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A Novel Glutamate Transport System in Poly(γ-Glutamic Acid)-Producing Strain *Bacillus subtilis* CGMCC 0833

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Abstract *Bacillus subtilis* CGMCC 0833 is a poly(γ-glutamic acid) (γ-PGA)-producing strain. It has the capacity to tolerate high concentration of extracellular glutamate and to utilize glutamate actively. Such a high uptake capacity was owing to an active transport system for glutamate. Therefore, a specific transport system for L-glutamate has been observed in this strain. It was a novel transport process in which glutamate was symported with at least two protons, and an inward-directed sodium gradient had no stimulatory effect on it. K_m and V_m for glutamate transport were estimated to be 67 μM and 152 $nmol^{-1}$ min^{-1} mg^{-1} of protein, respectively. The transport system showed structural specificity and stereospecificity and was strongly dependent on extracellular pH. Moreover, it could be stimulated by Mg^{2+}, NH_4^+, and Ca^{2+}. In addition, the glutamate transporter in this strain was studied at the molecular level. As there was no important mutation of the transporter protein, it appeared that the differences of glutamate transporter properties between this strain and other *B. subtilis* strains were not due to the differences of the amino acid sequence and the structure of transporter protein. This is the first extensive report on the properties of glutamate transport system in γ-PGA-producing strain.

Keywords *Bacillus subtilis* CGMCC 0833 · Glutamate transport · Poly(γ-glutamic acid) · Secondary transport system

Abbreviations

γ-PGA	Poly(γ-glutamic acid)
Δp	Proton motive force
ΔpNa	Sodium gradient
ΔpH	Transmembrane proton gradient
ΔΨ	Transmembrane electrical potential

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Introduction

Poly(γ-glutamic acid) (γ-PGA) is a homo-polyamide made of D- and L-glutamic acid monomers via γ-amide linkages. It is water soluble, biodegradable, edible and nontoxic toward humans and the environment, and has broad applications in fields of medicine, foods, plastics, and many others [1]. It is produced by several *Bacillus* species as an extracellular polymer [1, 2]. Recently, researchers have investigated the nutrient requirements for γ-PGA production and found that the nutrient requirements varied according to the strain used. According to the nutrient requirements, γ-PGA-producing bacteria are divided into two groups: glutamate-dependent bacteria and glutamate-independent bacteria. In the former bacteria, exogenous glutamate is essential for the production of γ-PGA, while in the latter ones, glutamate is not required. Now, production of γ-PGA is most extensively studied in the former bacteria due to the low-productivity of γ-PGA in the latter ones [1].

B. subtilis CGMCC 0833 is a glutamate-dependent γ-PGA-producing strain; it shows an absolute requirement for extracellular glutamate to produce γ-PGA [3]. The production of γ-PGA by *B. subtilis* CGMCC 0833 has been previously investigated [3, 4]. In this strain, γ-PGA is produced directly from the intracellular glutamate, while the intracellular glutamate comes from two parts: glutamate which is transported into the cell from extracellular glutamate and glutamate which is converted from extracellular glucose, and it has been determined that more than 90% units of the polymer comes from the part of extracellular glutamate [5]. Our previous study also showed that enhancing the transport of extracellular glutamate into cells of this strain would increase the supply of intracellular glutamate, and then stimulate the production of γ-PGA [4]. As the cell membrane is a natural barrier of microbes, and the glutamate transport system within the membrane of this strain regulates the uptake of glutamate, therefore, the transport of glutamate plays an important part in γ-PGA production process. Then it is necessary for us to pay attention to glutamate transport in this strain. However, most of the studies to date have focused on the cultivation conditions to improve the yield of γ-PGA [6–9], and only few studies have focused on uptake of extracellular substrate in these producing strains. The little attention of glutamate import has severely limited further improvement of γ-PGA production.

Generally, amino acid transport is a ubiquitous phenomenon and serves a variety of functions, such as for cell growth and some metabolites productions [10]. Transport of amino acids is carried out by adenosine triphosphate (ATP)-binding cassette-type transport system or secondary transport system. In the former system, solute transport is driven by ATP hydrolysis. While in the latter, transport is driven by transmembrane electrochemical gradients of another solute or ion [11].

Bacillus is an important Gram-positive genus, and extensive studies suggested that the activities of solute transport systems depend directly on the rate of electron transfer in this genus [12–14].

