Journal of Microbiology and Biotechnology
Vol. 5. No. 4, 188-193, 1995

Cloning and Expression of the Gene Encoding Glucose Permease of the Phosphotransferase System from *Brevibacterium flavum* in *Escherichia coli*

KWON, IL, KYU NAM LEE, JUNG KEE LEE, JAE GU PAN, TAE KWANG OH, HYUNG HOAN LEE[1], AND KI HONG YOON*

Korea Research Institute of Bioscience and Biotechnology, KIST, P.O. Box 115, Yoosung, Taejon 305-600,
[1]Department of Biology, Kon-Kuk University, Seoul 133-701, Korea

A *Brevibacterium flavum* gene coding for glucose permease of the phosphoenolpyruvate-dependent phosphotransferase system (PTS) was cloned by complementing the *Escherichia coli* ZSC113 mutations affecting a *ptsG* gene with the *B. flavum* genomic library. From the *E. coli* clone grown as red colony on a MacConkey plate supplemented with glucose as an additional carbon source, a recombinant plasmid was isolated and named pBFT93. The plasmid pBFT93 was identified as carrying a 3.6-kb fragment of *B. flavum* chromosomal DNA which enables the *E. coli* transformant to use glucose or mannose as a sole carbon source in an M9 minimal medium. The non-metabolizable sugar analogues, 2-deoxy-D-glucose (2-DG) and methyl-α-D-glucopyranoside (MeGlc) affected the growth of ZSC113 cells carrying the plasmid pBFT93 on minimal medium supplemented with non-PTS carbohydrate, glycerol, as a sole cabon source, while the analogues did not repress the growth of ZSC113 cells without pBFT93. It was also found that both 2-deoxy-D-[U-^{14}C]glucose and methyl-α-D-[U-^{14}C]glucopyranoside could be effectively transported into ZSC113 cells transformed with plasmid pBFT93. Several *in vivo* complementation studies suggested that the *B. flavum* DNA in pBFT93 encodes a glucose permease specific for glucose and mannose.

In a variety of Gram-negative and Gram-positive bacteria several carbohydrates are transported into the cells and concomitantly phosphorylated by the phosphoenolpyruvate:carbohydrate phosphotransferase system (PTS, 12, 17, 18, 22, 25). The system consists of four energy-coupling proteins, enzyme I (EI), histidine-containing phosphocarrier protein (HPr), enzyme II (EII) and enzyme IIA (EIIA). The general constitutive cytoplasmic phosphoproteins, EI and HPr, are commonly required for the transport of PTS carbohydrates, while sugar-specific permeases, EII and EIIA, determine the sugar specificity of cells (5, 6, 12, 18, 22). The cytoplasmic phosphoproteins sequentially transfer a phosphoryl group from phosphoenolpyruvate (PEP) to membrane-bound EII, which catalyzes the concomitant transport and phosphorylation of carbohydrates. In addition, the fructose-specific hybrid phosphotransfer protein (FPr) is capable of replacing HPr in *Escherichia coli* and *Salmonella typhimurium* mutants lacking HPr (7). Many genes encoding the carbohydrate-specific PTS proteins were recently cloned and sequenced. The various EIIs contain three or four structural domains IIA, IIB, IIC and IID, and are divided into four groups (17, 23). Among them, EII proteins specific for glucose, sucrose or β-glucoside belonging to the same group contain three domains IIA, IIB and IIC. The domain IIA, that interacts with phosphorylated-HPr, exists as a separate protein or as another domains-linked protein.

