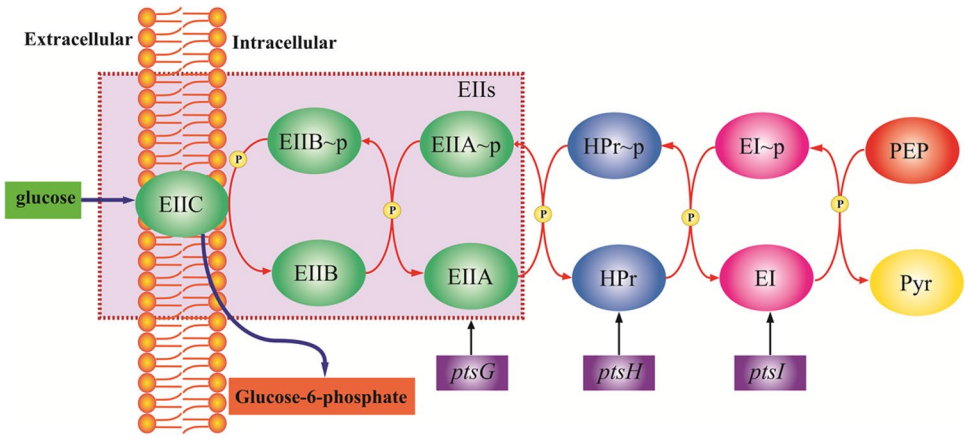

Fig. 2 Pathway of phosphate group to glucose and composition of PTS^{Glc} system

phosphorylated by glucokinases. The glucokinases in GPGS are the same as in IPGS (Ikeda et al. 2015). Since GPGS was only found in mutant strain derived from *C. glutamicum* ATCC 31833, its application remains to be explored.

Regulatory mechanisms of genes in glucose uptake systems

The regulatory mechanisms in PTS^{Glc}

Regulations of the DeoR-type transcriptional regulators

When *C. glutamicum* was cultured in medium with a mixed carbon source, the consumption rate of glucose was decreased (Wendisch et al. 2000). This is because SugR, an acetic acid-mediated deoxyribonucleotide repressor (DeoR)-type transcriptional regulator, inhibits gene expression in the PTS^{Glc} (Gaigalat et al. 2007). SugR is encoded by the gene *sugR*, which inhibits the expression of many genes in *C. glutamicum*, such as 6-phosphate fructokinase, fructose-1, 6-bisphosphate aldolase, enolase, pyruvate kinases and L-lactate dehydrogenase (Engels et al. 2008). In the PTS, SugR binds to a specific region in the promoter of the key genes *ptsH* and *ptsI*, thereby suppressing the expression of these genes (Tanaka et al. 2008a). The specific region is a GTTGCACA sequence or a TG(T)$_{2\text{-}5}$G sequence (Tanaka et al. 2008b). In addition, a 21 bp fragment of the *ptsI–fruR* promoter region is also binded by SugR (Gaigalat et al. 2007).

FruR is another DeoR-type transcription regulator to regulate the PTS, and its coding gene *fruR* is located at the upstream of the *ptsF* gene (Liu et al. 2016). Its expression level is increased with the increase of intracellular PTS sugar concentration, thus inhibiting the expression of the genes *ptsI* and *ptsH* (Tanaka et al. 2008b). In *E. coli*, FruR also plays an important role in regulating a large number of key genes in carbon metabolism, such as *pckA* (encoding PEP carboxykinase), *edd* (encoding 6-phosphogluconate dehydratase) (Liu et al. 2017). However, no reports indicated that FruR in *C. glutamicum* inhibits the expression of genes except the PTS-related genes. These results indicated that FruR in *C. glutamicum* seems to play a role in regulating the expression of genes involved in the PTS, thereby preventing the excessive intake of sugar by PTS system.

