Cloning, Expression, and Nucleotide Sequencing of the Gene Encoding Glucose Permease of Phosphotransferase System from *Brevibacterium ammoniagenes*

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Abstract A *Brevibacterium ammoniagenes* gene coding for glucose/mannose-specific enzyme II (EII^{Glc}) of the phosphoenolpyruvate-dependent phosphotransferase system (PTS) was cloned by complementing an *Escherichia coli* mutation affecting a *ptsG* gene, and the complete DNA nucleotide sequence was determined. The cloned gene was identified to be a *ptsG*, which enables the *E. coli* transformant to use glucose more efficiently than mannose as the sole carbon source in an M9 minimal medium. The *ptsG* gene of *B. ammoniagenes* consists of an open reading frame of 1,983 nucleotides putatively encoding a polypeptide of 661 amino acid residues and a TAA stop codon. The deduced amino acid sequence of the *B. ammoniagenes* EII^{Glc} shows, at 46%, the highest degree of sequence similarity with the *Corynebacterium glutamicum* EII specific for both glucose and mannose. In addition, the EII^{Glc} shares approximately 30% sequence similarities with sucrose-specific and β-glucoside-specific EIIs of the several bacteria belonging to the glucose-PTS class. The 161-amino-acid C-terminal sequence of EII^{Glc} is also similar to that of *E. coli* enzyme IIA^{Glc}, specific for glucose ($EIIA^{Glc}$). The *B. ammoniagenes* EII^{Glc} consists of three domains; a hydrophobic region (EIIC) and two hydrophilic regions (EIIA, EIIB). The arrangement of structural domains, IIBCA, of the EII^{Glc} is identical to those of EIIs specific for sucrose or β-glucoside. While the domain IIA was removed from the *B. ammoniagenes* EII^{Glc}, the remaining domains IIBC were found to restore the glucose and mannose-utilizing capacity of *E. coli* mutant lacking EII^{Glc} activity with $EIIA^{Glc}$ of the *E. coli* mutant. EII^{Glc} contains a histidine residue and a cysteine residue which are putative phosphorylation sites for the protein.

Key words: *Brevibacterium ammoniagenes*, glucose-specific Enzyme II, phosphotransferase system, *ptsG* gene, cloning, nucleotide sequence

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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) [18]. The system consists of two general constitutive cytoplasmic phosphoproteins and sugar-specific permeases consisting of either one or several polypeptide chains, commonly designated Enzyme II (EII). The cytoplasmic phosphoproteins, Enzyme I (EI) and Histidine-containing phosphocarrier protein (HPr), are required for the transport of PTS carbohydrates. 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 [11].

The various EIIs contain three or four structural domains, namely, IIA, IIB, IIC, and IID [25], and have been grouped into four distinct classes on the basis of the alignments of their amino acid sequences as follows: glucose-PTS, mannose-PTS, lactose-PTS, and mannitol-PTS [22]. Among them, EII proteins specific for glucose, sucrose, or β-glucoside belonging to the glucose-PTS class 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. The previously reported EIIs including the sucrose-specific EII (EII^{Scr}) of *Streptococcus mutans* [28], the β-glucoside-specific EII (EII^{Bgl}) of *E. coli* [3], and the EII^{Bgl} and the glucose-specific EII (EII^{Glc}) of *Bacillus subtilis* [32] contain IIA, IIB, and IIC domains in a single polypeptide with a molecular weight of 65,000~75,000 daltons. The EII^{Glc} [9] and the EII^{Scr} [7] of *E. coli* have similar molecular weights when the IIBC and separate protein IIA pairs are considered together.

The mannose-specific EII (EII^{Man}) proteins of the mannose-PTS class are involved in glucose transport as

well as mannose transport in bacteria including *E. coli* [10], *Lactobacillus curvatus* [30], and *Vibrio furnissii* [2]. The EII^{Man} proteins are composed of four domains, IIA, IIB, IIC, and IID, and their amino acid sequences are not homologous to those of $EIIs^{Glc}$ while they have the specificity for glucose.

The gram-positive corynebacteria, including *Brevibacterium lactofermentum*, *B. flavum*, *Corynebacterium glutamicum*, and *B. ammoniagenes* have been used for industrial production of various amino acids and nucleotides. The presence of two PTS systems specific for glucose or fructose was reported in *B. flavum* [20] and *C. glutamicum* [17], respectively. Recently, the genes encoding EII specific for both glucose and mannose were cloned from *B. flavum* [12] and *C. glutamicum* [15], and the EII gene of *C. glutamicum* was sequenced [16]. There were no reports on the glucose transport system of *B. ammoniagenes* although glucose has been used as a fermentative sugar for nucleotide production by *B. ammoniagenes*.

In order to characterize the sugar-specific permease which plays a major role in the utilization of the fermentative sugar for production of nucleotides, we have undertaken the cloning and sequencing of the gene encoding the glucose-specific permease from *B. ammoniagenes*. The present work describes the cloning, identification, and sequencing of a complete *ptsG* gene that codes for an EII^{Glc}, and compares the deduced amino acid sequence with those of other EIIs.