B. flavum, an industrial coryneform bacterium producing various aromatic amino acids, can efficiently utilize monosaccharides or disaccharides including glucose, fructose, sucrose and maltose as carbon sources (1). Amino acid production is greatly affected by sugar metabolism in the organism. The presence of two PTS systems specific for glucose or fructose was reported in *B. flavum* (15, 16) and *Corynebacterium glutamicum* (11), respectively. It was also reported that sucrose is transported into *B. flavum* cells by PTS system (26). In *B. flavum*, glucose is mainly transported by glucose-specific PTS and the uptaken glucose is metabolized through the Embden-Meyerhof Pathway (EMP, 16). ATP-dependent glucokinase is also related to the uptake of glucose, but

*Corresponding Author
Key words: *Brevibacterium flavum*, phosphotransferase system, glucose permease gene, cloning

the relative activity of this enzyme is very low and negligible as compared to that of PTS. Recently, the gene encoding EII specific for glucose and mannose was cloned and sequenced from *C. glutamicum* (10).

In this work, we describe the molecular cloning of the gene encoding a *B. flavum* glucose permease, and investigate the physiological properties of *E. coli* cells transformed with the cloned gene.

MATERIALS AND METHODS

Bacterial Strains, Plasmids and Media

The bacterial strains and plasmids used in this work are listed in Table 1. *B. flavum* KCTC 1738 was used as a source of the gene coding for glucose permease of the PTS. For *in vivo* complementation studies, *E. coli* mutant strains ZSC113, JLV86 and KCTC 2300 were used. MacConkey indicator plates containing glucose (1%) or fructose (1%) were used to investigate the sugar-fermenting capacities of *E. coli* transformants. *E. coli* and *B. flavum* were cultured at 37°C in LB broth (10 g of tryptone, 5 g yeast extract, 10 g of NaCl per liter, pH 7.0). An M9 minimal medium (6 g Na_2HPO_4, 3 g KH_2PO_4, 0.5 g NaCl, 1 g NH_4Cl, 0.5 g $MgSO_4$, 0.01 g $CaCl_2$ per liter, pH 7.4) containing sugars was used for investigating the effect of sugars on the growth of *E. coli* cells. The minimal medium consisting of 7 g $(NH_4)_2SO_4$, 2 g urea, 0.5 g K_2HPO_4, 0.5 g KH_2PO_4, 0.5 g NaCl, 1 g casamino acid, 0.5 g $MgSO_4$ $7H_2O$, 6 mg $FeSO_4$, 6 mg $MnSO_4$ $6H_2O$, 200 μg biotin, 200 μg thiamin HCl and 1% carbon source per liter (pH 7.5) was used for *B. flavum.*

Chemicals, Enzymes and Isotopes

Restriction endonucleases, calf intestinal alkaline phosphatase, T4 DNA ligase and RNase were obtained from Boehringer Mannheim, and were used as recommended by the manufacturer. The 2-deoxy-D-[U-^{14}C]glucose ([^{14}C]-2DG) and methyl-α-D-[U-^{14}C] glucopyranoside ([^{14}C]-MeGlc) were from Du Pont. Glucose, fructose, sucrose and mannose were purchased from Sigma Chemical Co.

DNA Preparations and Manipulations

For rapid isolation of plasmids from *E. coli* cells, the alkaline lysis method described by Birnboim and Doly (2) was employed. The chromosomal DNA was isolated from the cells of *B. flavum* grown exponentially in LB medium supplemented with glycine (2%) according to the preparative method described by Rodriquez and Tait (19).

Construction of a *B. flavum* Gene Library

Fifty micrograms of the purified *B. flavum* chromosomal DNA was partially digested with *Sau*3AI, and DNA fragments ranging from 2 to 10 kb were isolated by sucrose gradient centrifugation for 20 h at 25,000 rpm in a Beckman SW40 rotor. The Sau3AI-generated chromosomal DNA fragments were ligated to *Bam*HI-digested, dephosphorylated pUC9. The ligation mixture was used to transform *E. coli* ZSC113.

Table 1. Bacterial strains and plasmids.