Regulations of the GntR-type transcriptional regulators

GntR-type transcriptional regulators are a family of important transcriptional regulators, which are widespread in bacteria and actinomycetes (Lambrecht et al. 2018). The structure of GntRs generally consists of a conserved N-terminal helix-turn-helix motif and a variable C-terminal effector binding/oligomerization domain (Taw et al. 2015). There are two GntR-type transcription regulators in *C. glutamicum*, called GntR1 and GntR2, respectively (Tanaka et al. 2012). Similar in *E. coli* (Porco et al. 1997) and *B. subtilis* (Reizer et al. 1991), GntR-type transcriptional regulators in *C. glutamicum* strongly inhibit the expression of genes involved in the regulation of gluconic acid catabolism, i.e., *gntP* (encoding glycosyl peroxidase), *gntK* (encoding gluconate kinase) and *gnd* (encoding gluconate dehydrogenase) (Tanaka et al. 2012). However, the difference is that these transcriptional regulators in *C. glutamicum* activate the expression of the key gene *ptsG* in the PTS^{Glc}, thus increasing the glucose uptake rate (Frunzke et al. 2008; Teramoto et al. 2011). One possible reason is that GntR1/2 binding site of the genes *gntP and gntK* is similar to the starting position of the *ptsG* gene (Teramoto et al. 2011). This may be why *C. glutamicum* can co-utilize glucose and other carbon sources but *E. coli* or *B. Subtilis* can not do it (Stansen et al. 2005).

The regulatory mechanisms in non-PTS^{Glc}

Regulations of the RpiR-like transcriptional regulators

As mentioned above, the IPGS is consisting of *myo*-inositol permeases (i.e., IolT1 and IolT2) and endogenous glucokinase (i.e., GlK, PpgK). Klaffl et al. (2013) found that a RpiR-Like transcriptional regulatory factor IolR locates at the upstream of the coding genes of IolT1 and IolT2 (i.e., *iolT1* and *iolT2*, respectively) in the opposite direction. In addition, the binding site of the IolR was found in the promoter region of *iolT1* and *iolT2* (Klaffl et al. 2013). Previous researches indicated that that the mRNA level of the *iol* gene in the IolR-mutated strain was significantly increased (> 100-fold). These results indicated that IolR suppresses the expression of *iol* gene and presumably regulated by negative autoregulation. Moreover, Zhou et al. (2015) found that IolR may inhibits the expression of two glucokinase genes (i.e., *glk* and *ppgk*). However, the inhibition mechanism of IolR on glucokinase needs to be further explored.

Regulations of the EII component of the β-glucoside-PTS

As the key component of the β-glucoside-PTS, EII^{bgl} was encoded by gene *bglF*, which located in the gene cluster *bglF-bglA-bglG* (Tanaka et al. 2009). This gene cluster is responsible for encoding genes related to β-glucoside transport. Similar in *Streptococcus mutans*, β-glucoside-PTS-related genes in *C. glutamicum* are sensitive to the presence of glucose (Cote et al. 2000). However, the transcription level of *bglA* has no significant effect on *bglF* expression (Tanaka et al. 2011). Experimental results showed that a ribonucleic antiterminator (RAT) sequence exists in the upstream of *bglF* (Tanaka et al. 2009). The role of the RAT

sequence was activated during the presence of glucose, and then to disable the anti-termination mechanism of the gene cluster *bglF-bglA-bglG*, thus inhibiting *bglF* expression (Kotrba et al. 2003). It should be noted that the regulatory effect of the RAT sequence always exists, though *C. glutamicum* depends on PTS^{Glc} to transport glucose. Interestingly, glucose also has a weak inhibitory effect on *bglF* expression in the strain with a deletion of the RAT sequence. It may be that glucose is able to weakly block the initiation of *bglF* gene transcription. In contrast, an antiterminator protein BglG, encoded by gene *bglG*, can relieve the inhibition of glucose on *bglF* gene expression (Tanaka et al. 2011).