MATERIALS AND METHODS

Chemicals, Enzymes and Isotopes

Restriction endonucleases, DNA polymerase (Klenow fragment), T4 DNA ligase, and RNase were obtained from Boehringer Mannheim (Mannheim, Germany), and were used as recommended by the manufacturers. Sequenase, a modified T7 DNA polymerase, was obtained from U.S. Biochemical Co. (Cleveland, OH, U.S.A.). The $[\alpha^{-35}S]$ dATP (1,000 Ci/mmol) was purchased from Amersham Co. (Buckinghamshire, England). Ampicillin, mannose, and glucose were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). MacConkey agar base was purchased from Difco (Detroit, MI, U.S.A.).

Bacterial Strains, Plasmids and Media

B. ammoniagenes ATCC 6872 was used as a source of the gene coding for EII^{Glc} of PTS. *E. coli* JM109 (*recA*1 *supE*44 *endA*1 *hsdR*17 *gyrA*96 *relA*1 *thi* Δ (*lac-proAB*) F'[*traD*36 *proAB*+ *lacI*^{q} *lacZ*ΔM15]) was used as a host for cloning experiments. For *in vivo* complementation studies, *E. coli* mutant strain ZSC113 [5] carrying the *ptsG*, *ptsM*, and *glk* mutations was used. The plasmids

pBR322 and pUC19 were used for all cloning and sequencing experiments. MacConkey indicator plates containing glucose or mannose were used to investigate the sugar-fermenting capacities of *E. coli* transformants. 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) supplemented with thiamine and sugars was used for investigating the effect of sugars on growth of *E. coli* cells.

DNA Manipulation and Construction of a *B. ammoniagenes* Genomic Library

The standard procedures of Sambrook *et al.* [26] were used for DNA manipulation. The chromosomal DNA was isolated from *B. ammoniagenes* cells grown exponentially in LB medium supplemented with 1% glycine according to the preparative method described by Rodriquez and Tait [24]. Fifty micrograms of the isolated *B. ammoniagenes* chromosomal DNA was partially digested with *Sau*3AI, and DNA fragments ranging from 2 to 10 kb were fractionated by sucrose gradient centrifugation for 20 h at 25,000 rpm in a Beckman SW40 rotor. The *Sau*3AI-generated chromosomal DNA fragments were ligated to *Bam*HI-digested, dephosphorylated pBR322. The ligation mixture was used to transform *E. coli* ZSC113 by the electroporation method [6].

DNA Sequencing and Computer Analysis

Restriction endonuclease-generated DNA fragments of the *B. ammoniagenes* chromosomal DNA were subcloned into pUC19. The nucleotide sequences of the fragments were determined using the dideoxy-chain termination method [27] with a Sequenase ver. 2.0 Kit and double-stranded DNAs as templates. The reverse primer from Phamacia (Uppsala, Sweden) was used for sequencing double-stranded DNAs. The DNA and protein sequences were analyzed by using the DNASIS program.

RESULTS AND DISCUSSION

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

A glucose permease gene was cloned from *B. ammoniagenes* by *in vivo* complementation of a *E. coli* mutant strain lacking EII^{Glc}. Identification of *E. coli* ZSC113 transformants, which the expressed EII^{Glc} enzyme, was based on the ability of the cells to utilize glucose on MacConkey plates supplemented with glucose (1%) as an additional carbon source. Colonies growing on these agar plates are expected to become red when the EII^{Glc} activity of *E. coli* ZSC113 is functionally complemented by the glucose permease gene of *B. ammoniagenes*, and
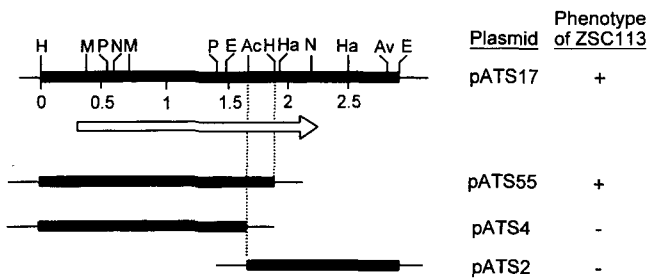
Fig. 1. Restriction endonuclease map and characterization of the deletion derivatives of pATS17 containing the *B. ammoniagenes* chromosomal DNA.

Phenotype of *E. coli* ZSC113 transformed with these plasmids was determined by checking the color of their colonies grown overnight on MacConkey-glucose plates; +, generated red colonies; -, generated white or pale-red colonies. Box and line indicate the DNA regions corresponding to the *B. ammoniagenes* DNA and pBR322, respectively. Below the box of recombinant plasmids, the bold lines denote the 1,983 nucleotides encoding the glucose-specific EII with the arrow indicating the direction of transcription. Restriction site abbreviations are as follows: Ac, *Acc*I; Av, *Ava*I; E, *Eco*RI; H, *Hin*dIII; Ha, *Hpa*I; M, *Mlu*I; N, *Nco*I; P, *Pvu*II.

Table 1. Sugar utilization of *E. coli* ZSC113 transformants.

Sugars	Concentration (%)	Phenotype[a] of strain ZSC113 carrying plasmid	
		pBR322	pATS17
Glucose	0.1	-	-
	0.2	-	+/-
	0.3	-	+
	0.4	-	++
	0.5	-	++
	2.0	-	++
Mannose	0.1	-	-
	0.2	-	-
	0.3	-	+/-
	0.4	-	+
	0.5	-	+
	2.0	-	+

[a]Phenotype of *E. coli* ZSC113 was scored by the color of colonies formed overnight on MacConkey agar containing various amounts of sugars as an additional carbon source. ++, deep-red colonies; +, red colonies; +/-, faint red/pink colonies; -, white colonies.

are expected to become white without the gene or when its complementation is ineffective.

