Identification and application of a different glucose uptake system that functions as an alternative to the phosphotransferase system in *Corynebacterium glutamicum*

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Abstract *Corynebacterium glutamicum* uses the phosphoenolpyruvate-dependent sugar phosphotransferase system (PTS) to uptake and phosphorylate glucose; no other route has yet been identified. Disruption of the *ptsH* gene in wild-type *C. glutamicum* resulted, as expected, in a phenotype exhibiting little growth on any of the PTS-sugars: glucose, fructose, and sucrose. However, a suppressor mutant that grew on glucose but not on the other two sugars was spontaneously isolated from the PTS-negative strain WTΔptsH. The suppressor strain SPH2, unlike the wild-type strain, exhibited a phenotype of resistance to 2-deoxyglucose which is known to be a toxic substrate for the glucose-PTS of this microbe, suggesting that strain SPH2 utilizes glucose via a different system involving a permease and native glucokinases. Analysis of the *C. glutamicum* genome sequence using *E. coli* galactose permease, which can transport glucose, led to the identification of two candidate genes, *iolT1* and *iolT2*, both of which have been reported as *myo*-inositol transporters. When cultured on glucose medium supplemented with *myo*-inositol, strain WTΔptsH was able to consume glucose, suggesting that glucose uptake was mediated by one or more *myo*-inositol-induced transporters. Overexpression of *iolT1* alone and that of *iolT2* alone under the *gapA* promoter in strain WTΔptsH rendered the strain capable of growing on glucose, proving that each transporter played a role in glucose uptake. Disruption of *iolT1* in strain SPH2 abolished growth on glucose whereas disruption of *iolT2* did not, revealing that *iolT1* was responsible for glucose uptake in strain SPH2. Sequence analysis of the *iol* gene cluster and its surrounding region identified a single-base deletion in the putative transcriptional regulator gene Cgl0157 of strain SPH2. Introduction of the frameshift mutation allowed strain WTΔptsH to grow on glucose, and further deletion of *iolT1* abolished the growth again, indicating that inactivation of Cgl0157 under a PTS-negative background can be a means by which to express the *iolT1*-specified glucose uptake bypass instead of the native PTS. When this strategy was applied to a defined lysine producer, the engineered strain displayed increased lysine production from glucose.

Key words : glucose uptake bypass, phosphotransferase system, *myo*-inositol transporter, *Corynebacterium glutamicum*, lysine production

Introduction

Corynebacterium glutamicum is an industrially important microorganism that is widely used for the production of various amino acids (Kinoshita and Nakayama 1978). Worldwide production of amino acids has increased yearly, and nowadays more than 2 million tons of amino acids are produced annually using modified *C. glutamicum* strains (Ikeda 2003). The feedstocks for the fermentation are sugars from agricultural crops such as cane molasses and beet molasses and starch hydrolysates from corn and cassava. Since these sugars include glucose, fructose and sucrose, the system by which this microbe takes up each of these sugars has been intensively studied and reviewed (Parche et al. 2001; Yokota and Lindley 2005; Moon et al. 2007; Arndt and Eikmanns 2008; Blombach and Seibold 2010). Recently, however, starch hydrolysates (mainly glucose) have been successfully used for amino acid fermentation; these tend to have fewer impurities and less variation among lots compared with molasses.

C. glutamicum uses the phosphoenolpyruvate (PEP)-dependent sugar phosphotransferase system (PTS) to uptake and phosphorylate glucose, as well as fructose and sucrose (Mori and Shiio 1987; Parche et al. 2001). The glucose-PTS consists of the glucose-specific EIIABC component encoded by *ptsG* (Cgl1360, NCgl1305) and the two general components EI and HPr encoded by *ptsI* (Cgl1933, NCgl1858) and *ptsH* (Cgl1937, NCgl1862), respectively. In most studies concerning *C. glutamicum*, the glucose uptake has been generally assumed to involve the PTS (Parche et al. 2001; Yokota and Lindley 2005). However, due to residual growth on glucose and the residual

glucose-transporting activity of PTS-negative mutants, and due to a discrepancy between measured levels of PTS activity and the observed specific glucose consumption rates during a glucose-limited chemostat culture, glucose uptake by diffusion or minor contribution of unknown permease(s) to glucose uptake has been postulated (Cocaign-Bousquet et al. 1996; Parche et al. 2001; Gourdon et al. 2003), but the existence of an alternative glucose transport system has not yet been demonstrated.

On the other hand, glucokinase activity catalyzing ATP-dependent phosphorylation of glucose into glucose-6-phosphate has been reported for this microbe (Park et al. 2000). The enzyme GLK, which is encoded by *glk* (Cgl2185, NCgl2105), has been shown to be involved in the metabolism of the disaccharide maltose, which is hydrolyzed to free glucose inside the cell (Park et al. 2000). Very recently, an additional glucokinase encoded by *ppgK* (Cgl1910, NCgl1835) has been identified (Lindner et al. 2010). The enzyme PPGK was shown to strongly prefer polyphosphate over ATP as a phosphoryl donor, and thus is assumed to be important especially under growth conditions depending on high intracellular polyphosphate concentrations. It has also been shown that the polyphosphate-dependent PPGK plays a role in maltose catabolism, as does the ATP-dependent GLK.

Our laboratory aims at a better understanding of the entire cellular system of *C. glutamicum* and also exploitation of new methods to fully draw out the potential of this microbe for amino acid production. In the present study, we observed suppressor mutants emerging from a PTS-disrupted mutant on glucose agar plates. This finding let us to predict the existence of a potential glucose uptake bypass in this microbe and simultaneously anticipate that this different glucose uptake system, if it really functions as an alternative to the PTS system, may have an impact on amino acid production. In this report, we describe the identification of the alternative glucose uptake route which is expected to exist in the suppressor mutant and show how to create

a *C. glutamicum* strain expressing the bypass route instead of the original PTS. Amino acid production from glucose through the bypass route is also demonstrated using lysine fermentation as a model.