The amino acid transporters in *Bacillus stearothermophilus* and *Bacillus caldotenax* studied to date facilitate an electrogenic symport reaction, in which the transport of glutamate and aspartate is driven by the proton motive force (Δp) but also by an inwardly directed Na^+ gradient (ΔpNa) [12, 14, 15]. While in *B. subtilis* 6GM, glutamate transport is a proton-motive force, rather than a sodium-motive force-driven process [16].

As a γ-PGA-producing strain, *B. subtilis* CGMCC 0833 has physiological properties noticeably different from other strains of *B. subtilis* species [3]. In this paper, we identified and characterized the glutamate transporter in this strain, with special emphasis on its

functional properties. This is the first time to extensively study the properties of glutamate transport system in γ-PGA-producing strain. It would not only complement the knowledge of glutamate transport system in *Bacillus* genus, but also would be helpful for us to understand the biosynthesis mechanism of γ-PGA deeply, and then to improve substrate utilization for γ-PGA production.

Materials and Methods

Culture Conditions

B. subtilis CGMCC 0833 was isolated from soil sample [3]. It was deposited in China General Microbiological Culture Collection Center. Cells were first inoculated into 50 ml of seed medium containing glucose, 20 $g l^{-1}$; yeast extract, 5 $g l^{-1}$; $K_2HPO_4 \cdot 3H_2O$, 2 $g l^{-1}$; L-glutamic acid, 10 $g l^{-1}$; $MgSO_4$, 0.1 $g l^{-1}$ in 500-ml flask and aerobically incubated at 32.5 °C for 16 h with shaking at 220 rpm. Seed culture (1.2 ml) was then transferred to a 500-ml flask containing 80 ml of basal medium comprising glucose, 40 $g l^{-1}$; $(NH_4)_2SO_4$, 5 $g l^{-1}$, $K_2HPO_4 \cdot 3H_2O$, 2 $g l^{-1}$, L-glutamic acid, 40 $g l^{-1}$; $MgSO_4$, 0.1 g l^{-1}, $MnSO_4$, 0.03 $g l^{-1}$, and the final pH was adjusted to 7.0. The flask culture was incubated at 32.5 °C in a rotary shaker at 220 rpm for different times.

When the effect of metabolic inhibitors on growth of strain was studied, the inhibitors were added at 24 h of cultivation, respectively, and the time profile of cell growth was determined. The final concentrations of inhibitors were as follows: carbonyl m-chlorophenylhydrazone (CCCP), 100 μM; *N*,*N*-dicyclohexylcarbodiimide (DCCD), 500 μM; valinomycin, 20 μM; and vanadate, 500 μM, as the first three were water insoluble, they were dissolved in ethanol. The blanks were ethanol treated in order to rule out the effect of ethanol. The inhibitors were also stable during the incubation times.

Glutamate Transport Assays

Exponentially growing cells were harvested by centrifugation (8,000×*g*, 10 min, 4 °C) and washed twice in potassium phosphate buffer (50 mM, pH 6.5). The cell pellet was resuspended in the same buffer to achieve a concentration of 5 to 10 mg of protein per ml. Aliquots (200 μl) of cell suspension were placed into tubes in a shaking water bath at 32.5 °C, and transport was initiated by the addition of L-[U-^{14}C] glutamate to a final concentration of 100 μM. After 0 to 30 s, transport was terminated by the addition of ice-cold LiCl (2 ml, 100 mM) and rapid filtration (0.22-μm-pore-size cellulose-nitrate filter). The filters were washed once with 2 ml of LiCl, dried for 15 min at 105 °C, and counted by liquid scintillation (1450LSC&Luminescene Counter, PerkinElmer).

When the effect of metabolic inhibitors on glutamate transport was studied, the inhibitors were added to transport assay buffer 10 min before L-[U-^{14}C] glutamate, respectively, and the reaction was terminated and determined at different times. The final concentrations of inhibitors were as follows: CCCP 10 μM, DCCD 100 μM, valinomycin 5 μM, and vanadate 500 μM, as the first three were water insoluble, they were dissolved in ethanol. The blanks were ethanol treated in order to rule out the effect of ethanol. The inhibitors were stable during the incubation times. The results of experiments were expressed as the mean of three determinations. The experimental errors were less than 10%.