A library of *B. ammoniagenes Sau*3AI DNA fragments was constructed in the vector pBR322, and transformed into *E. coli* ZSC113. The cells were plated on MacConkey-glucose plate containing ampicillin. Among approximately 10,000 transformants, one glucose-utilizing colony was obtained by scoring its deep-red color. The plasmid DNA was isolated from this clone, and its insert DNA was reduced by subcloning a 2.8-kb *Hin*dIII and *Eco*RI fragment capable of complementing the *ptsG* mutation of ZSC113 into pBR322, named pATS17. A physical map of the *B. ammoniagenes* chromosomal DNA fragment in pATS17 was determined using several restriction enzymes (Fig. 1). To locate the glucose permease gene on the 2.8-kb *B. ammoniagenes* DNA fragment of pATS17, three subclones were constructed by using the restriction enconucleases, *Hin*dIII for pATS 55, *Hin*dIII and *Acc*I for pATS4, and *Eco*RI and *Acc*I for pATS2, respectively. The resultant plasmids and phenotypes of *E. coli* ZSC113 transformed with them on MacConkey-glucose plates are shown in Fig 1.

Utilization of *E. coli* ZSC113 Transformant

To investigate the sugar utilization of *E. coli* transformants, *E. coli* ZSC113 (pATS17) and *E. coli* ZSC113 (pBR322) were streaked on MacConkey plates containing various amounts of glucose or mannose (0~2.0%). After incubating the cells for 24 h, the colors of the colonies were observed as described in Table 1. The strain ZSC 113 carrying plasmid pATS17 utilized both glucose and mannose to become red colonies on MacConkey-glucose or MacConkey-mannose plates, while ZSC113 with

pBR322 formed white colonies. The intensity of the red color produced by the colony of ZSC113 (pATS17) increased with the amount of sugars added to the MacConkey plate. The color of colonies became deep-red on the plates containing glucose of over 0.3%, but their color remained red on the plates containing 2.0% mannose. The ZSC113 (pATS55) was identified to show the same phenotypes on the MacConkey-glucose and MacConkey-mannose plates as ZSC113 (pATS17) (data not shown).
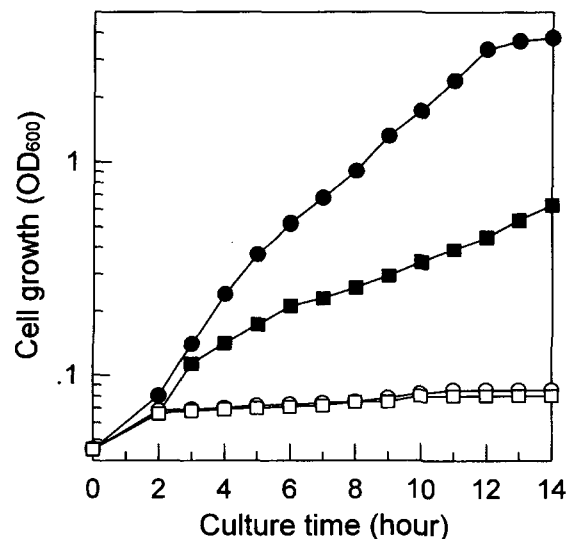

Fig. 2. Growth of *E. coli* ZSC113 carrying pBR322 (open) and pATS17 (closed).

The cells were grown in M9 minimal medium supplemented with 0.4% glucose (circle) and 0.4% mannose (square), respectively.

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AAGCTTGCTGGTGGCGAGAACTAATTCTCACCGCTAAATAGCTAATAGCAACTAGCTAAGAAAACGCCCGCCGCTTCATTGCGGTGGGGTTTTTCTCTTG  100

CCAAAACCTGAAAATTTAATTAAATGTTCAATAACAGTTGATTTTATCTGCACAGTCTGCTTATTATGTAACCTACAACACCCACAGGGTTGTTACTCGT  200

AATGGAGGCAAGACCTGAGTGGCTTTTCTATTGTGAATTGCCGCTCAGGTCTTTTCGTGTTTTACCGAGAATGAAAGAAGGTACATCTCATGTCCAAGAC  300
                                                                                M  S  K  T

TGAGATGCGCACCGCCGCAGAGGAAATCCTCGCAGGCATTGGCGGGGCGGATAATATCGCATCATTTACGCACTGCGCAACGCGTTTACGCTTTGAGCTT  400
 E  M  R  T  A  A  E  E  I  L  A  G  I  G  G  A  D  N  I  A  S  F  T  H  C  A  T  R  L  R  F  E  L

AACGATGCGTTCAAGGCTGACAAAGACAAGCTCGACGCCATCCCCAAGGTCATGGGCGCTGTCCCACAGGGTGGGCGCAACTACCAGGTTGTTATCGGTG  500
 N  D  A  F  K  A  D  K  D  K  L  D  A  I  P  K  V  M  G  A  V  P  Q  G  G  R  N  Y  Q  V  V  I  G

GCGATGTTGCCAGCGTGTACAACGAAATGGTGCAGCTGCCAGGCGTTCGCAAGTCTGATGCTGACGTCAAAGCCGCGAGCCGCGCGAAGACACAAGGCAA  600
 G  D  V  A  S  V  Y  N  E  M  V  Q  L  P  G  V  R  K  S  D  A  D  V  K  A  A  S  R  A  K  T  Q  G  K