Materials and methods

Bacterial strains and plasmids

The wild-type strain of *C. glutamicum* used in this study is strain WT-96, a single-colony derivative of *C. glutamicum* ATCC 31833. The lysine-producing strain used for evaluation of the practical functionality of the *iolT1*-specified glucose uptake bypass is *C. glutamicum* AHP-3, which carries three specific mutations (*hom59*, *lysC311*, and *pyc458*) on its genome (Ohnishi et al. 2002). *Escherichia coli* DH5α was used as a host for DNA manipulation. Plasmid pCS299P (Mitsuhashi et al. 2004), a *C. glutamicum*-*E. coli* shuttle vector, was used to construct a genomic library and also to clone the PCR products. Plasmid pESB30 (Mitsuhashi et al. 2004), which is nonreplicative in *C. glutamicum*, is a vector for gene replacement in *C. glutamicum*. Plasmids pCiolT1 and pCiolT2 were used to constitutively express the *iolT1* and *iolT2*genes, respectively, under the promoter of the endogenous *gapA* gene. For the construction of pCiolT1, the cording region of *iolT1* was PCR amplified using primers Cgl0181FusF and Cgl0181RXhoI with WT-96 genomic DNA as a template. On the other hand, the genomic region from -1 to -972 bp upstream of the *gapA* gene, which comprises its promoter, was amplified using primers CGLgapAup1000F and Cgl0181FusR. These two fragments were fused by PCR with the primers CGLgapAup1000F and Cgl0181RXhoI. The resulting 2.9-kb fragment was digested with *Xho*I and then ligated to *Sal*I-digested pCS299P to yield pCiolT1. Similarly, for the construction of pCiolT2, the cording region of *iolT2* was amplified

using primers Cgl3058FusF and Cgl3058RBamHI. On the other hand, the same promoter region upstream of *gapA* was amplified using primers CGLgapAup1000F and Cgl3058FusR. These two fragments were fused by PCR with the primers CGLgapAup1000F and Cgl3058RBamHI. The resulting 2.7-kb fragment was digested with *Bam*HI and then ligated to *Bam*HI-digested pCS299P to yield pCiolT2. The sequences of the primers used in this study are listed in Table 1. All primers were designed on the genome sequences of *C. glutamicum* (BA000036).

Media and culture conditions

Complete medium BY and minimal medium MM were used for growth of *C. glutamicum* strains (Takeno et al. 2007). For growth test in liquid culture, a 0.05-ml amount of the seed culture grown aerobically at 30ºC in BY medium for 12 h was inoculated into 5 ml of medium in a L-type test tube and cultivated at 30ºC using a Monod shaker at 40 strokes/min. For lysine production, a 4.0-ml sample of the seed culture grown at 30°C in BY medium containing 2% glucose (w/v) and 1% $CaCO_3$ (w/v) was inoculated into 40 ml of LFG1 medium in a 500-ml Sakaguchi flask and cultivated aerobically at 30°C until the sugar was consumed. LFG1 medium consisted of (per liter): 50 g of glucoses, 10 g of corn steep liquor, 45 g of $(NH_4)_2SO_4$, 2 g of urea, 0.5 g of KH_2PO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.3 mg of biotin, and 30 g of $CaCO_3$ (pH 7.0).

Recombinant DNA techniques

Standard protocols (Sambrook and Russell 2001) were used for the construction, purification, and analysis of plasmid DNA and for the transformation of *E. coli*. Extraction of *C. glutamicum* chromosomal DNA and transformation of *C. glutamicum* by electroporation were carried out as

described previously (Takeno et al. 2007). PCR was performed using a DNA thermal cycler (GeneAmp PCR System 9700, Applied Biosystems, USA) using Pyrobest DNA Polymerase (Takara Bio, Japan). Sequencing of the *iol* gene cluster and its surrounding region was performed using an ABI PRISM^{TM} 377 DNA sequencer from Applied Biosystems, with ABI PRISM Big Dye Terminator Cycle Sequencing Kit (Perkin Elmer, USA). The subsequent electrophoresis analysis was carried out by Pageset SQC-5ALN 377 (Toyobo, Japan).

Strain construction

For the chromosomal deletion of *ptsH* (Cgl1937), *iolT1* (Cgl0181), *iolT2* (Cgl3058), and Cgl0157, plasmids pCptsHd, pCiolT1d, pCiolT2d, and pC0157d which contained the corresponding genes with internally deleted, respectively, were used to replace the wild-type chromosomal genes with the deleted genes. For the construction of plasmid pCptsHd, the 5'-region of the *ptsH* gene was amplified by PCR using primers ptsHup800F and ptsHFusR with WT-96 genomic DNA as a template. Similarly, the 3'-region of the gene was amplified using primers ptsHFusF and ptsHdown800R. Fusion PCR was then performed using the purified 5'- and 3'-region fragments as templates and the primers ptsHup800F and ptsHdown800R. The resulting 1.6-kb fused fragment was digested with *Bcl*I and then ligated to *Bam*HI-digested pESB30 to yield pCptsHd. Likewise, for the construction of plasmid pCiolT1d, the 5'- and 3'-regions of the *iolT1* gene were amplified using two pairs of primers: the pair comprising Cgl0181delFt and Cgl0181delFusR and the pair comprising Cgl0181delFusF and Cgl0181delR, respectively. Two resulting fragments were fused by PCR and then ligated to pESB30 to yield pCiolT1d. For the construction of plasmid pCiolT2d, the 5'- and 3'-regions of the *iolT2* gene were amplified using two pairs of primers: the pair comprising Cgl3058delF and

Cgl3058delFusR and the pair comprising Cgl3058delFusF and Cgl3058delR, respectively. Two fragments were fused by PCR and then ligated to pESB30 to yield pCiolT2d. On the other hand, plasmid pCgl0157d was constructed as follows. A 2.3-kb fragment comprising Cgl0157 gene was amplified using primers Cgl0157Bam3F and Cgl0157Bam3R, and then ligated to pT7Blue T-Vector (Novagen, Madison, WI) by TA-cloning method (Sambrook and Russell 2001) to yield pT7Cgl0157. Inverse PCR with the pT7Cgl0157 as a template was performed using 5'-phosphorylated inverse primers Cgl0157delF and Cgl0157delR. The resulting 4.6-kb fragment was self-ligated to yield pT7Cgl0157d. A 1.8-kb fragment which contained the internally deleted Cgl0157 was obtained by digestion of pT7Cgl0157d with *Bam*HI, and ligated to *Bam*HI-digested pESB30 to yield pC0157d. Defined chromosomal deletion of the individual gene was accomplished using each plasmid via two recombination events as described previously (Ohnishi et al. 2002).

For the generation of a strain carrying the frameshift mutation in the Cgl0157 gene of strain SPH2, plasmid pC0157delA was constructed as followed. The mutated Cgl0157 gene region was PCR amplified using primers Cgl0157BamF and Cgl0157BamRN with SPH2 genomic DNA as a template. The PCR product was cloned into pESB30 to yield pC0157delA. This plasmid was used to replace the wild-type chromosomal Cgl0157 gene with the mutated allele.