Artificial Membrane Potentials

Cells were washed twice in potassium phosphate buffer (50 mM, pH 6.5) and subsequently incubated in potassium phosphate buffer (50 mM, pH 6.5) containing 50 mM potassium acetate for 30 min at 0 °C. After centrifugation for 5 min at 8,000×*g*, cells were resuspended in reaction buffer to a concentration of approximately 10 mg of protein per milliliter listed in Table 1, and all the buffers used were adjusted to pH 6.5. The transport reaction was the same with the above statements. The results of experiments were expressed as the mean of three determinations. The experimental errors were less than 10%.

Analytical Procedures

Biomass was determined from 10-ml cell suspensions that were harvested by centrifugation, washed with distilled water, and dried at 80 °C for 24 h to a constant weight. Glutamate uptake into the strain during cultivation was determined by the decreasing of glutamate in medium, and the concentration of glutamate in medium was measured directly using a bioanalyzer (SBA-40C, Shandong Academy of Sciences). The volumetric yield of γ-PGA was measured by gel permeation chromatography system following the method reported previously [4]. Protein concentration was determined by means of Bradford [17], using bovine serum albumin as the standard. Every experiment was repeated for three times. The errors for biomass were less than 6%, for glutamate concentration were less than 10%.

Cloning and Sequencing of the Glutamate Transporter Gene

The glutamate transporter gene (*gltP*) in *B. subtilis* CGMCC 0833 was amplified by PCR with the primers of sense sequence: 5 ′TCTAAAGGGGGATTCATTTGAAAAA 3′ and antisense sequence: 5 ′CGTATTGAGGTGTTCTTTTTTTCTA 3′. The reaction mixture

Table 1 Glutamate uptake driven by artificial gradients in *B. subtilis* CGMCC 0833

Reaction buffer	Valinomycin	Forces	Glutamate uptake (nmol min^{-1} mg^{1} protein)
50 mM potassium phosphate 50 mM potassium acetate	−	None	0
50 mM choline phosphate	+	$\Delta p(\Delta\Psi+\Delta pH)$	45±4
50 mM choline phosphate 50 mM acetate	+	$\Delta\Psi$	20±2
50 mM choline phosphate	−	ΔpH	28±2
50 mM sodium phosphate 50 mM sodium acetate	−	ΔpNa	0
50 mM sodium phosphate 50 mM sodium acetate	+	$\Delta pNa+\Delta\Psi$	22±2
50 mM sodium phosphate 50 mM sodium chloride	−	$\Delta pNa+\Delta pH$	30±3
50 mM sodium phosphate 50 mM sodium chloride	+	$\Delta pNa+\Delta p(\Delta\Psi+\Delta pH)$	47±4

(100 μl) consisted of 8 μmol of Tris-HCl buffer (pH 8.3), 2 μmol of $(NH_4)_2SO_4$, 0.3 μmol of $MgCl_2$, 20 nmol of each dNTP, 2.5 units of Taq DNA polymerase (TaKaRa Co., Ltd.), 0.5 μg of chromosomal DNA of *B. subtilis* CGMCC 0833, and each 100 pmol of primers. The thermal cycling program was: denaturation for 5 min at 95 °C, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 54 °C for 1 min and extension at 72 °C for 1 min, and a final extension step at 72 °C for 10 min. The amplified DNA fragment (1.2 kb) was ligated into pGEM-T vector. *E. coli* DH5α was directly transformed with the ligation mixture. The transformants were selected on LB plates containing ampicillin. The nucleotide sequence of the gene was determined by Shanghai Bioasia Biotechnology Co,. Ltd.

Chemicals

L-[U-^{14}C] Glutamate (01 mCi ml^{-1}) was purchased from China Isotope Corp. DCCD, CCCP and valinomycin were purchased from Sigma Chemical Co. Other chemicals were reagent grade and were obtained from commercial sources.

Results and Discussion

Importance of Glutamate Transport for γ-PGA Production

B. subtilis CGMCC 0833 showed dependence on extracellular glutamate during γ-PGA cultivation, as it would not produce γ-PGA without glutamate in medium. As shown in Fig. 1, the glutamate uptake increased with the increasing of extracellular glutamate concentration, and the increase of glutamate uptake increased the cellular glutamate content. It seemed that as a supplier of intracellular glutamate, the glutamate transporter played an important role in γ-PGA production.