Manipulation strategy for improving the glucose conversion ratio in glucose uptake systems

The strategies based on manipulated PTS^{Glc}

Introducing the exogenous substances to increase the glucose uptake rate

Maltose is an incomplete hydrolysis product of starch liquefaction and saccharification. Previous researches indicated that *C. glutamicum* can use maltose as the sole carbon source to produce amino acids (Xu et al. 2016). Maltose was generally catalyzed by 4-α-glucan transferase to produce maltodextrin and glucose (Kalebina et al. 2008). Then, the glucose enters in the glycolytic pathway catalyzed by glucokinases, whereas the maltodextrin is degraded to produce glucose-1-phosphate catalyzed by maltodextrin phosphorylase (MalP) (Seibold et al. 2009). Unlike *E. coli*, *C. glutamicum* doses not exhibit a diauxic growth during use of maltose and glucose as a mixed carbon source (Xu et al. 2016). Interestingly, the growth rate of bacteria was significantly accelerated during growth on mixture of maltose and glucose as compared with on glucose as a single carbon source, though the content of total carbon source in culture medium is unchanged (Henrich et al. 2013). In addition, Krause et al. (2010) pointed out that the transcription level of *ptsG* was increased during addition of maltose, indicating that maltose promotes glucose metabolism in *C. glutamicum*. Further studies conclusively showed that maltose was transported into cells by the MusEFGK2I ABC transport system, resulting in increasing the transcription level of *ptsG* (Henrich et al. 2013). Therefore, addition of maltose to medium is an effective method to increase the glucose uptake rate in bacteria. However, maltose is expensive, thus limiting its application in industry. To solve this problem, addition of starch may be a good choice during use of a recombinant strain with heterologous expression of α-amylase. Previous research indicated that a recombinant *C. glutamicum* strain with overexpression of AmyA from *Streptococcus bovis* 148 on its cell surface can hydrolyze starch to maltose, thus promoting the expression of *ptsG* (Tateno et al. 2007). This may provide an interesting idea for improving the glucose uptake rate of strain. However, this strategy was merely a technological speculation, it takes a long time to realize industrial production manner. Generally speaking, the strategy based on addition of exogenous substance to increase the glucose uptake rate may not be a optimum approach in industry because of the high-cost production.

Increasing the activity of enzymes/proteins in PTS

PTS^{Glc} consists of two cytoplasmic protein components (i.e., EI and HPr) and one membrane-bound glucose-specific EIIBCA component (i.e., EII^{Glc}) (Xu et al. 2019). In theory, the glucose uptake rate of strain can be increased by overexpression of key genes in PTS^{Glc}. For example, Krause et al. (2010) indicated that the glucose consumption rate of recombinant strain *C. glutamicum* (pBB1-*ptsG*) with overexpression of *ptsG* gene was significantly increased as compared with the origin strain. The similar results were also found in other research reported by Lindner et al. (2013), in which overexpression of *ptsG* gene in lysine-producing strain *C. glutamicum* DM1729 Δ*pgi* increased glucose consumption rate, cell growth rate and L-lysine production on glucose medium. The above-mentioned researches show that improvement of the enzymes/proteins in PTS^{Glc} is beneficial to increase the glucose uptake rate of strain. However, the positive effects are very limited because the genes in the PTS pathway are regulated by many factors, such as the transcriptional regulator SugR (Engels et al. 2008; Lindner et al. 2013). Therefore, it is difficult to significantly improve the glucose uptake rate by simple overexpression of genes in PTS^{Glc}.

Eliminating the negative transcriptional regulators in PTS

SugR is mediated by acetic acid and inhibits the expression of genes in PTS, especially the key genes *ptsG*, *ptsI* and *ptsH* in PTS^{Glc} (Gaigalat et al. 2007). Therefore, eliminating or weakening the effect of SugR is an effective strategy to increase the expression of genes in PTS, thereby increasing the glucose uptake rate in bacteria. And researchers have made preliminary attempts in this regard. For example, Engels and Wendisch (2007) deleted SugR-coding gene *sugR* in *C. glutamicum* and found that the growth rate of this recombinant strain was increased during growth on PTS sugar (e.g., glucose, fructose and sucrose), because the expression level of genes in PTS was increased. In addition, Gaigalat et al. (2007) found that the negative effect of SugR on *ptsG* gene was weakened when fructose-1-phosphate (F-1-P), 1,6-fructose diphosphate (F-1,6-P) or glucose