Analysis

Cell growth was monitored by measuring OD_{660} of the culture broth with a Miniphoto 518R spectrophotometer (Taitec, Japan). Glucose concentration was determined using Determinar GL-E (Kyowa Medex, Japan). L-lysine titer was determined as L-lysine hydrochloride by HPLC system

(Shimazu, Japan) after derivatization with *o*-phthalaldehyde.

Results

Isolation of suppressor mutants from a PTS-negative strain

We first aimed at destroying the overall function of the PTS that are required for the import of every PTS-sugar. Disruption of the *ptsH* gene could meet the purpose because it encodes a general component of the PTS. Thus, we constructed a PTS-negative strain, WTΔptsH, through in-frame deletion of the *ptsH* inner sequence from *C. glutamicum* wild-type strain WT-96. As expected, and in agreement with earlier studies (Kotrba et al. 2001; Parche et al. 2001), strain WTΔptsH exhibited a phenotype of little growth on any of the PTS-sugars, namely, glucose, fructose, and sucrose, when these were the sole carbon sources in MM agar plates. When 10^8 cells of strain WTΔptsH were spread on an MM agar plate with glucose, however, colonies appeared spontaneously at a frequency of 10^{-5} after approximately five days of cultivation. One of the suppressor mutants that arose from the PTS-negative strain was designated strain SPH2 and further characterized. Strain SPH2 was capable of growth on glucose but not on either of the other two PTS-sugars in MM agar plates. Growth experiments using liquid MM medium with 1% glucose showed that strain SPH2 actually consumed glucose and grew well on it as shown in Fig. 1, although its growth rate was not completely restored to the wild-type level.

Growth response of strain SPH2 to 2-deoxyglucose

It is generally known for bacteria that 2-deoxyglucose, one of the glucose analogues, is taken up

and phosphorylated to a non-metabolizable toxic phosphate ester by the glucose-PTS owing to its relatively broad substrate specificity, thus inhibiting cell growth under the conditions without glucose (Curtis and Epstein 1975). The glucose analogue has also been shown to be a toxic substrate for the glucose-PTS of *C. glutamicum* (Mori and Shiio 1987). If strain SPH2 uptakes glucose exclusively by a different system from the PTS, the strain may exhibit a phenotype of resistance to 2-deoxyglucose. To examine this possibility, we compared the sensitivity to the glucose analogue between strains WT-96 and SPH2 using MM medium with glucose, ribose, or acetate as the sole carbon source. As shown in Fig. 2, wild-type WT-96 showed clear sensitivity to 0.1% 2-deoxyglucose on the carbon sources other than glucose under both conditions of agar plates (A) and liquid cultures (B), while strain SPH2 exhibited a phenotype of resistance to the analogue on any of the carbon sources. These results indicate that strain SPH2 uptakes and phosphorylates glucose by a 2-deoxyglucose-resistant non-PTS system, probably involving a particular permease and the native glucokinases, either or both of which are assumed to have low affinity for the glucose analogue.

In silico analysis of possible genes for glucose uptake

It is known that the *galP*-encoded galactose permease of *E. coli* transports glucose by a proton symport mechanism (Flores et al. 1996; Chen et al. 1997). Although *C. glutamicum* does not harbor such a galactose permease system, any proteins with homology to the *galP* product, that may be present in *C. glutamicum*, are likely to act as glucose transporters. Based on this idea, the genome sequence of *C. glutamicum* was searched for *galP* homologues. This analysis identified two candidate genes, *iolT1* (Cgl0181) and *iolT2* (Cgl3058), the products of which showed 33 and 30% sequence identity to the *galP* product, respectively. Both genes have recently been reported to

encode *myo*-inositol transporters and to share a high degree of identity with each other (55%) (Krings et al. 2006).

If *myo*-inositol acts as an inducer of the gene(s) and the gene product(s) also functions as a glucose transporter(s), the PTS-negative WTΔptsH strain should be able to consume glucose when cultured in a glucose medium supplemented with *myo*-inositol. As shown in Fig. 3, the co-presence of 0.1% *myo*-inositol with 0.5% glucose in the culture of strain WTΔptsH did cause glucose consumption by the PTS-negative strain and thereby resulted in additional growth, suggesting that glucose uptake was mediated by one or more *myo*-inositol-induced transporters.

Expression of *iolT1* and *iolT2* in the PTS-negative strain

To substantiate the glucose-uptake capabilities of the *iolT1* and *iolT2* gene products, we examined the effect of their individual expression on growth and glucose metabolism in strain WTΔptsH. For this purpose, the coding regions of both genes were separately cloned on a multi-copy vector so as to be constitutively expressed under the promoter of the endogenous *gapA* gene encoding glyceraldehyde 3-phosphate dehydrogenase; the resulting plasmids, pCiolT1 and pCiolT2, were then introduced into strain WTΔptsH. As shown in Fig. 4, either plasmid could confer the ability to grow in MM medium with glucose as the sole carbon source on the PTS-negative strain, proving that each transporter played a role in glucose uptake.

Disruption of *iolT1* and *iolT2* in strain SPH2

Considering the finding, described above, that both of the *myo*-inositol transporters encoded by the *iolT1* and *iolT2* genes mediate glucose uptake, the suppressor strain SPH2 is most likely to depend

on either or both of the transporters for its ability to grow on glucose. In order to identify the transporter(s) functioning in strain SPH2, we disrupted the *iolT1* and *iolT2* genes individually through in-frame deletion of the corresponding inner sequences in the strain and examined the effects of their disruption on the ability to grow on glucose. As shown in Fig. 5, disruption of the *iolT1* gene in strain SPH2 thoroughly abolished growth on glucose, whereas the *iolT2* disruption did not affect growth at all, revealing that *iolT1* was responsible for glucose uptake in strain SPH2.