On the other hand, this strain had the capacity to tolerate high concentration of glutamate, while few strain could grew with more than 40 gl^{-1} of glutamate in the medium, and this was mostly owing to an active glutamate transporter in this strain. It appeared that the strain showed an increasing uptake rate of extracellular glutamate when the concentration of glutamate increased from 0 to 60 gl^{-1}, and the maximum specific uptake rate reached 0.05 mM min^{-1} g^{-1} DCW, which was a relative high value compared with other *B. subtilis* strains [16, 18]. Therefore, it was necessary to investigate such an active transport system for glutamate in this strain, which would be benefit for the improvement of γ-PGA production.

Effect of Metabolic Inhibitors on Cell Growth and Glutamate Transport

The effect of specific metabolic inhibitors on *B. subtilis* CGMCC 0833 was tested to determine the energy source required for cell growth and glutamate transport, and the results were shown in Figs. 2 and 3.

Vanadate, an inhibitor of ATP-driven transport systems, had no effect on either cell growth or glutamate transport of this strain.

The effect of DCCD on the transport rate was investigated to verify the involvement of H^+-ATPase on the transport of glutamate. The biomass decreased a little after the addition of this inhibitor, however, after several hours, the strain began to grow gradually, while the biomass was still less than that of the control. In addition, DCCD also showed inhibition of

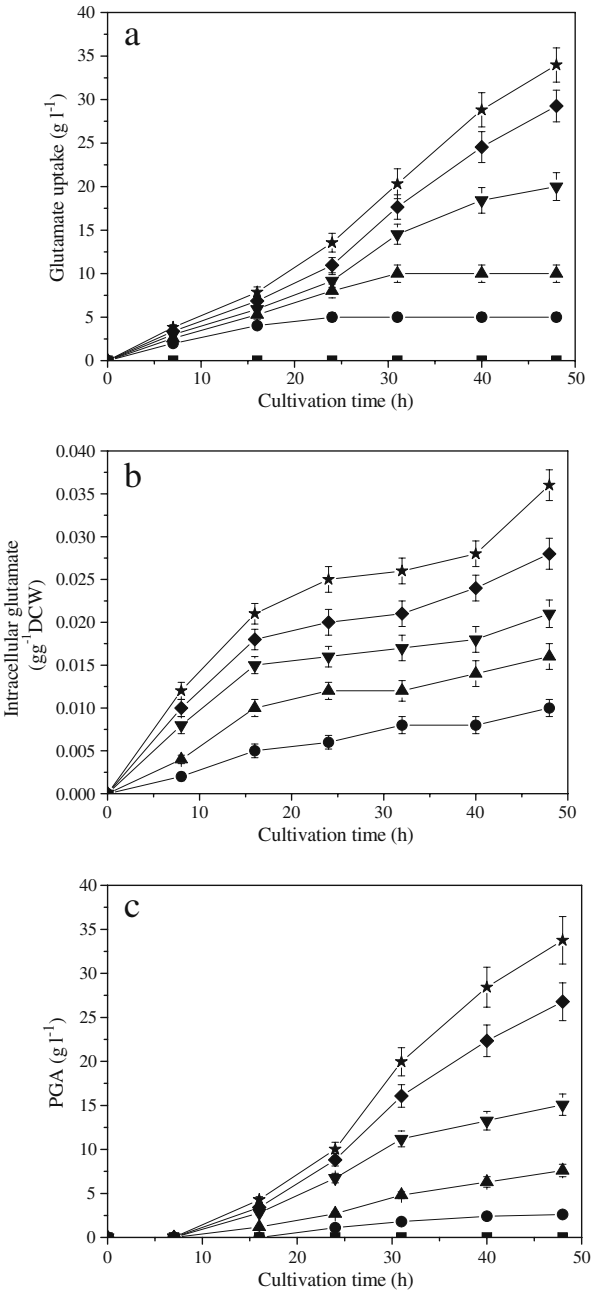

Fig. 1 Dependence of *B. subtilis* CGMCC 0833 on extracellular glutamate during cultivation. **a** Glutamate utilization of the strain, **b** intracellular glutamate of the strain, and **c** γ-PGA production by the strain. Strain without glutamate (*squares*), with 5 gl^{-1} glutamate (*circles*), with 10 gl^{-1} glutamate (*triangles*), with 20 gl^{-1} glutamate (*inverted triangles*), with 40 gl^{-1} glutamate (*diamonds*), and with 60 gl^{-1} glutamate (*stars*)