6-phosphate (G-6-P) are present, because F-1-P, F-1,6-P and G-6-P keep SugR from binding with the promoter of *ptsG*. Interestingly, the transcription level of genes in PTS were increased in SugR-deficient *C. glutamicum* strain, both on PTS sugar and non-PTS sugar medium (Tanaka et al. 2008a). There results indicated that the regulatory relationships between SugR and PTS are very complex. Maybe the third part factors intervene in the process of regulation. However, we don't know what the total number of factors and what they would be.

It should be noted that improvement of PTS^{Glc} helps to increase the glucose consumption rate, but it also causes the unbalance between glycolytic pathway and tricarboxylic acid cycle, resulting in overflow metabolism and deficient cell growth (Becker et al. 2011; Lara et al. 2008; Xu et al. 2020). The strain with high efficient of PTS^{Glc} not only improve the yield of the target product, but also increase the content of by-products. These results implied that increase of the glucose conversion ratio is not satisfied with simply improving the PTS^{Glc}.

The strategies based on manipulated non-PTS^{Glc}

Increasing the expression level of the enzymes/proteins in non-PTS^{Glc}

Aside from engineering PTS to increase the glucose uptake rate, many studies pointed out the important role of non-PTS^{Glc} for cell growth and yield of target products. Up to now, there are two types of non-PTS^{Glc}, i.e., IPGS and GPGS (Xu et al. 2019). Glucose assimilation through non-PTS^{Glc} is regulated by permeases (i.e., IolT1, IolT2 and EII^{Bgl}) and glucokinase (i.e., GlK, PpgK and Cgl2647) (Fig. 1). Therefore, changing the expression level of these protein-coding genes in non-PTS^{Glc} can regulate the glucose uptake rate in this system. Zhou et al. (2015) pointed out that co-overexpression of *ppgk* and *iolT1* and knock-out of *iolR* in a PTS-deficient *C. glutamicum* strain completely restore the glucose uptake rate and increase the yield of succinic acid. Similar results was also found in another study reported by Zhang et al. (2015), in which the strain with insertion of the *iolT2-ppgk* cassette at the *ptsI* locus showed the similar glucose uptake rate as original strain, but showed a high L-phenylalanine production and a low by-products. In addition, Xu et al. (2019) have pointed out that the recombinant strain *C. glutamicum* ZL-92 with introducing two point mutations in the promoter of iolT1 and replacing the nature promoter of *iolT2* and *ppgK* by the *tuf* promoter showed a quick growth and a high glucose consumption rate and L-lysine production. As compared with overexpression of genes in PTS^{Glc}, increasing the expression level of genes in non-PTS^{Glc} exhibited the best glucose uptake system for producing L-lysine. These studied have a common point that the recombinant strain showed the increase of the intracellular PEP concentration because of inactivation of PTS^{Glc}.