Identification of the suppressor mutation

Based on the result mentioned above, the suppressor mutation carried by strain SPH2 was assumed to exist on somewhere in the regions that could affect the expression of the *iolT1* gene, most likely in the probable promoter-operator region of *iolT1*or the putative transcriptional regulator genes Cgl0157, Cgl0170, and Cgl0179, within or near the *iol* gene cluster (Fig. 6). To identify the suppressor mutation, we sequenced the relevant regions of strain SPH2. Although there was no mutation in the *iolT1* gene itself, we found a single-base deletion (320delA) in a putative transcriptional regulator gene Cgl0157 (Fig. 6). The single-base deletion causes a frameshift after Lys106 in the polypeptide to create a stop codon 89 residues further downstream, and thus is assumed to impair the function of the gene product. Introduction of the frameshift mutation into strain WTΔptsH actually allowed the resulting strain to grow on glucose: the growth profile in MM medium with glucose as the sole carbon source was the same as that of strain SPH2 (data not shown). When the *iolT1*gene was further disrupted in the carrier of the frameshift mutation, the resulting mutant unquestionably lost the ability to grow on glucose (data not shown). Based on these results, we identified the suppressor mutation as 320delA in the Cgl0157 gene. We also examined the effect of in-frame deletion of the Cgl0157 inner sequence on the growth of strain

WTΔptsH; the resulting growth profile was the same as that observed in the case of strain WTΔptsH harboring the frameshift mutation (data not shown). This result indicates that the inactivation of Cgl0157 enables a PTS-negative strain to grow on glucose through the *iolT1*-specified glucose uptake system.

Lysine production by the PTS-independent glucose uptake system

The results so far obtained show that disruption of the *ptsH* gene and subsequent introduction of the frameshift mutation into the Cgl0157 gene can create a *C. glutamicum* strain expressing the bypass route instead of the original PTS. We applied this method to a defined lysine-producing strain, *C. glutamicum* AHP-3, to assess the practical performance of a strain harboring the bypass route. The resulting strain, designated strain AHPΔptsH320delA, was compared with the reference strain AHP-3 in terms of their production ability. As shown in Fig. 7, strain AHPΔptsH320delA required slightly more time to complete its fermentation than strain AHP-3, but the engineered strain accumulated approximately 20% higher lysine than the reference strain, suggesting the potential of the newly identified glucose uptake system for industrial applications.

Discussion

This study was inspired by the observation that colonies emerged from a PTS-disrupted mutant of *C. glutamicum* on glucose agar plates. This phenomenon was unexpected because, even now, more than ten years after the determination of the whole genome sequence of this microbe, no glucose uptake route other than the PTS is known. The present results not only show that *C. glutamicum* has the potential to utilize other glucose uptake systems as alternatives to the PTS system, but also

illustrate the possibility that the functions of such systems could be applied to improve amino acid production yields. Since worldwide amino acid production from glucose is assumed to depend exclusively on the original PTS in all *C. glutamicum* strains, the discovery of the endogenous PTS-independent glucose uptake routes will introduce new options for the development of more efficient production strains.

The present study has established a strategy for engineering *C. glutamicum* to express the *iolT1*-specified glucose uptake bypass instead of the original PTS. This is comprised of just two steps, (i) disruption of the *ptsH* gene and (ii) introduction of the frameshift mutation (320delA) or deletion of the Cgl0157 gene. There is no doubt that the engineered strain depends exclusively on the *iolT1* gene product, namely *myo*-inositol transporter, for its growth on glucose, because disruption of the *iolT1* gene in the strain thoroughly abolished growth on glucose. The lysine-producing strain thus constructed produced the desired amino acid effectively, demonstrating the usefulness of this engineering strategy in strain improvement. Based on these original results, we have applied for an international patent, which is now open to the world (Ikeda et al. 2010). Although we applied this strategy to lysine fermentation as an example, it should be generally applicable to any producers derived from *C. glutamicum*, regardless of the substances produced.

It is interesting to note that switching the glucose transport systems from the PTS to the *iolT1*-specified bypass offered an advantage in terms of lysine production. The metabolic consequences of different glucose uptake systems have been studied for *E. coli* from the viewpoint of the connection between glucose uptake and PEP utilization (Flores et al. 1996; Chen et al. 1997). An *E. coli* mutant that uses a galactose-proton symport system for glucose uptake instead of the native PTS system has been shown to have the advantage of avoiding PEP depletion for glucose uptake, making more carbon available for aromatic amino acid production that are limited by PEP availability. In theory, the concept that elimination of PTS removes the major pathway of PEP

consumption and thereby increases the availability of PEP for other biosynthetic reactions should be true for *C. glutamicum*. Based on the established knowledge that lysine production by normal PTS strains of *C. glutamicum* is often limited by the pyruvate carboxylase-catalyzed anaplerotic reaction (Peters-Wendisch et al. 2001), it is probable that increased availability of PEP relative to pyruvate in the non-PTS strain of *C. glutamicum* contributes to a better-balanced supply of carbon from central metabolism into the lysine-biosynthetic pathway through the two anaplerotic reactions involving pyruvate carboxylase and PEP carboxylase.

The potential practical advantage of the non-PTS route for glucose uptake is not limited to saving PEP for other biosynthetic reactions, and other advantages can be reasonably expected, considering the known characteristics of the *C. glutamicum* PTS system, *e.g.*, its relative sensitivity to increased osmolality (Gourdon et al. 2003). In addition, we have obtained original preliminary data suggesting that the PTS limits growth of wild-type *C. glutamicum* at elevated temperatures in MM medium containing glucose as the sole carbon source (Ikeda et al. 2010). Based on these findings, the non-PTS route could increase the robustness of *C. glutamicum* strains; this possibility is currently under investigation as our next step after the present study.

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Figure legends

Fig. 1 Growth profile of suppressor mutant SPH2 in MM medium containing 1% glucose as the sole carbon source. For comparison, the profiles of wild-type strain WT-96 and its PTS-negative strain WTΔptsH are shown as controls. Solid and open symbols represent growth and residual glucose, respectively. Symbols: circle, strain WT-96; triangle, strain SPH2; square, strain WTΔptsH. Values are means of duplicated cultures.

Fig. 2 Growth response of suppressor mutant SPH2 to 2-deoxyglucose. (A) Appropriate dilutions (ca. 10^4/ml) of cultures of strain SPH2 and wild-type WT-96 were spotted onto MM agar plates containing 1% of the indicated carbon sources supplemented with (+) and without (-) 0.1% 2-deoxyglucose. Growth was assessed after two days of incubation at 30ºC. (B) Strains SPH2 and WT-96 were grown in MM medium containing 1% of the indicated carbon sources supplemented with (hatched columns) and without (white columns) 0.1% 2-deoxyglucose at 30ºC under aerobic conditions. Values are means of duplicated cultures.

Fig. 3 Effect of *myo*-inositol supplementation on growth and glucose metabolism in PTS-negative strain WTΔptsH. Cultivation was carried out in MM medium containing 0.5% glucose in the presence (circle) and absence (square) of 0.1% *myo*-inositol. Solid and open symbols represent growth and residual glucose, respectively. For reference, growth in MM medium containing only 0.1% *myo*-inositol as the sole carbon source is shown (triangle). Values are means of duplicated cultures.