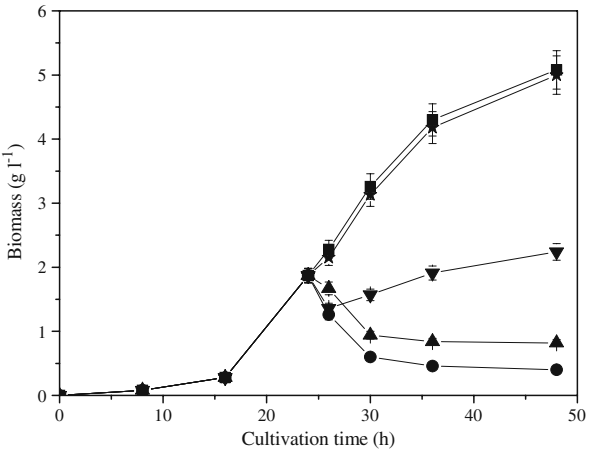

Fig. 2 Effect of metabolic inhibitors on growth of *B. subtilis* CGMCC 0833. Inhibitors were added at 24 h of cultivation. Biomass without inhibitors (*squares*), with 100 μM CCCP (*circles*), with 20 μM valinomycin (*triangles*), with 500 μM DCCD (*inverted triangles*), and with 500 μM vanadate (*stars*)

glutamate transport. This indicated the involvement of the H^+-translocating ATPase in the maintenance of transmembrane proton gradient (ΔpH).

CCCP is a weak acid and decreases the proton electrochemical gradient across the cytoplasmic membrane of bacterial cells. Cells challenged with CCCP in the exponential phase of growth were immediately inhibited, and the biomass decreased to one fourth of the original value. Moreover, the initial rate of glutamate transport was nearly completely blocked in the presence of CCCP. This suggests that Δp influences glutamate uptake.

Similarly, as an ionophore which dissipates transmembrane electrical potential (Δψ), valinomycin also inhibited cell growth and glutamate uptake. Cells challenged with valinomycin immediately ceased to grow, and the biomass decreased to half of the original

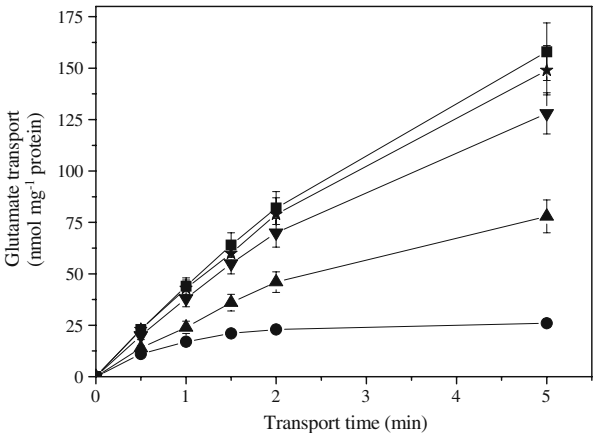

Fig. 3 Effect of metabolic inhibitors on glutamate transport. Inhibitors were added to transport assay buffer 10 min before glutamate. Glutamate transport without inhibitors (*squares*), with 10 μM CCCP (*circles*), with 5 μM valinomycin (*triangles*), with 100 μM DCCD (*inverted triangles*), and with 500 μM vanadate (*stars*)

value. The initial rate of glutamate transport was also strongly reduced in the presence of valinomycin, indicating Δψ also functioned as a driving force for glutamate transport.

The sensitivity of glutamate transport to metabolic inhibitors CCCP and valinomycin implied that a proton motive force (Δp) and Δψ both acted as the driving force for transport. Therefore, it was concluded that transport of glutamate in this strain belonged to a secondary transport system with an electrogenic process.

Glutamate Uptake Driven by Artificial Gradients

Since L-glutamate is in an anionic species at pH 6.5, the electrogenic nature of the transport process suggests that at least two cations are symported with the substrate. To examine the nature of the cotransported cations more closely and to find out which component of Δp (Δψ or ΔpH) was the driving force for glutamate transport, we carried out experiments in which glutamate uptake was driven by artificially applied ion gradients.