Strains/Plasmids	Relative properties	Source/Reference
B. flavum KCTC 1738	Gene source	KCTC
E. coli ZSC113	*ptsG, ptsM, glk*	4
E. coli JLV86	*crr, nagE, manI, manA*	29
E. coli KCTC 2300	*HfrKL-16, ptsF*	KCTC
pUC9	Cloning vector	28
pBFT93	Glc$^+$, Man$^+$	This study

Transport Experiment

E. coli cells were grown to late logarithmic phase in M9 minimal medium supplemented with 0.4% non-PTS carbohydrate (glycerol) as a sole carbon source. After centrifugation, cells were washed twice with M9 minimal medium that does not contain the carbon source, and were resuspended in the same medium at $O.D._{600}$=0.5 (0.3 mg dry weight/ml). To decrease the uptake rate of 2-DG in cells, the cell suspension was incubated in ice for 30 min. To 5 ml of the ice-cold cell suspension the [^{14}C]2-DG (0.15 μCi/μmol) or [^{14}C]-MeGlc (0.15 μCi/μmol) was added. Samples of 0.5 ml were taken at 10 sec intervals, filtered through membrane filters (0.45 μm porosity), and washed quickly with the ice-cold suspension medium. Filters with cells were placed in 10 ml of scintillation fluid and counted in a liquid scintillation spectrometer (Beckman).

PTS-Mediated Repression Test

E. coli cells were grown overnight in M9 minimal medium supplemented with 0.2% of glycerol as a sole carbon source, and transferred into the same medium. At the late-logarithmic phase, they were transferred to the same medium, again. When the cultures reached an optical density of approximately 0. 15 at 600 mm, and after adding MeGlc or 2-DG with a final concentration of 10 mM, the cell growth was investigated.

RESULTS AND DISCUSSION

Cloning of a Glucose Permease Gene from *B. flavum*

We cloned a glucose permease gene of *B. flavum* by *in vivo* complementation of a mutant strain, *E. coli* ZSC113, lacking both glucose permease (EII^{Glc}) and mannose permease (EII^{Man}). Identification of *E.*

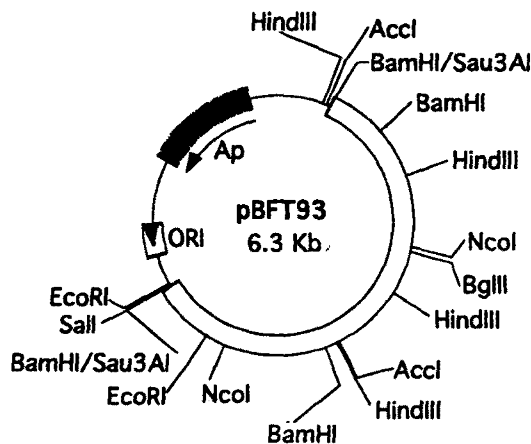

Fig. 1. Restriction endonuclease map of pBFT93.
The open bar represents the glucose permease gene of *B. flavum*.

coli ZSC113 transformants which expressed the EII^{Glc} enzyme was based on the ability of the cells to ferment glucose on MacConkey plate supplemented with glucose (1%) as an additional carbon source. Colonies growing on these plates are expected to become red when the EII^{Glc}-deficient mutation of *E. coli* ZSC113 is functionally complemented by the glucose permease gene of *B. flavum*, and are expected to become white without the gene or when its complementation is ineffective.

A library of *B. flavum Sau*3AI DNA fragments was constructed in the vector pUC9, and was introduced into *E. coli* ZSC113. The transformed cells were plated on MacConkey-glucose plates containing 50 μg/ml of ampicillin. Among approximately 8,000 transformants, one glucose-fermenting colony was obtained as identified by its deep-red color.

From the glucose-fermenting clone, plasmid DNA was found to carry the 3.6-kb DNA fragment of *B. flavum*, and was named pBFT93. A physical map of the plasmid was determined using several restriction enzymes as shown in Fig. 1. The restriction map of the cloned gene in pBFT93 was different from that of the gene encoding *C. glutamicum* EII protein (10) for glucose and mannose although both the *B. flavum* gene of pBFT93 and *C. glutamicum* gene of pCTS3 could transform *E. coli* ZSC113 on MacConkey plates to ferment glucose and mannose, respectively.