GATGCCATGGTTGGATACCGCGTTTGAGTATCTATCCGACTCCTTCCGCCCGATCCTGGGTGTGCTGCTGGGCGCGTCGCTAATCATCGCGTTTACTGCG  700
 M  P  W  L  D  T  A  F  E  Y  L  S  D  S  F  R  P  I  L  G  V  L  L  G  A  S  L  I  I  A  F  T  A

GTGATGGAGGCTTTCGGTGTCGTCGATACGCGTGCCGATGACAAGGCTGCCATCTGGTTCTTCATCGATGCCATGTGGCGCTCGGTGTTTTACTTCCTGC  800
 V  M  E  A  F  G  V  V  D  T  R  A  D  D  K  A  A  I  W  F  F  I  D  A  M  W  R  S  V  F  Y  F  L

CCGTGATGGTGGCCTATAACGCCGGTAATAAACTGCGCATTGACCCCTGGGTTCCTGCCGCGGTTATGCTCGCGTTATTTACGCCGGAGTTCTTAGGGCT  900
 P  V  M  V  A  Y  N  A  G  N  K  L  R  I  D  P  W  V  P  A  A  V  M  L  A  L  F  T  P  E  F  L  G  L

GAAAGAAAACCCTGTTGCCAACTGCGTGGTTGATGAAGCACTAGGCACTGAGACCTGTTCCATTGACTTGCTGGGCATTAACGTGGCTTTGCCGGACTAC  1000
 K  E  N  P  V  A  N  C  V  V  D  E  A  L  G  T  E  T  C  S  I  D  L  L  G  I  N  V  A  L  P  D  Y

GGCGGAAACGTCTTTGTACCGCTGATTATGGCACTGGTTGCTGCCGGTATTTACAAGGGCTTCCAGAAGATCATTCCATCCGCTGTGCACATGGTCTTCG  1100
 G  G  N  V  F  V  P  L  I  M  A  L  V  A  A  G  I  Y  K  G  F  Q  K  I  I  P  S  A  V  H  M  V  F

TGCCATTTTTGACCCTGCTGTTTACCATCCCAATTACTGCTGTCCTGATTGGACCTTTCGGCGTCTGGCTCGGTAGCACCATCGGCGTGGGCCTGGCATG  1200
 V  P  F  L  T  L  L  F  T  I  P  I  T  A  V  L  I  G  P  F  G  V  W  L  G  S  T  I  G  V  G  L  A  W

GATGAACGGTAACGCACCATTCGTCTTCGCCATCCTGATTCCAATGCTGTATCCATTCCTGGTTCCACTCGGTCTGCACTGGCCACTCAACGCACTGATG  1300
 M  N  G  N  A  P  F  V  F  A  I  L  I  P  M  L  Y  P  F  L  V  P  L  G  L  H  W  P  L  N  A  L  M

CTCGTCAACATCGAGGCTTTGGGCTACGACTTCATCCAAGGTCCAATGGGCGCCTGGAACTTCGCTTGTTTCGGTGCAACTGCTGCGGTCTTGGCCATCT  1400
 L  V  N  I  E  A  L  G  Y  D  F  I  Q  G  P  M  G  A  W  N  F  A  C  F  G  A  T  A  A  V  L  A  I

CCATGCGTGAGAAAGACCCAGAAATGCGCCAGACGTCCGGTTCGGCACTGGCAGCTGGACTCTTCGGTGGTATTTCCGAACCGTCTCTCTACGGAATTCA  1500
 S  M  R  E  K  D  P  E  M  R  Q  T  S  G  S  A  L  A  A  G  L  F  G  G  I  S  E  P  S  L  Y  G  I  H

CCTGCGCTTTAAGCGCATTTACCCGCGCATGCTGGTCGGTTGTTTCGCTGGTGGTCTTACCATCGCTATCTTGGGAACCGCCTCCGGCGGCGTTACCACC  1600
 L  R  F  K  R  I  Y  P  R  M  L  V  G  C  F  A  G  G  L  T  I  A  I  L  G  T  A  S  G  G  V  T  T

AACGCTTTCGTGTTTACGTCCTTGCTTACCATCCCGGTCTTTTCCCCAATGCTGACGTATACCATCGCGGTTGCTGTCGCTTTCGCTGTTGCCTTCCTGC  1700
 N  A  F  V  F  T  S  L  L  T  I  P  V  F  S  P  M  L  T  Y  T  I  A  V  A  V  A  F  A  V  A  F  L

TGATTTACTTCACTGACTACCGCACCGCAGAGGAAAAGGAAGCATCCCGTGAGCGCGCAGCAGAAGCAGGATTAGTTCCGAGCGGAACTAATCCTGAAAC  1800
 L  I  Y  F  T  D  Y  R  T  A  E  E  K  E  A  S  R  E  R  A  A  E  A  G  L  V  P  S  G  T  N  P  E  T

AGAAGCCAGCACAACTACTGAGACCGCTGACTTGGTCTCACCAGTCGATGGCATGGCCGTCAAGCTTGAAAATATCGACGACAAGGTCTTCGCCAGCGGA  1900
 E  A  S  T  T  E  T  A  D  L  V  S  P  V  D  G  M  A  V  K  L  E  N  I  D  D  K  V  F  A  S  G