As shown in Table 1, Δp as well as its components Δψ and ΔpH were able to drive L-glutamate uptake, and both Δψ and ΔpH played important roles in glutamate uptake. On the other hand, ΔpNa alone could not drive glutamate transport independent of the presence of an artificially generated, also it had no influence on transport whether or not in addition to an artificially generated Δp, Δψ or ΔpH. Therefore, the Na gradient was not a driving force for glutamate transport. In addition, we determined the effect of external NaCl (with concentration ranging from 1 to 100 mM) on transport, and there was no stimulation effect either. Therefore, the mechanism of transport in *B. subtilis* CGMCC 0833 was glutamate-H^+ symport, in accordance with previous observations in *B. subtilis* 6GM [16]. Thus, it appeared that L-glutamate transport in *B. subtilis* was coupled to protons whereas sodium ions and protons were used in the related thermophiles *B. stearothermophilus* and *B. caldotenax* [14].

Kinetic Properties of Glutamate Transport System

Transformation of the experimental data into Eady–Hofstee plots yielded a single straight line (Fig. 4), indicating the presence of only one kinetically distinguishable glutamate transport system. From the initial rates of transport estimated over a concentration range of 0–400 μM, the Michaelis constant (K_m) and the maximum velocity (V_m) for glutamate transport in *B. subtilis* CGMCC 0833 were estimated to be 67 μM and 152 $nmol^{-1}$ min^{-1} mg^{-1} of protein, respectively. Such a V_m value was relatively high in *B. subtilis* species ever reported [13, 16, 18]. Moreover, the system in this strain showed new functional properties with respect to its own growth environment and growth characteristics. The presence of such a low-affinity but unusual high-capacity transport system provides this organism with a specific physiological advantage, as the strain grew under the condition when the glutamate supply is in excess, and it was in large requirement for extracellular glutamate for γ-PGA production. This was a specific characteristic in glutamate transport system, which also made the system different with other systems.

We also compared the growth of γ-PGA-producing strain and nonproducing strain with different glutamate concentration in medium. It was discovered that the nonproducing strain grew even worse, and it did not grow when glutamate concentration was more than 80 gl^{-1}, while *B. subtilis* CGMCC 0833 could still survive and grow. It was deduced that it was the stronger capacity of glutamate transport in this strain that more efficiently transport glutamate into cells, which alleviate the inhibition of glutamate on cells, which made the strain survive in high concentration of glutamate.

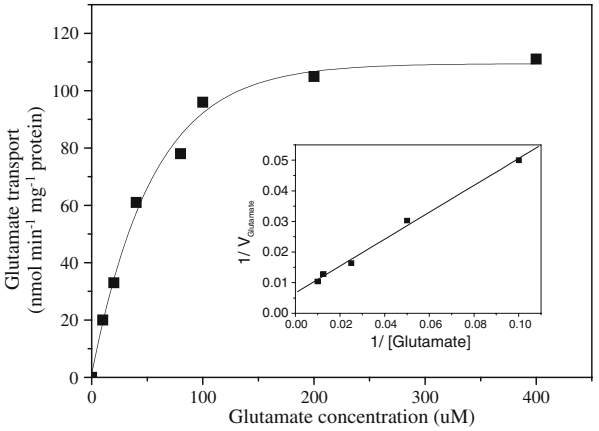

Fig. 4 Kinetics of glutamate transport in *B. subtilis* CGMCC 0833. The experiment was carried out at pH 6.5

Dependence of Glutamate Transport on Extracellular pH

The rate of glutamate transport was studied over a range of pH values, as shown in Fig. 5. A strong pH dependence was observed, with a maximum initial uptake rate of 45 $nmol^{-1}$ min^{-1} mg^{-1} of protein at pH 6.5. In addition, it is interesting that the optimal pH for transport differed from that for cell growth (pH 7.0), while the optimum pH for cell growth and glutamate uptake appeared to be the same in *B. subtilis* W23 and *B. subtilis* 168. The pH disagreement of growth and transport was the special feature of this strain, which might be expressed by the inconsistency of the physiological adaptability of the whole cell and the membrane protein. It would render γ-PGA cultivation sub-optimal, and consideration should be further studied.