Fig. 4 Effect of *iolT1* and *iolT2* expression on growth and glucose metabolism in PTS-negative strain WTΔptsH. Cultivation was carried out in MM medium containing 1% glucose as the sole carbon source. For comparison, the profiles of wild-type strain WT-96 and its PTS-negative strain WTΔptsH are shown as controls. Solid and open symbols represent growth and residual glucose, respectively. Symbols: circle, strain WT-96; square, strain WTΔptsH; diamond, strain WTΔptsH carrying plasmid pCiolT1; triangle, strain WTΔptsH carrying plasmid pCiolT2. Values are means of duplicated cultures.

Fig. 5 Effect of *iolT1* and *iolT2* disruption on growth of suppressor mutant SPH2. Cultivation was carried out under the same conditions as Figure 4. For comparison, the profiles of wild-type strain WT-96 and its PTS-negative strain WTΔptsH are shown as controls. Symbols: solid circle, strain WT-96; solid square, strain WTΔptsH; solid triangle, strain SPH2; open diamond, strain SPH2ΔiolT1; open circle, strain SPH2ΔiolT2. Values are means of duplicated cultures.

Fig. 6 Structural organization of the *C. glutamicum iol* gene cluster and its surrounding region. The *iol* genes which are assumed to be involved in the proposed pathway for *myo*-inositol degradation are shown on the corresponding genes. The three genes, Cgl0157, Cgl0170, and Cgl0179, are annotated as putative transcriptional regulators in the *C. glutamicum* genomic database (accession number: BA000036), but there is as yet no report about their functions. The specific mutation (320delA) identified on Cgl0157 of strain SPH2 is indicated (triangle) in the gene.

Fig. 7 Comparison of the defined lysine producer AHP-3 (A) and the newly developed strain AHPΔptsH320delA (B) in terms of ability to produce lysine from glucose. Fermentation was

carried out at 30°C using 500-ml Sakaguchi flasks. Symbols: solid circle, lysine; solid square, growth; solid diamond, glucose. Values are means of triplicated cultures, which differed from each other by <5%. The standard deviations from the means are indicated as error bars.

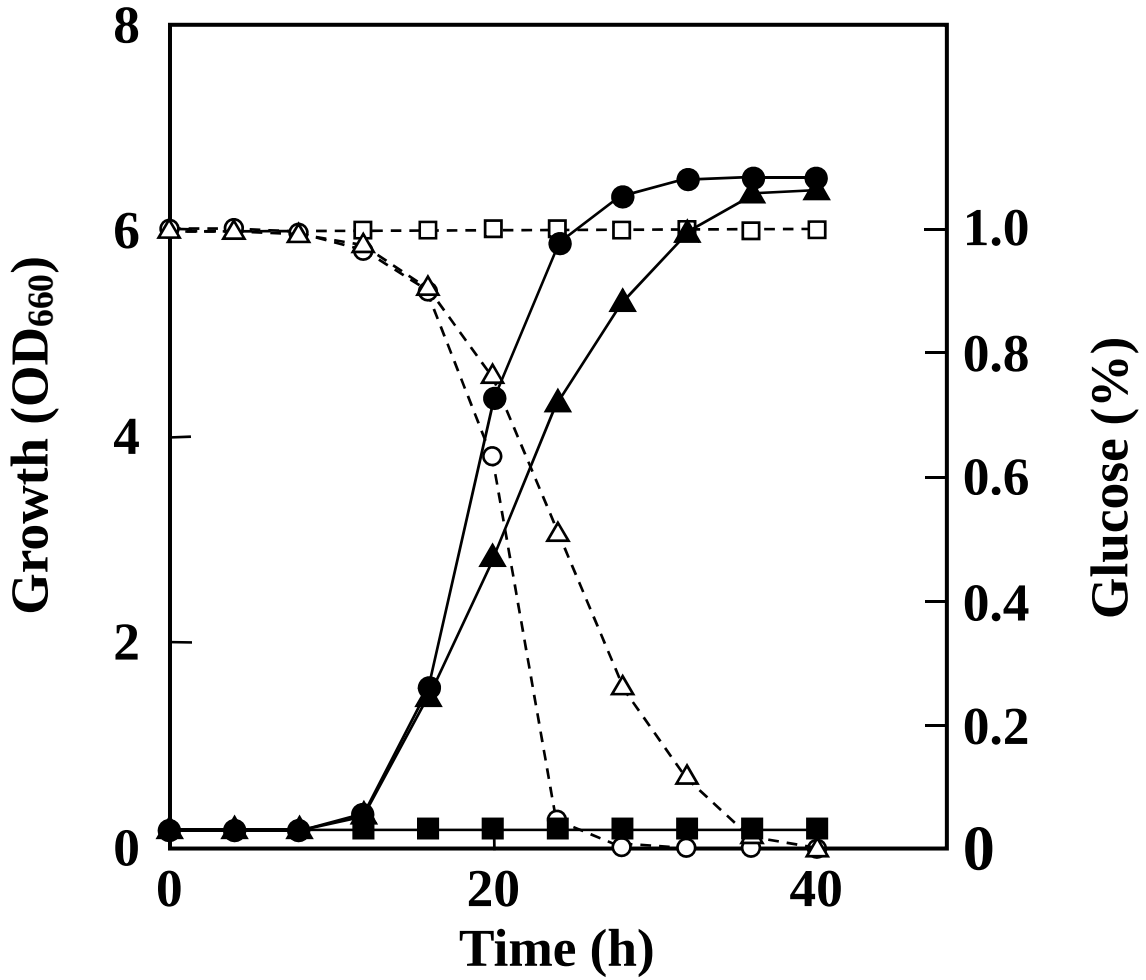
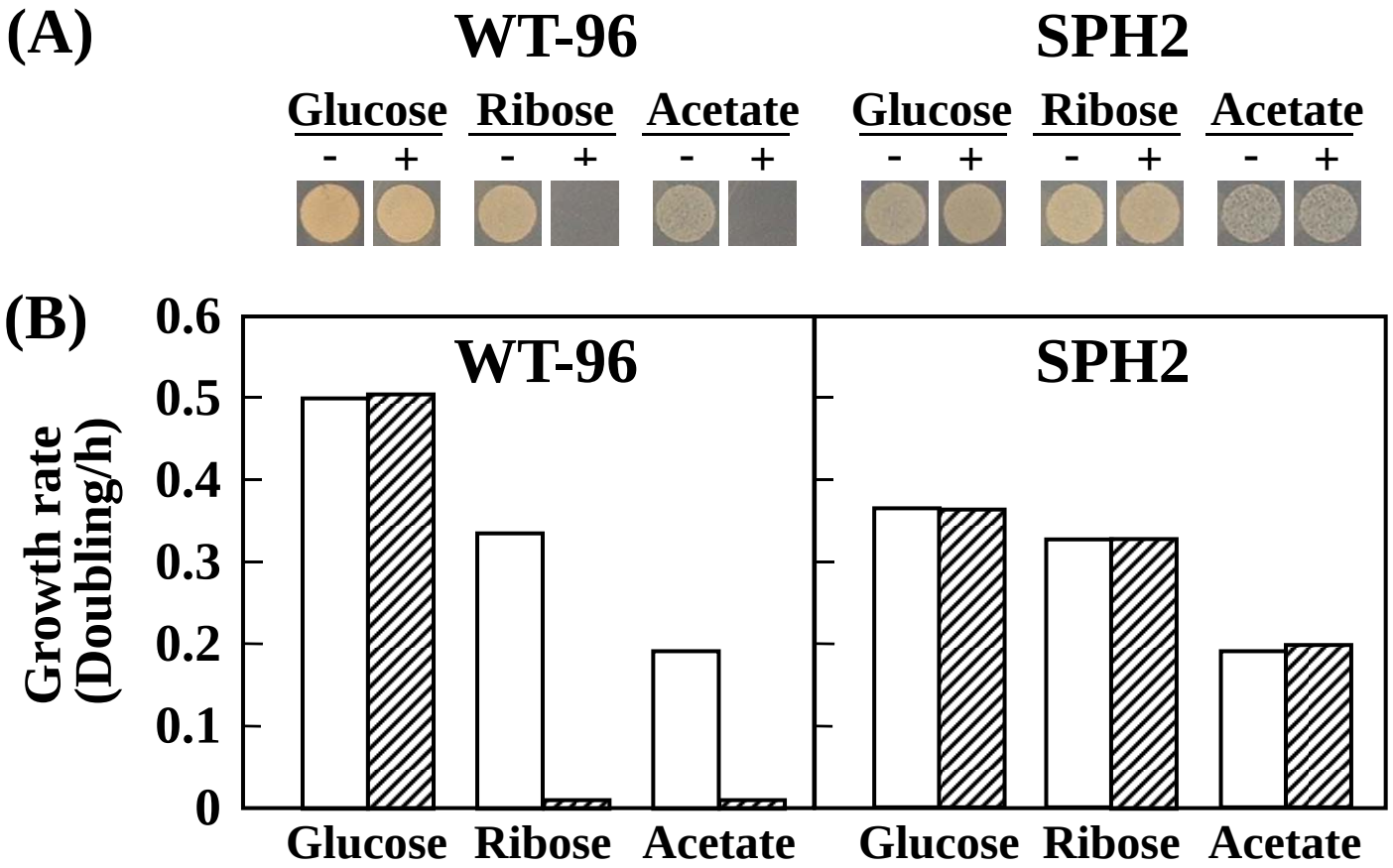