ACACTGGGCAATGGCATCGGCATCGTGCCAGTTAACGGCAATATCCAAAGCCCAGTCGCCGGCACCATCGCGACGGTCACCAAGACAGGCCACGCCTTCG  2000
 T  L  G  N  G  I  G  I  V  P  V  N  G  N  I  Q  S  P  V  A  G  T  I  A  T  V  T  K  T  G  H  A  F

GTATCAAGACTGATGATGGGGTAGAGGTTCTAGTCCACATCGGTATTAACACCGTGCGAATGAAGGGCGAAGGCTTTGAACCCAAGGTCGCCAAGGGAGA  2100
 G  I  K  T  D  D  G  V  E  V  L  V  H  I  G  I  N  T  V  R  M  K  G  E  G  F  E  P  K  V  A  K  G  D

CGTCGTCAGCATCGGCACGCCACTGGCCGAAGTTGACCTGGACAAGATCAAGGAAGCCGGCTACGACAACACCGTCGTTGTCACCGTCACCAACACGAAA  2200
 V  V  S  I  G  T  P  L  A  E  V  D  L  D  K  I  K  E  A  G  Y  D  N  T  V  V  V  T  V  T  N  T  K

GCCATGGGGGAGGTCGCAAGCATCGCCGAAGGAGCAGTCAAGGCTGGTGAGGCGGTCGTTGCCATTAAGCGCTAACCTGGTTATCGATGCAGATCCTTCG  2300
 A  M  G  E  V  A  S  I  A  E  G  A  V  K  A  G  E  A  V  V  A  I  K  R  ***

AGTATTTAATAACAACGTCGTCCTCGCACGCCGCGGGGACGAGGAAGTTATTGTCACAGGTCGCGGTCTGGGTTTTAAAGCCCAGCACGGCGAAGAGATT  2400

GACGGCAATCGCATTGCCCAGGTTTTTACACCGTCGCTTGGCCGCGACCCGGACCACCTCGCCATCATGTTGGCGGCGTTGAGCCCGGACTATATCGACA  2500

AAGTTGTAACCGCGCTAACGGCGGTTAACGTCGAAGCCGAGCTCACGCTGGTGGTCGCGCTCGCAGACCACATCCAGTCCGCAGTCCAAAGAACCCGCGC  2600

GGGCCACATCGTGGATTACCCGCTGCGCGCCGAGGTCGAACACCTTTATCCAGAGGACTACCAGCGCGCCGTCAAGACGCTAGAAGTCATCAACGCCGAG  2700

CTGGATACGCCTTTACCGGATTCAGAGGCGATTGCTTTGGCGCTGCATTTGGTCAACGCGGGCTTTGCCTCGGGGGATCTGTCCCAGACCTATCGCATGA  2800

CGGGGCTGATTCAGCAGCTCATTGAAATCATCGGGGAATTC 2841
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Fig. 3. Nucleotide sequence of the 2,841-nucleotide *B. ammoniagenes* DNA fragment and deduced amino acid sequence.
The underlined sequence preceding the ATG start codon of *ptsG* gene is a putative ribosome-binding site. The TAA stop codon is indicated with asterisks below the sequences. Numbers at the end of each line correspond to the nucleotide position. The sequence data have been submitted to the GenBank nucleotide sequence database under accesion number AF045481.

In order to compare the effects of glucose and mannose on the growth of *E. coli* ZSC113, the ZSC113 (pATS17) and ZSC113 (pBR322) grown in M9 minimal broth supplemented with glycerol (0.4%), non-PTS sugar, were transferred into M9 minimal broth containing glucose (0.4%) or mannose (0.4%, w/v) as the sole carbon source, and followed by measuring their growth (Fig. 2). The ZSC113 (pATS17) could grow by fermenting the glucose and mannose as a carbon source, while ZSC 113 (pBR322) hardly grew at all. It was also observed that the specific growth rate of ZSC113 (pATS17) on glucose was higher than that on mannose, although the cell growth became identical on both glucose and mannose after incubating for 24 h (data not shown). From these results, it can be concluded that the gene product is more active on glucose than on mannose. Until now, it was not clear whether the gene product of pATS17 is either EII^{Glc} or EII^{Man} since the EII^{Man} specific for both glucose and mannose have been reported in other bacteria [2, 10, 30].

Nucleotide Sequence of the *B. ammoniagenes ptsG* Gene

The 2.8-kb *B. ammoniagenes* DNA fragment of pATS17 was completely sequenced (Fig. 3). The 1,986-nucleotide ORF, beginning at position 290 by an ATG codon and terminating at position 2,275 by the ochre stop codon TAA, was identified in the nucleotide sequence. The ORF encodes a single polypeptide of 661 amino acid residues with a calculated M_r of 70,109. This value is comparable to those of large EII^{Glc} proteins consisting of domains IIA, IIB, and IIC. It was also found that eight bases upstream from the ATG start codon is an AGAAGG at nucleotide position 276~281, which might be the Shine-Dalgarno ribosomal recognition site for the *B. ammoniagenes* gene.