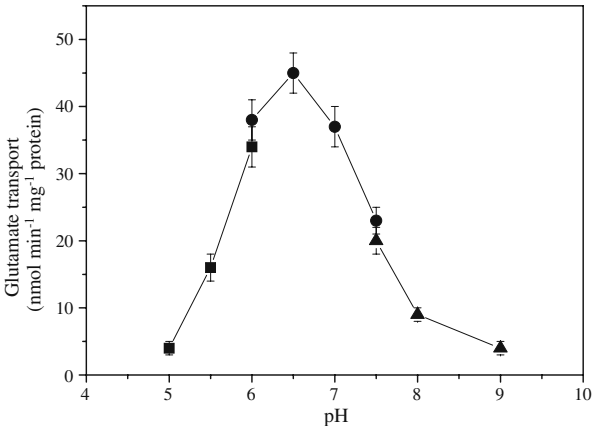

Fig. 5 Effect of extracellular pH on the initial rate of glutamate transport. The following 50 mM buffers were used: disodium hydrogen phosphate-citric acid buffer (*squares*), potassium phosphate buffer (*circles*), and Tris-HCl buffer (*triangles*)

Substrate Specificity

The specificity of glutamate transport system was determined by measuring the initial uptake rate of L-[U-^{14}C] glutamate in the presence of a tenfold excess of other unlabeled amino acids. As shown in Table 2, L-glutamate uptake was specifically inhibited by L-aspartate, while L-glutamine and D-glutamate had slight effect on L-glutamate uptake. This suggests that glutamate transport in this strain exhibited structural specificity (L-glutamate vs. L-aspartate) as well as stereospecificity.

Effect of Cations on Glutamate Transport

The effects of several monovalent and divalent cations on glutamate transport were examined. In particular, Mg^{2+}, NH_4^+, and Ca^{2+} enhanced the uptake of glutamate (Fig. 6). Moreover, these cations had no effect on the affinity (data not shown), therefore they might promote the enzyme activity directly as a cofactor in the transport process. This is the first report in the literature of NH_4^+ and Ca^{2+} stimulating glutamate transport. Also, the difference between this strain and other *B. subtilis* strains might have resulted from the adaptation of the bacterium to a different growth environment. The effect of NH_4^+ on transport discovered in this work might be a good explanation for a published report that NH_4^+ could stimulate γ-PGA biosynthesis [19].

Important Factors for Glutamate Transport

There were several factors in cultivation broth of *B. subtilis* CGMCC 0833, including glucose and γ-PGA, which were in abundance in cultivation broth. It was found that both glucose and γ-PGA could effectively stimulate glutamate transport in this strain (Fig. 7). It was deduced that glucose might be used as an energy source, while the effect of γ-PGA is related to its structure and characteristics. Since γ-PGA was a linear polymer molecular, the reaction broth with γ-PGA was more viscous. Therefore, glutamate could be concentrated and gathered outside of the cell surface, which could increase glutamate transport. Moreover, the stimulation of the two factors was beneficial in γ-PGA production by this strain (data not shown). Thus indicating glucose and γ-PGA in the cultivation broth could be useful for glutamate transport and γ-PGA production.

Table 2 Effect of competitive substrates on glutamate transport in *B. subtilis* CGMCC 0833

Substrate (1 mM)	Inhibition (%)
L-Glutamate	86±7
L-Glutamine	30±3
L-Aspartate	78±6
D-Glutamate	18±2
L-Lysine	7±1
L-Arginine	8±1
L-Threonine	0
α-Ketoglutarate	8±1

The level of inhibition was expressed as the percent inhibition of the initial rate of uptake compared with control (nominally, 100%) in the absence of competitive substrates

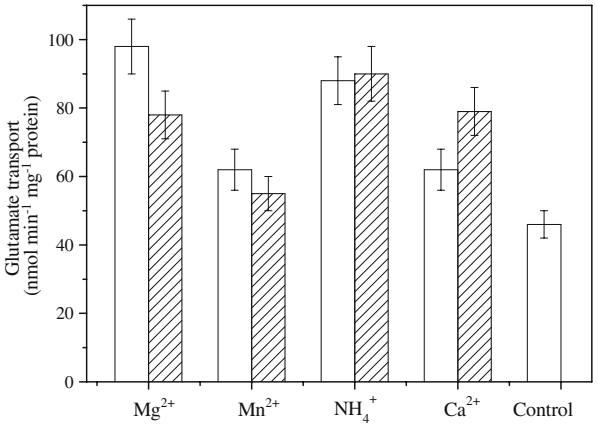

Fig. 6 Effect of cations on glutamate transport. Transport of glutamate was measured in buffer containing $MgCl_2$, $MnCl_2$, NH_4Cl, and $CaCl_2$, respectively. Cations with concentration of 5 (□) and 10 μM (▨)