Fermentability of *E. coli* ZSC113 Transformant

To investigate the sugar fermentability of the *E. coli* transformant, *E. coli* ZSC113 (pBFT93) and *E. coli* ZSC113 (pUC9) were streaked on MacConkey plates containing various amounts of glucose or mannose (0%~0.5%). After incubating the cells for one day, the color of the colonies was observed as described in Table 2. The strain ZSC113 carrying plasmid pBFT 93 fermented both glucose and mannose to grow red colonies on MacConkey-glucose or MacConkey-mannose plates, while ZSC113 with pUC9 formed white colonies. The intensity of red color produced by the colony of ZSC113 (pBFT93) was increased with the amount of sugars added to the MacConkey plate. The color of colonies became deep-red on the plates containing glucose over 0.3%, but their color was deep-red on the plates containing mannose over 0.5%. Preadapted *E. coli* ZSC113 (pBFT93) and *E. coli* ZSC113 (pUC9) were inoculated in M9 minimal broth containing glucose or mannose as a sole carbon source, and followed by measuring their growth for 20 h (Fig. 2). *E. coli* ZSC113 (pBFT93) could grow in all kind of media except the media lacking a carbon source, but *E. coli* ZSC113 (pUC9) barely grew in any media. It was also observed that the growth in the medium containing 0.4% glucose was more effective than growth in the medium containing 0.4% mannose with shorter lag time. From these results, it can be suggested that the product of the cloned gene is more active on glucose than on mannose.

Table. 2. Sugar fermentation of *E. coli* ZSC113 transformants.

Sugars	Concentration (%)	Fermentability[a] of *E. coli* ZSC113 carrying	
		pUC9	pBF93
Glucose	0.05	-[b]	-
	0.1	-	+[c]
	0.2	-	++
	0.3	-	+++
	0.4	-	+++
	0.5	-	+++
Mannose	0.05	-	-
	0.1	-	-
	0.2	-	+
	0.3	-	+
	0.4	-	++
	0.5	-	+++

[a]Fermentabilities were determined by the color of colonies formed for 24 h on MacConkey agar plates supplemented with various concentration of sugars as an additional carbon source. [b]-, white color by the colonies. [c]+, intensity of red color by the colonies.

To determine whether the cloned 3.6-kb DNA fragment of *B. flavum* complements a *crr* mutation of *E. coli* corresponding to the EIIA protein of the glucose-PTS, pUC9 and pBFT93 was introduced into a *crr* mutant strain, *E. coli* JLV86, respectively. The resulting strain *E. coli* JLV86 (pBFT93) formed red colonies on MacConkey-glucose plates while *E. coli* JLV86 (pUC 9) formed white colonies, indicating that the cloned *B. flavum* gene of pBFT93 complements *crr* mutation

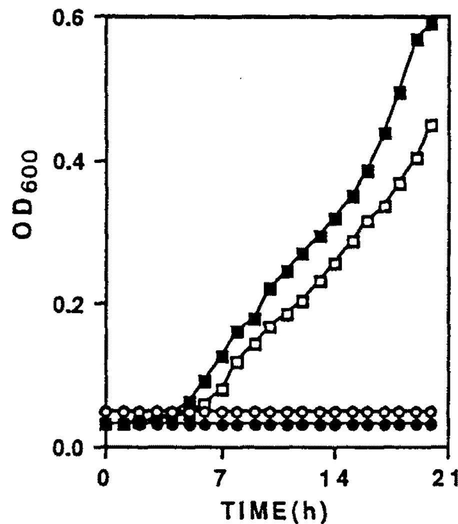

Fig. 2. Growth of *E. coli* ZSC113 carrying pUC9 (circle) and pBFT93 (square).
The cells were grown on M9 minimal medium supplemented with 0.4% glucose (closed) or 0.4% mannose (open).