A hydropathy of the deduced amino acid sequence was calculated by the method of Kyte and Doolittle [13] as shown in Fig. 4. It was found that the gene product contains a hydrophobic region (IIC domain) corresponding to amino acid residues from 122 to 475, consisting of six subregions with an average hydropathy exceeding 1.0, each comprised of 18~62 amino acid residues capable of spanning the cytoplasmic membrane. Both the amino-terminal part (1-110 aa) and carboxy-terminal part (476-661 aa) are relatively hydrophilic. They correspond to IIB domain and IIA domain, respectively, indicating that the EII^{Glc} of *B. ammoniagenes* has an arrangement of IIBCA domains belonging to the sucrose and β-glucosidase subgroup of the glucose-PTS class. As shown in Fig. 1, the 135-amino-acid carboxy-terminal region including the EIIA domain could be deleted from *B. ammoniagenes* EII^{Glc} without affecting the remaining IIBC domain of the EII^{Glc} capable of complementing the function of
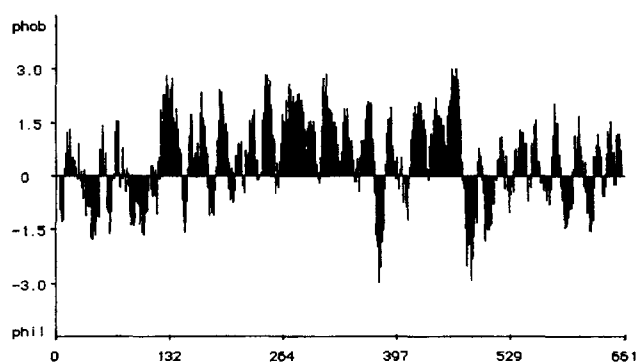

Fig. 4. Hydropathy plot of the deduced enzyme II^{Glc} amino acids sequence.
Hydropathy was calculated by the method of Kyte and Doolittle with a window size of 9 amino acid residues [13].

glucose uptake of *E. coli* ZSC113. This suggests that the IIBC domain of *B. ammoniagenes* EII^{Glc} interacts efficiently with the EIIA^{Glc} of *E. coli* in the cells as shown by Vogler *et al.* [31]. With respect to sugar utilization and domain structure of the gene product, it is assumed that the *B. ammoniagenes* gene corresponds to a *ptsG* gene encoding EII^{Glc}.

Comparison of the *B. ammoniagenes* EII^{Glc} Sequence with Other EII Sequences

When the deduced amino acid sequence of the *B. ammoniagenes* EII^{Glc} was compared with the sequences of other EIIs in the NCBI database using the BLAST search program [1], the EII^{Glc} was homologous to the EII^{Man} of *C. glutamicum* with the highest score of 46% sequence similarity (Fig. 5). Gaps were introduced to optimize the alignment. It was also found that the EII^{Glc} is similar to the β-glucoside-specific EIIs and sucrose-specific EIIs of the glucose-PTS class, while the EII^{Glc} shows no similarity with EIIs of the mannose-PTS class. The intact EII^{Glc} exhibits 33% similarity with the EII^{Scr} of *S. mutans*, 30% similarity with EII^{Scr} of *Streptococcus sobrinus* [4], and 31~33% similarities with the EII^{Bgl} proteins of *B. subtilis* [29], *E. coli* [3], *Erwinia chrysanthemi* [8], and *Klebsiella oxytoca* [14]. It has recently been thought that the EII^{Man} of *C. glutamicum* belongs to the sucrose/β-glucoside subgroup of the glucose-PTS class on the basis of sequence alignments, although its sugar specificity was similar to that of the *E. coli* EII^{Man}. Furthermore, the *C. glutamicum* EII^{Man} showed no sequence similarity with EIIs of mannose-PTS class reported so far. The degree of sequence similarity between the EII^{Glc} of *B. ammoniagenes* and EII^{Man} of *C. glutamicum* is comparable to the 44% similarity between the EII^{Scr} proteins of *S. mutans* and *S. sobrinus*. In addition, it was found that the 161-amino-acid C-terminal region of the *B. ammoniagenes* EII^{Glc} exhibits 29% homology with the EIIA^{Glc} of *E. coli*.

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BAM    1  MSKTEMRTAAEEILAGIGGADNIASFTHCATRLRFELNDAFKADKDKLDAIPKVMGAVPQ
CGL    1  MASKLT*TSQH**ENL**P***T*M********QVK*QSIV*QQEI*SD*S*L*V***

BAM   61  GGRNYQVVIGGDVASVYNEMVQLPGVRKSDADVKAASRAKTQ-----GKMPWLDTAFEY
CGL   61  *STGM***M**S**NY*Q*ILK*D*-M*HF**GE*TESSSKKEYGGVR**YS*I*Y***F

BAM  115  LSDSFRPILGVLLGASLIIAFTAVMEAFGVVDTRA--DDKAAIWFFIDAMWRSVFYFLPV
CGL  120  ***T*****WA********TLLVLADT**LQ*F**PM*EQPDTYV*LHS*********I

BAM  173  MVAYNAGNKLRIDPWVPAAVMLALFTPEFLGLKENPVANCVVDEALGTETCSIDLLGINV
CGL  180  **GAT*AR**GANE*IG**IPA**L****-------------***SAGDTVTVF*LPM

BAM  233  ALPDYGGNVFVPLIMALVAAGIYKGFQKIIPSAVHMVFVPFLTLLFTIPITAVLIGPFGV
CGL  225  V*N**S*Q**P***A*IGLYWVE**LK****E**Q******FS**IM**A**F*L****I