Cloning and Sequence Analysis of gltP

In order to observe the difference of glutamate transporters in this strain and other *Bacillus* species, the *gltP* was cloned and sequenced, and the amino acid sequence deduced from the gene was compared with the sequence from other *Bacillus* species using Vector NTI 8.0. The *gltP* in *B. subtilis* CGMCC 0833 shows 99.4% identity to the reported $gltP_{Bsu}$ (GenBank accession no. **Z99105**). Although there were eight nucleotides different (204C, 382G, 528C, 600A, 609G, 786C, 1098A, and 1134G) in *gltP* of this strain, only one amino acid was mutated. We did the molecular simulation of the deduced protein. It was found that the mutant amino acid would not altered the kinetic properties of the transporter, this indicates that the differences between this glutamate transport system and other *Bacillus* species might not be due to the differences in the amino acid sequence of GltP.

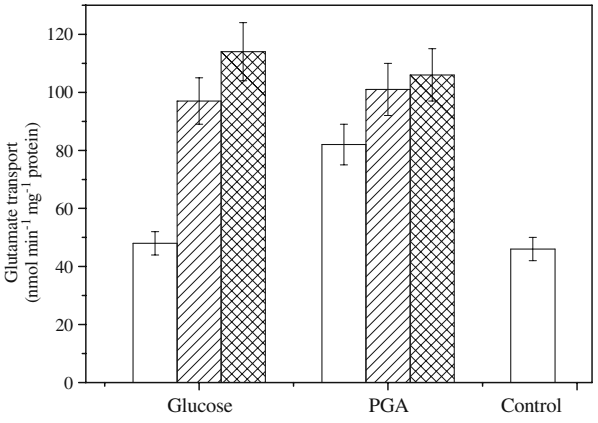

Fig. 7 Stimulation of glucose and γ-PGA in glutamate transport. Glucose, 0.2 (□), 1 (▨), and 5 g/l (▩); γ-PGA, 0.1 (□), 0.5 (▨), and 1 g/l (▩)

Conclusion

B. subtilis CGMCC 0833, a γ-PGA-producing strain, showed an absolute requirement for extracellular glutamate to produce γ-PGA. It had the capacity to tolerate high concentration of extracellular glutamate and to utilize glutamate actively. Such a high capacity was owed to an active transport system for glutamate. L-Glutamate was an anionic species at the optimal pH 6.5, which implied that the amino acid was cotransported with at least two cations. Among possible candidates for cotransport with L-glutamate, namely Na^+ and H^+, ΔpNa could not drive glutamate transport. It was therefore concluded that it was an electrogenic process in which glutamate was symported with at least two protons. Systems driven by proton motive force presumably require less metabolic energy per solute transported (one third to one half ATP equivalent) [20] than the binding protein-dependent systems (one ATP). *B. subtilis* CGMCC 0833 appears to have such as proton motive force-dependent transport system for glutamate, which implies that such an energy-saving, relative low-affinity but high-capacity transport system provided this organism with a specific physiological advantage under conditions when the extracellular glutamate supply was in excess.

Glutamate transport in this strain was strongly dependent on extracellular pH. The optimal pH for transport (pH 6.5) differed from that for cell growth (pH 7.0). This raises the question about the cause of this difference. Further studies will be needed to determine the optimal pH for γ-PGA cultivation. Moreover, the transport system could be stimulated by many factors, such as some cations (Mg^{2+}, NH_4^+, and Ca^{2+}), glucose and the product γ-PGA. This was significant for γ-PGA production, as these factors could be employed in cultivation to enhance production.

In order to observe the difference of glutamate transporters in this strain and other γ-PGA-nonproducing strain of *Bacillus* species, the transport system was studied at the molecular level, but there was no important mutation of GltP in this strain, indicating that the differences between these glutamate transporters might not be due to the differences in the amino acid sequence of GltP. As the V_m value of the transporter in this strain was much higher than that in *B. subtilis* W23 when they were determined under the same conditions, it was deduced that the more active capacity of glutamate uptake might be due to the much more amount of transporter protein existed in this strain, and this could be partly verified by the stronger ability of glutamate transport with the membrane vesicles in this strain compared with that in *B. subtilis* W23.

This is the first extensive study of the properties of glutamate transport in a γ-PGA-producing strain. The glutamate transport system in *B. subtilis* CGMCC 0833 studied in this paper would not only facilitate an electrogenic symport reaction, but also would be helpful for a better understanding of the biosynthesis mechanism of γ-PGA.

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