of *E. coli* (Data not shown.). It was, however, found that pBFT93 could not complement a *ptsF* mutation of *E. coli* KCTC 2300. From these results, it could be suggested that the cloned 3.6-kb insert of pBFT93 carries the gene encoding glucose permease of PTS specific for glucose and mannose, which is capable of complementing *E. coli* mutations affecting *ptsG, ptsM* and *crr* gene.

Two PTSs, which are active on glucose and mannose, have been reported in enteric bacteria (12). One is a glucose-PTS including EII^{Glc}, which interacts with EIIA, more active on glucose than mannose. The other is a mannose-PTS including EII^{Man} active both on mannose and glucose. In fact, it is known that *B. flavum* grows fully on glucose as a sole carbon source, but not on mannose (16). It is therefore assumed that the *B. flavum* gene in pBFT93 corresponds to a *ptsG* gene encoding EII^{Glc}, though it is not clear whether the gene product is a single polypeptide or not

PTS-Mediated Repression by Non-metabolizable Sugar Analogues

In many kinds of heterotrophic bacteria, PTS-mediated repression by non-metabolizable sugar analogues have been reported (3, 13). Phosphorylated forms of sugar analogues, which are not metabolized in cells, cause the cells grown on non-PTS carbohydrate to cease cell growth owing to their toxic effects on the living cells (11, 17).

When the non-metabolizable sugar analogue, MeGlc or 2-DG (10 mM of final concentration), was added to *E. coli* ZSC113 (pBFT93) or *E. coli* ZSC113 (pUC9) growing in M9 minimal medium containing

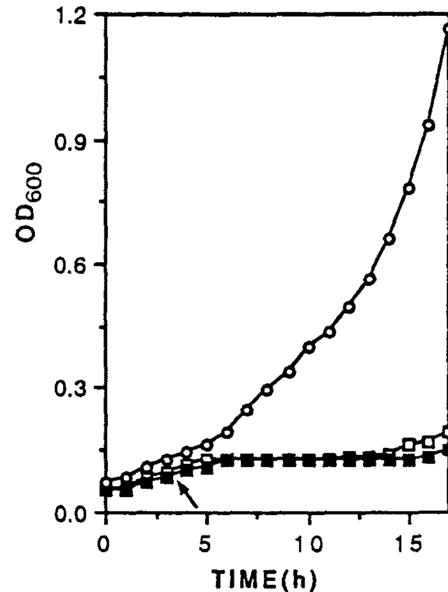

Fig. 3. Effect of non-metabolizable sugar analogues on the growth of *E. coli* ZSC113 carrying pBFT93.
MeGlc (-□-) or 2-DG (-■-) was added to be a final concentration of 10 mM at the point indicated by arrows. Open circle represents the growth of ZSC113 (pBFT93) without the addition of analogues.

non-PTS sugar, glycerol (0.2%), respectively, the growth of *E. coli* ZSC113 (pBFT93) was seriously repressed for 9~10 h (Fig. 3), but *E. coli* ZSC113 (pUC9) showed normal growth on the same medium (data not shown). This indicates that the EII of *B. flavum* is a kind of wide-range permease active on both MeGlc and 2-DG. After MeGlc-mediated growth repression for 9 h, *E. coli* ZSC113 (pBFT93) began to grow, again. It is assumed that the growth is due to the passive efflux of free MeGlc from the cells, in which MeGlc-6-phosphate is hydrolyzed by the intracellular phosphatase (12, 17).