BAM  293  WLGSTIGVGLAWMNGNAPFVFAILIPMLYPFLVPLGLHWPLNALMLVNIEALGYDFIQGP
CGL  285  GV*NG*SNL*EAI*NFS**ILS*V**L***************I*IQ**NT*********

BAM  353  MGAWNFACFGATAAVLAISMREKDPEMRQTSGSALAAGLFGGISEPSLYGIHLRFKRIYP
CGL  345  **********LVTG*FLL*IK*RNKA***V*LGGML***L*********VL****KT*F

BAM  413  RMLVGCFAGGLTIAILGTASGGVTTNAFVFTSLLTIPVFSPMLTYTIAVAVAFAVAFLLI
CGL  405  *L*P**L***IVMG*-----FDIKAY**********AMD*W*G***GI****F*SMF*V

BAM  473  YFTDYRTAEE----------KEASRERAAEAGLVPSGTNPETEASTTT-----------
CGL  460  LAL***SN**RDEARAKVAAD*Q*EEDLK***NAT*AAPVAAAG*GAGAGAGAAAGAATA

BAM  511  ----------ETADLVSPVDGMAVKLENIDDKVFASGTLGNGIGIVPVNGNIQSPVAGTI
CGL  520  VAAKPKLAAG*VV*I***LE*K*IP*SEVP*PI**A*K**P**A*Q*TGNTVVA*ADA*V
ECO    1  MGLFDKLKSLVSD*KKDTGTIEI*NI*DVP*V***EKIV*D**A*K*TGNKMVA**D***

BAM  561  ATVTKTGHAFGIKTDDGVEVLVHIGINTVRMKGEGFEPKVAKGDVVSIGTPLAEVDLDKI
CGL  580  IL*Q*S***VALRL*S***I***V*LD**QLG****TVH*ERRQQ*KA*D**ITF*A*F*
ECO   61  GKIFE*N***S*ES*S***LF**F**D**EL*****KRIAEE*QR*KV*DTVI*F**PLL

BAM  621  KEAGYDNTVVVTVTNTKAMGEVASIAEGAVKAGEAVVA--IKR   (661)
CGL  640  RSKDLPLITP*V*S*AAKF**IEG*PADQANSSTT*IKVNG*NE  (683)
ECO  121  E*KAKSTLTP*VIS*MDEIK*-LIKLS*S*TV**TP*IR-**K   (161)
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Fig. 5. Comparison of the *B. ammoniagenes* EII^{Glc} (BAM) with *C. glutamicum* EII^{Man} (CGL) and *E. coli* EIIA^{Glc} (ECO). The amino acid sequences of three polypeptides are given in the one-letter code and have been aligned by introducing gaps (hyphens) to maximize similarities. Residues identical to the EII^{Glc} amino acid sequence of *B. ammoniagenes* are indicated by asterisks in other sequences. The histidine and cysteine residues, which may be phosphorylated, are indicated by the closed circles above the sequences. Numbers at the beginning of each line correspond to the amino acid position in the protein.

The EII^{Glc} contains two amino acid residues which mediate phosphate transfer from phospho-HPr to glucose. One is a histidyl residue at position 583 homologous to the active site, His, of *E. coli* EIIA^{Glc} which is phosphorylated by the phospho-HPr protein [23]. The amino acid sequences surrounding the His residue are conserved. The other is assumed to be a cystidyl residue (29 aa) in the IIB domain of the protein. The EII^{Glc} of *E. coli* is phosphorylated at a Cys residue (421 aa) by a soluble phospho-EIIA^{Glc} [19]. The amino acid sequence conservation around the Cys residue of EII^{Glc} of *B. ammoniagenes* is also shared with other EIIs specific for

glucose, sucrose, and β-glucoside. It has also been reported that the Cys residue (348 aa) is a phosphorylation site of the mannitol-specific EII (EII^{Mtl}) from *E. coli* [21]. It is worth noting that the active Cys residue is in the hydrophilic region of all EIIs analyzed thus far [22].

Acknowlegments

REFERENCES

1. Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* **215:** 403–410.

2. Bouma, C. L. and S. Roseman. 1996. Sugar transport by the marine chitinolytic bacterium *Vibrio furnissii. J. Biol. Chem.* **271:** 33468–33475.

3. Bramley, H. F. and H. L. Kornberg. 1987. Nucleotide sequence of *bglC*, the gene specifying Enzyme II^{Bgl} of the PEP: sugar phosphotransferase system in *Escherichia coli* K12, and overexpression of the gene product. *J. Gen. Microbiol.* **133:** 563–573.

4. Chen, Y. Y., L. N. Lee, and D. J. LeBlanc. 1993. Sequence analysis of *scrA* and *scrB* from *Streptococcus sobrinus* 6715. *Infect. Immunol.* **61:** 2602–2610.

5. 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.

6. Dower, W. J., J. F. Miller, and C. W. Ragsdale. 1988. High efficiency transformation of *Escherichia coli* by high voltage electroporation. *Nucleic Acids Res.* **16:** 6127–6145.

7. Ebner, R. and J. W. Lengeler. 1988. DNA sequence of the gene *scrA* encoding the sucrose transport protein Enzyme II^{Scr} of the phosphotransferase system from enteric bacteria: Homology of the Enzyme II^{Scr} and Enzyme II^{Bgl} proteins. *Mol. Microbiol.* **2:** 9–17.