Optimizing the configuration of non-PTS^{Glc}

Although non-PTS^{Glc} participates the glucose assimilation, PTS^{Glc} is the main glucose-specific uptake system in *C. glutamicum* (Xu et al. 2020). This is because permease, a key compenent in non-PTS^{Glc}, is not the ubiquitous protein in *C. glutamicum* (Ikeda 2012). In addition, these permeases normally show a low affinity to glucose (Lindner et al. 2011) and a low expression level (Ikeda 2012). In addition, different types of glucokinase use different substrates (i.e., ATP, ADP and inorganic polyphosphates) as phosphoryl donor (Lindner et al. 2011). Therefore, optimizing the configuration of non-PTS^{Glc} is also a good strategy besides overexpression of permease-coding genes. The expression of IolT1 is repressed by IolR (Klaffl et al. 2013), but knock-out of IolR causes some negative effects on strain (Brusseler et al. 2018). Fortunately, introduction of two point mutations at position − 113 (A→G) and − 112 (C→G) in the nature promoter of *iolT1* reliefs the repression of IolR and avoids the negative effects on strain (Brusseler et al. 2018). In addition, Ikeda et al. (2015) indicated that introduction of a point mutation at position 134 (G→T) in the upstream of *bglF* gene significantly activates the expression of EII^{Bgl}. Moreover, co-overexpression of ADP-GlK/PFK from *Methanococcus maripaludis* and ADP-GlK from *Bacillus subtilis* in L-lysine producer effectively improves glucose consumption rate, cell growth, L-lysine production and by-products accumulation either in shake-flasks or in fed-batch fermentation (Xu et al. 2020).

Knocking-out or site-directed mutating the negative transcriptional regulators in non-PTS^{Glc}

As a DeoR transcriptional regulator, IolR inhibits the expression of glucokinases (i.e., GlK and PpgK) and IolT1 (Klaffl et al. 2013; Zhou et al. 2015). In order to increase participation of non-PTS^{Glc} in glucose assimilation, many researches focus on genetically modifying the IolR. For example, Zhou et al. (2015) found that the transcription levels of key genes in non-PTS^{Glc} were significantly increased during knock-out of *iolR* gene, resulting in completely restoring the glucose consumption rate and increasing succinic acid production. Similar results were also found in other previous researches (Michiko and Shiio 1987), in which the glucose uptake rate and the production of target products were increased during knock-out of *iolR* gene. However, knock-out of IolR causes some negative effects on strain (Brusseler et al. 2018). So could we relief the repression of IolR and avoid the negative effects on strain by introducing point mutations in *iolR* gene? Therefore, further research is necessary.

In addition, the expression of the gene *bglF* is affected by the RAT sequence and the anti-termination protein BglG. Tanaka et al. (2011) pointed out that the inhibition of *bglF* expression by glucose will be able to somewhat relieved by over-expressing *bglG*. However, the most important limiting factor of *bglF* expression level is the negative effect of glucose on RAT sequence function. Ikeda et al. (2015) found that a mutant strain of *C. glutamicum* could grow on a medium with glucose as the sole carbon source, even there were no PTS and IPGS. Further study found that the expression level of *bglF* was increased by about 11 times than that of wild type because it had a mutation (T to G) at 134 bp upstream of *bglF* (Ikeda et al. 2015). It shoud be noted that the mutation $T134^{\text{Up of bglF}}G$ was located in the RAT sequence, precisely so this mutation hinders the binding of the RAT sequence to glucose. This also provides ideas for the strengthening of the GPGS approach.

Conclusions and future prospects

Glucose is mainly transported by PTS^{Glc} in *C. glutamicum*, but PTS^{Glc} uses PEP as phosphoryl donor, which affects the production of the PEP-derived products and by-products accumulation (Lindner et al. 2013; Xu et al. 2019). Therefore, researchers have been searching for other glucose uptake systems that do not depend on PEP as phosphoryl donor. Up to now, two types of non-PTS^{Glc}, i.e., IPGS and GPGS, were found in *C. glutamicum*. Many researches have proved that increasing the activity of enzymes/proteins in non-PTS^{Glc} not only increase the intracellular PEP content to improve the production of target products, but also reduce the accumulation of by-products (e.g., lactic acid, alanine, acetic acid) (Lara et al. 2008; Xu et al. 2019). Therefore, enhancing the participate of non-PTS^{Glc} in glucose assimilation is one of the best strategy to develop a high target product producing strain with high productivity and glucose conversion rate. However, it should be noted that the glucose transporting efficiency by non-PTS^{Glc} is not significantly increased and even slightly decreased as compared with PTS^{Glc} at present. Therefore, how to improve the glucose transporting efficiency by non-PTS^{Glc} is the important aspect to invest in the future.

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