Fig. 1. Ikeda et al.



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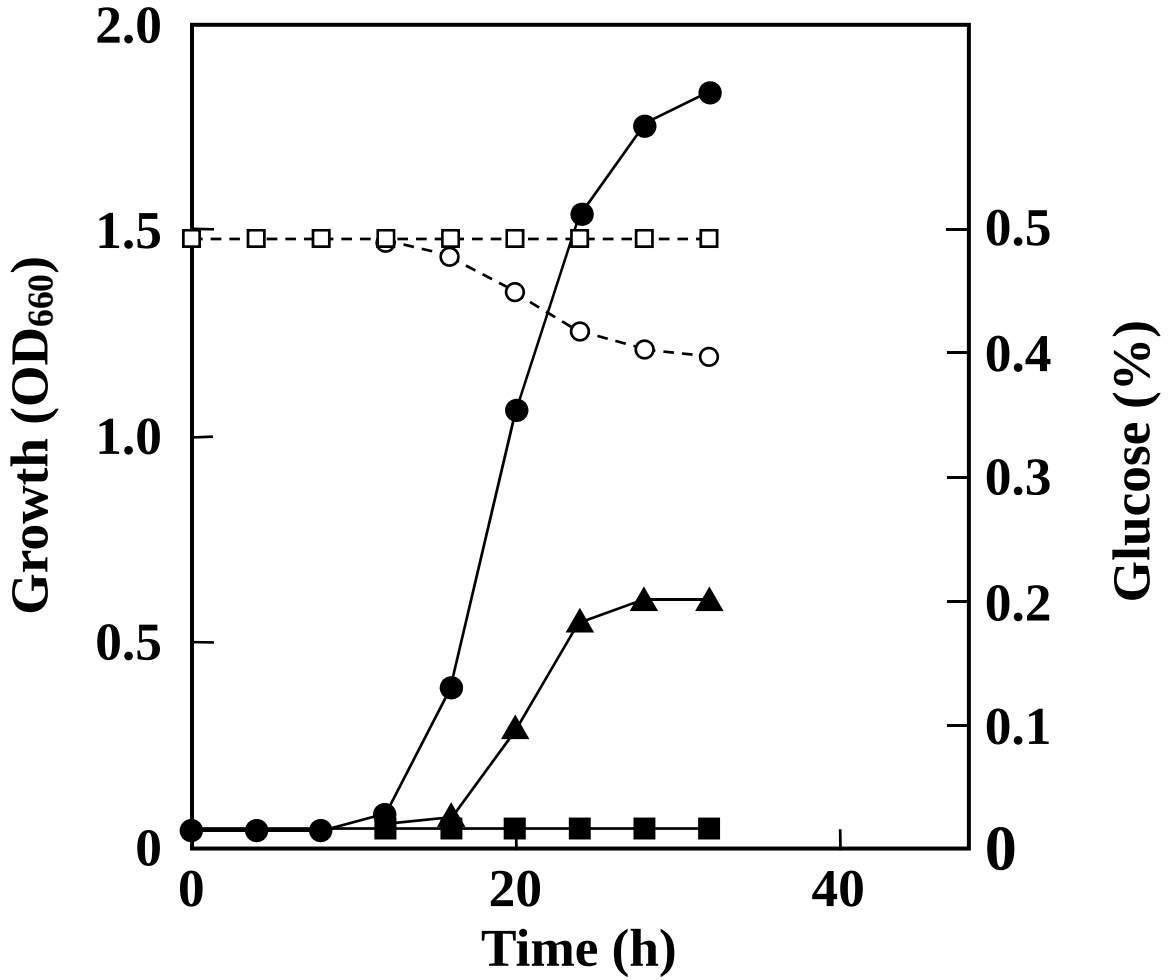