Transport of non-metabolizable Analogues

In order to determine the activity of *B. flavum* EII^{Glc} for glucose transport, the sugar uptake of *B. flavum* and *E. coli* cells carrying plasmids was tested using the structural analogues of glucose, $[^{14}C]$-MeGlc and $[^{14}C]$-2DG. Beacuse the analogues were transported and accumulated in the cells the intracellular amount of the transported analogues could be determined by measuring the redioactivities of the cells (9, 20, 21, 24). As shown in Fig. 4 the analogues were taken more effective by ZSC113 (pBFT93) than ZSC113 (pUC9), confirming that a *B. flavum* glucose permease gene of pBFT93 conferred the ability of sugar uptake on *E. coli* cells. Uptake of $[^{14}C]$-MeGlc by *E. coli* ZSC113 (pBFT93) reached saturation level within 20 sec of reaction time, and then the steady state of intracellular $[^{14}C]$-MeGlc continued for 40 sec. In

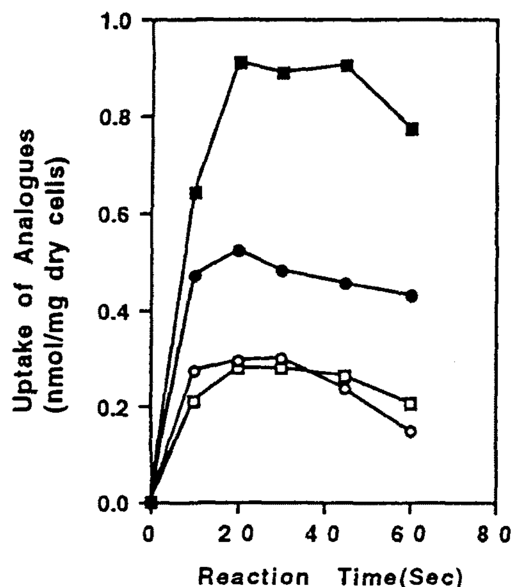

Fig. 4. Transport of [^{14}C]-MeGlc (square) and [^{14}C]-2DG (circle) by *E. coli* ZSC113 cells carrying pUC9 (open) or pBFT93 (closed).

E. coli ZSC113 (pUC9) was grown on 0.4% glycerol and *E. coli* ZSC 113 (pBFT93) was grown on 0.4% glucose as a sole carbon source, respectively.

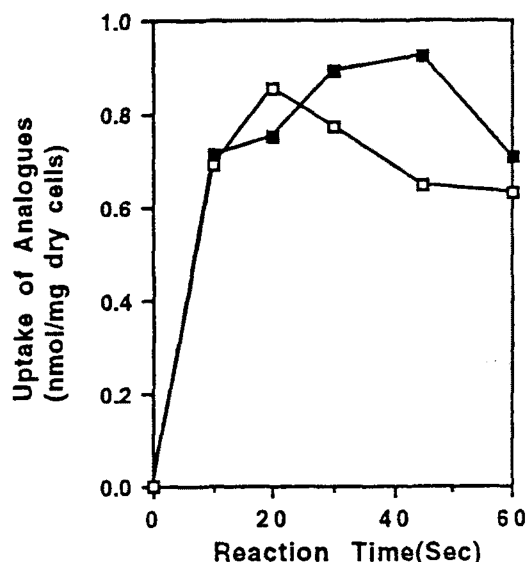

Fig. 5. Transport of [^{14}C]-MeGlc (-□-) and [^{14}C]-2DG (-■-) by *B. flavum* cells.

The cells were grown on minimal medium containing glucose (1%) as a sole carbon source.

case of [^{14}C]-2DG uptake of ZSC113 (pBFT93), the reaction time required to reach saturation level was similar to those of [^{14}C]-MeGlc but the steady state of intracellular [^{14}C]-2DG was rapidly destroyed, unlike [^{14}C]-MeGlc. Also, the uptake rate of [^{14}C]-MeGlc was about 1.8 times as high as that of [^{14}C]-2DG.