8. El Hassouni, M., B. Henrissat, M. Chippaux, and F. Barras. 1992. Nucleotide sequences of the *arb* genes, which control β-glucoside utilization in *Erwinia chrysanthemi*: Comparison with the *Escherichia coli bgl* operon and evidence for a new β-glycohydrolase family including enzymes from eubacteria, archaebacteria, and humans. *J. Bacteriol.* **174:** 765–777.

9. Erni, B. and B. Zanolari. 1986. Glucose permease of the bacterial phosphotransferase system. Gene cloning, overproduction, and amino acid sequence of enzyme II^{Glc} *J. Biol. Chem.* **261:** 16398–16403.

10. Erni, B., B. Zanolari, and H. P. Kocher. 1987. The mannose permease of *Escherichia coli* consists of three different proteins. Amino acid sequence and function in sugar transport, sugar phosphorylation, and penetration of phage lambda DNA. *J. Biol. Chem.* **262:** 5238–5247.

11. 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.

12. Kwon, I., K. N. Lee, J.-K. Lee, J.-G. Pan, T.-K. Oh, H. H. Lee, and K.-H. Yoon. 1995. Cloning and expression of the gene encoding glucose permease of the phosphotransferase system from *Brevibacterium flavum* in *Escherichia coli. J. Microbiol. Biotechnol.* **5:** 188–193.

13. Kyte, J. and R. F. Doolittle. 1982. A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* **157:** 105–132.

14. Lai, X., F. C. Davis, R. B. Hespell, and L. O. Ingram. 1997. Cloning of cellobiose phosphoenolpyruvate-dependent phosphotransferase genes: Functional expression in recombinant *Escherichia coli* and identification of a putative binding region for disaccharides. *Appl. Environ. Microbiol.* **63:** 355–363.

15. 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.

16. Lee, J.-K., M.-H. Sung, K.-H. Yoon, J.-H. Yu, and T.-K. Oh. 1994. Nucleotide sequence of the gene encoding the *Corynebacterium glutamicum* mannose enzyme II and analyses of the deduced protein sequence. *FEMS Microbiol. Lett.* **119:** 137–146.

17. Malin, G. M. and G. I. Bourd. 1991. Phosphotransferase-dependent glucose transport in *Corynebacterium glutamicum. J. Appl. Bacteriol.* **71:** 517–523.

18. Meadow, N. D., D. K. Fox, and S. Roseman. 1990. The bacterial phosphoenolpyruvate:glycose phosphotransferase system. *Annu. Rev. Biochem.* **59:** 497–542.

19. Meins, M., P. Jenö, P. Müller, W. J. Richter, J. P. Rosenbusch, and B. Erni. 1993. Cysteine phosphorylation of the glucose transporter of *Escherichia coli. J. Biol. Chem.* **268:** 11604–11609.

20. Mori, M. and I. Shiio. 1987. Phosphoenolpyruvate:sugar phosphotransferase system and sugar metabolism in *Brevibacterium flavum. Agric. Biol. Chem.* **51:** 2671–2678.

21. Pas, H. H., G. H. Meyer, W. H. Kruizinga, K. S. Tamminga, R. P. van Weeghel, and G. T. Robillard. 1991. ^{31}Phospho-NMR demonstration of phosphocysteine as a catalytic intermediate on the *Escherichia coli* phosphotransferase system EII^{Mtl}. *J. Biol. Chem.* **266:** 6690–6692.

22. Postma, P. W., J. W. Lengeler, and G. R. Jacobson. 1993. Phosphoenolpyruvate:carbohydrate phosphotransferase systems of bacteria. *Microbiol. Rev.* **57:** 543–594.

23. Presper, K. A., C.-Y. Wong, L. Liu, N. D. Meadow, and S. Roseman. 1989. Site-directed mutagenesis of the phosphocarrier protein, III^{Glc}, a major signal-transducing protein in *Escherichia coli. Proc. Natl. Acad. Sci. USA* **86:** 4052–4055.

24. Rodriquez, R. L. and R. C. Tait. 1983. *Recombinant DNA Techniques - An Introduction*. pp. 45–46. Addison-Wesley Pub. Co., Reading, MA.

25. Saier Jr, M. H. 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.

26. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular Cloning. A Laboratory Mannual.* 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

27. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74:** 5463–5467.

28. Sato, Y., F. Poy, G. R. Jacobson, and H. K. Kuramitsu. 1989. Characterization and sequence analysis of the *scrA* gene encoding enzyme II^{Scr} of the *Streptococcus mutans* phosphoenolpyruvate-dependent sucrose phosphotransferase system. *J. Bacteriol.* **171:** 263–271.

29. Tobisch, S., P. Glaser, S. Kruger, and M. Hecker. 1997. Identification and characterization of a new β-glucoside utilization system in *Bacillus subtilis*. *J. Bacteriol.* **179:** 496–506.

30. Veyrat, A., M. J. Gosalbes, and G. Perez-Martinez. 1996. *Lactobacillus curvatus* has a glucose transport system

31. 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.

32. Zagorec, M. and P. W. Postma. 1992. Cloning and nucleotide sequence of the *ptsG* gene of *Bacillus subtilis*. *Mol. Gen. Genet.* **234:** 325–328.

homologous to the mannose family of phosphoenolpyruvate-dependent phosphostransferase systems. *Microbiology* **142:** 3469–3477.