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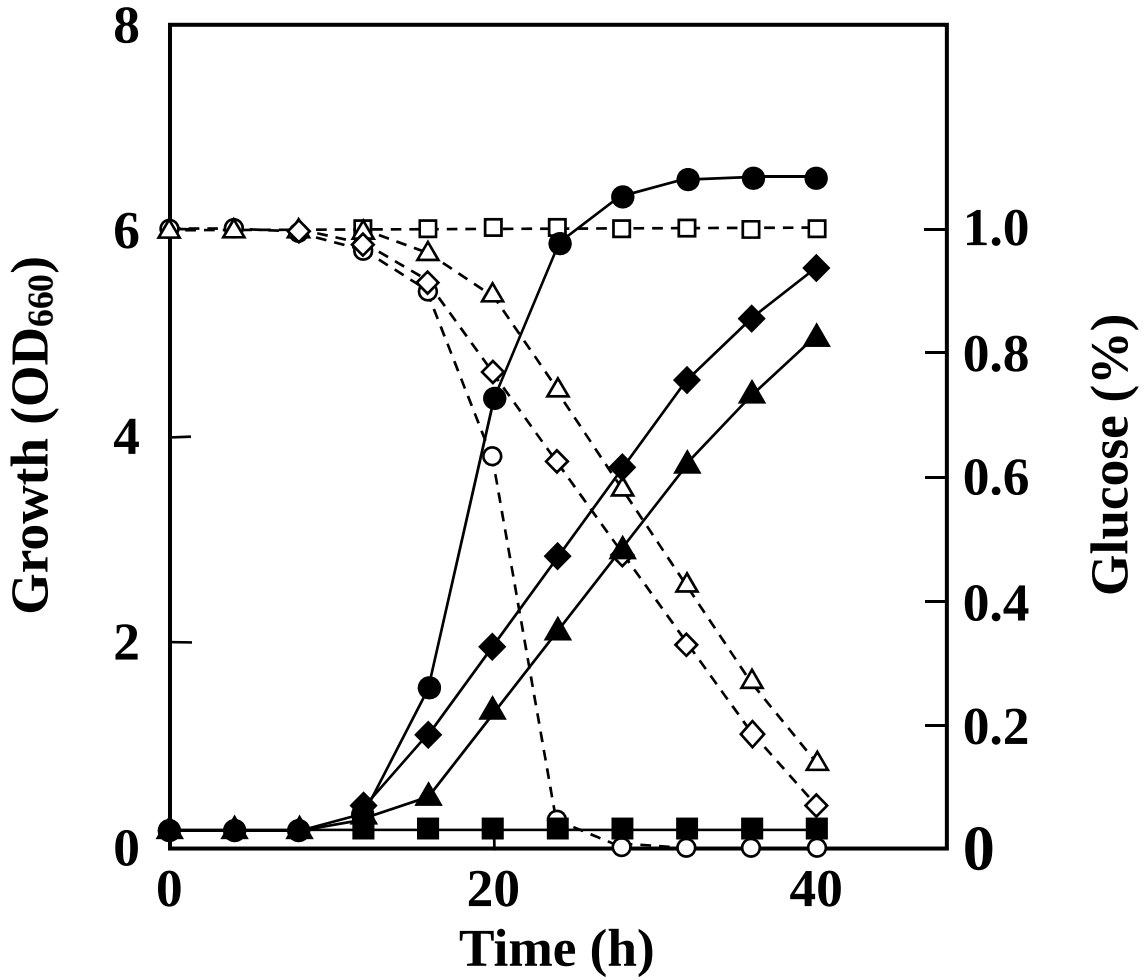

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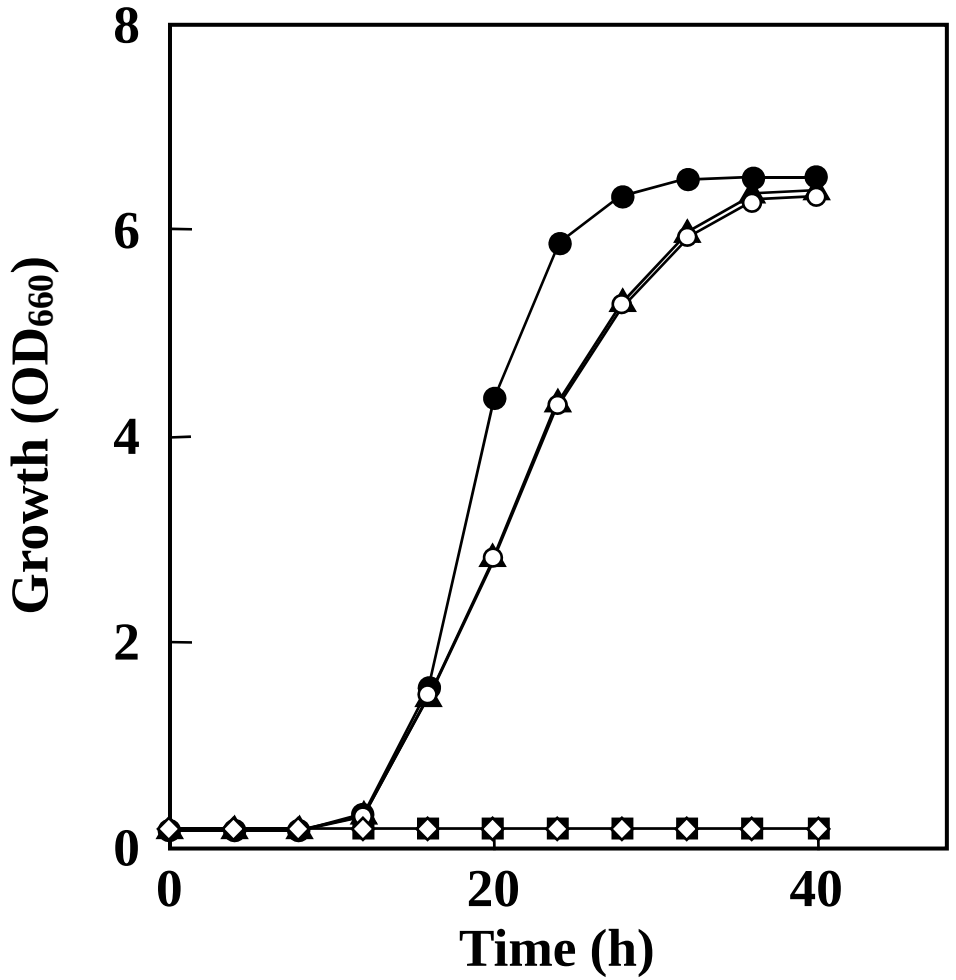