It was also found that the analogues were rapidly transported into cells of *B. flavum* grown in minimal medium supplemented with 1% sodium lactate or 1% glucose (Fig. 5). The intracellular amount of [^{14}C]-2DG was continuously maintained, even as high as that of the [^{14}C]-MeGlc in *B. flavum* cells. One possible explanation for the difference of 2-DG uptake level between *B. flavum* and ZSC113 (pBFT93) may be owing to conformational change of the *B. flavum* glucose permease in the membrane of *E. coli* because the enzyme is an integral protein.

REFERENCES

1. Abe, S. and K. Takayama. 1972. Amino acid-producing microorganisms: variety and classification, p. 3-38. *In* K. Yamada, S. Kinoshita, T. Tsunoda, and K. Aida (ed.), *The microbial production of amino acids.* p. 3-38. John Wiley & Sons, New York. London Sydney. Toronto.
2. Birnboim, H. C. and J. Doly. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.* 7: 1515-1523.
3. Bouma, C. L., N. D. Meadow, E. W. Stover, and S. Roseman. 1987. II-B^{Glc}, a glucose receptor of the bacterial phosphotransferase system: Molecular cloning of *ptsG* and purification of the receptor from an overproducing strain of *Escherichia coli. Proc. Natl. Acad. Sci.* **84**: 930-934.
4. Curtis, S. J. and W. Epstein. 1975. Phosphorylation of D-glucose in *Escherichia coli* mutants defective in glucose phosphotransferase, mannose phosphotransferase, and glucokinase. *J. Bacteriol.* **122**: 1189-1199.
5. Erni, B. and B. Zanolari. 1986. Glucose-permease of the bacterial phosphotransferase system. *J. Biol. Chem.* **261**: 16398-16403.
6. Erni, B. and B. Zanolari. 1985. The mannose-permease of bacterial phosphotransferase system. *J. Biol. Chem.* **260**: 15495-15503.
7. Geerse R. H., C. R. Ruig, A. R. J. Schuitema, and P. W. Postma. 1986. Relationship between pseudo-HPr and the PEP: fructose phosphotransferase system in *Salmonella typhimurium* and *Escherichia coli. Mol. Gen. Genet.* **203**: 435-444.
8. Gonzy-Treboul, G., J. H. de Waard, M. Zagorec, and P. W. Postma. 1991. The glucose permease of the phosphotransferase system of *Bacillus subtilis*: evidence for II^{Glc} and III^{Glc} domains. *Mol. Microbiol.* **5**: 1241-1249.
9. Liu, K. D. and S. Roseman. 1983. Kinetic characterization and regulation of phosphoenolpyruvate-dependent methyl-α-D-glucopyranoside transport by *Salmonella typhimurium* membrane vesicles. *Proc. Natl. Acad. Sci.* USA. **80**: 7142-7145.
10. Lee, J.-K., M.-H. Sung, K.-H. Yoon, J.-G. Pan, J.-H. Yu, and T.-K. Oh. 1993. Cloning and expression of the gene encoding mannose enzyme II of the *Corynebacterium glutamicum* phosphoenolpyruvate-dependent phosphotransferase system in *Escherichia coli.*

J. Microbiol. Biotechnol. **3**: 1-5.

11. Malin, G. M. and G. I. Bourd. 1991. Phosphotransferase-dependent glucose transport in *Corynebacterium glutamicum*. *J. Appl. Bacteriol.* **71**: 517-523.
12. Meadow, N. D., D. K. Fox, and S. Roseman. 1990. The bacterial phosphoenolpyruvate:glucose phosphotransferase system. *Annu. Rev. Biochem.* **59**: 497-542.
13. Misko, T. P., W. J. Mitcheii, N. D. Meadow, and S. Roseman. 1987. Sugar transport by the bacterial phosphotransferase system. *J. Biol. Chem.* **262**: 16261-16266.
14. Mitchell, J. W., D. W. Saffens, and S. Roseman. 1987. Sugar transport by the bacterial phosphotransferase system. *J. Bacteriol.* **252**: 16254-16260.
15. Mori, M., and I. Shiio. 1987. Pyruvate formation and sugar metabolism in an amino acid-producing bacterium, *Brevibacterium flavum*. *Agric. Biol. Chem.* **51**: 129-138.
16. Mori, M. and I. Shiio. 1987. Phosphoenolpyruvate: sugar phosphotransferase systems and sugar metabolism in *Brevibacterium flavum*. *Agric. Biol. Chem.* **51**: 2671-2678.
17. Postma, P, W., J. W. Lengeler, and G. R. Jacobson. 1993. Phosphoenolpyruvate:carbohydrate phosphotransferase systems of bacteria. *Microbiol. Rev.* **57**: 543-594.
18. Postma, P, W. and J. W. Lengeler. 1985. Phosphoenolpyruvate:carbohydrate phosphotransferase systems of bacteria. *Microbiol. Rev.* **49**: 232-269.
19. Rodriguez, R. L. and R. C. Tait. 1983. *Recombinant DNA techniques-An introduction*, p. 45-46. Addison-Weslley Pub. Co.
20. Romano, A. H., J. D. Trifone, and M. Brustolon. 1979. Distribution of the phosphoenolpyruvate:glucose phosphotransferase system in fermentative bacteria. *J. Bacteriol.* **139**: 93-97.
21. Romano, A. H., S. J. Eberhard, S. L. Dingle, and T. D. McDowell. 1970. Distribution of the phosphoenolpyruvate: glucose phosphotransferase system in bacteria. *J. Bacteriol.* **104**: 808-813.
22. Ruyter, G. J. G., P. W. Postma, and K. VAN Dam. 1991. Control of glucose metabolism by enzyme II^{Glc} of the phosphoenolpyruvate-dependent phosphotransferase system in *Escherichia coli*. *J. Bacteriol.* **173**: 6184-6191.
23. Saier, M. H. Jr. and J. Reizer. 1992. Proposed uniform nomenclature for the proteins and protein domains of the bacterial phosphoenolpyruvate: sugar phosphotransferase system. *J. Bacteriol.* **174**: 1433-1438.
24. Saier, M. H., Jr., B. U. Feucht, and W. K. Mora. 1977. Sugar phosphate: sugar transphosphorylation and exchange group translocation catalyzed by the enzyme II complexes of the bacterial phosphoenolpyruvate: sugar phosphotransferase system. *J. Biol. Chem.* **252**: 8899-8907.
25. Saier, M. H. Jr., D. F. Cox, and E. G. Moczydlowski. 1977. Sugar phosphate:sugar transphosphorylation coupled to exchange group translocation catalyzed by the enzyme II complexes of the phosphoenolpyruvate: sugar phosphotransferase system in membrane vesicles of *Escherichia coli*. *J. Biol. Chem.* **252**: 8908-8916.
26. Shiio, I., S. Sugimoto, and K. Kawamura. 1990. Effect of carbon source sugars on the yield of amino acid production and sucrose metabolism in *Brevibacterium flavum*. *Agri. Biol. Chem.* **54**: 1513-1519.
27. Stock, J. B., E. B. Waygood, N. D. Meadow, P. W. Postma, and S. Roseman. 1982. Sugar transport by the bacterial transport system: The glucose receptors of the *Salmonella typhimurium* phosphotransferase system. *J. Biol. Chem.* **257**: 14543-14552.
28. Vieira, J. and J. Messing. 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. *Gene* **19**: 259-268.
29. Vogler, A. P., C. P. Broekhuizen, A. Schuitema, J. W. Lengeler, and P. W. Postma. 1988. Suppression of III^{Glc}-defects by Enzyme II^{Nag} and II^{Bgl} of the PEP: carbohydrate phosphotransferase system. *Mol. Microbiol.* **2**: 719-726.

(Received April 11, 1995)