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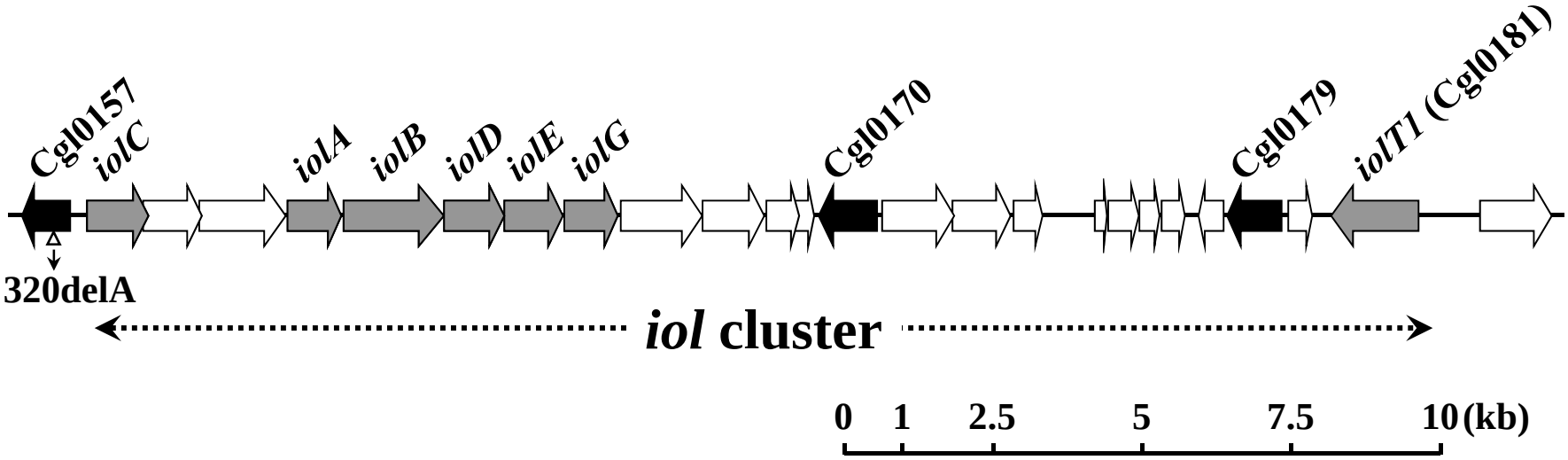

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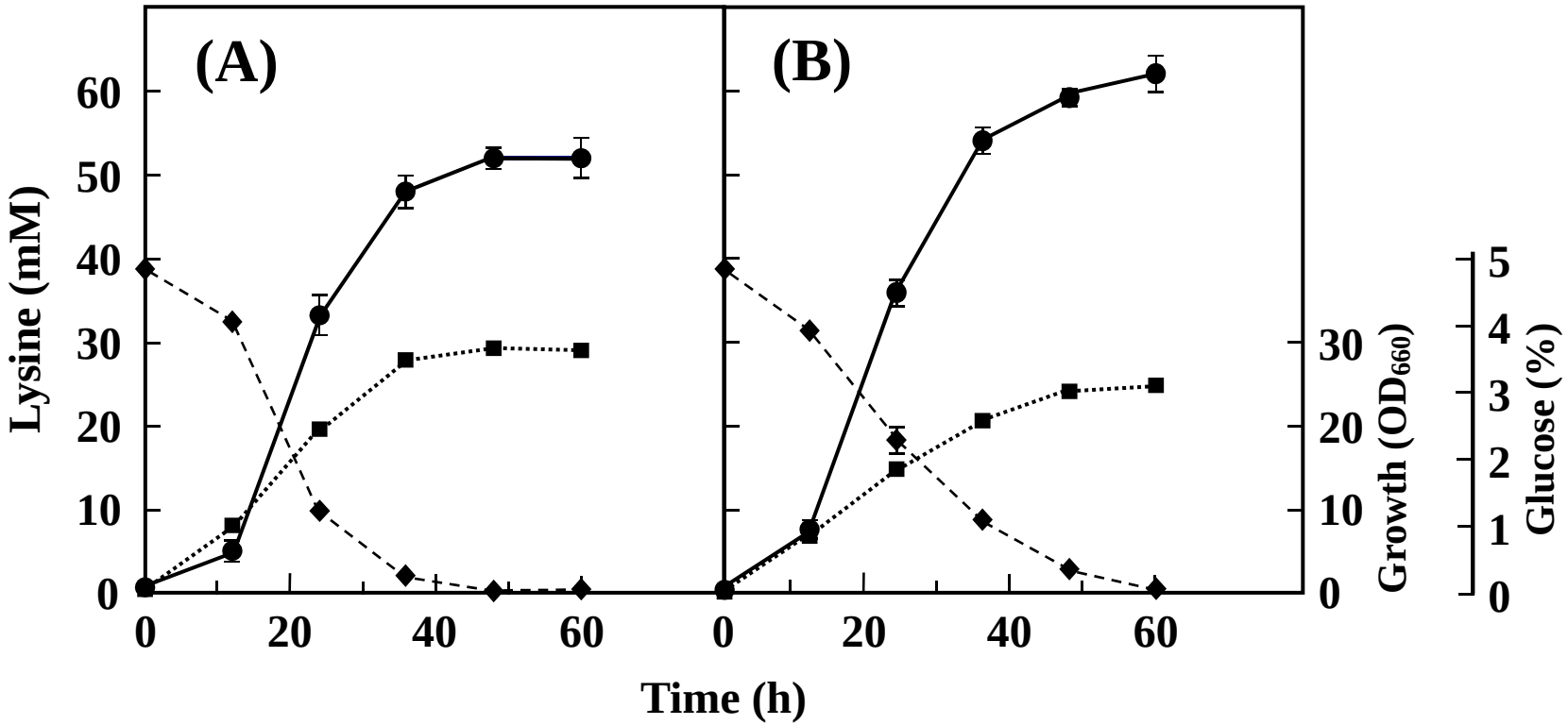

Fig. 7. Ikeda et al.