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ORIGINAL PAPER

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Characterization of salt-tolerant glutaminase from *Stenotrophomonas maltophilia* NYW-81 and its application in Japanese soy sauce fermentation

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Abstract Glutaminase from *Stenotrophomonas maltophilia* NYW-81 was purified to homogeneity with a final specific activity of 325 U/mg. The molecular mass of the native enzyme was estimated to be 41 kDa by gel filtration. A subunit molecular mass of 36 kDa was measured with SDS-PAGE, thus indicating that the native enzyme is a monomer. The N-terminal amino acid sequence of the enzyme was determined to be KEAETQQKLANVVILATGGTIA. Besides L-glutamine, which was hydrolyzed with the highest specific activity (100%), L-asparagine (74%), D-glutamine (75%), and D-asparagine (67%) were also hydrolyzed. The pH and temperature optima were 9.0 and approximately 60°C, respectively. The enzyme was most stable at pH 8.0 and was highly stable (relative activities from 60 to 80%) over a wide pH range (5.0–10.0). About 70 and 50% of enzyme activity was retained even after treatment at 60 and 70°C, respectively, for 10 min. The enzyme showed high activity (86% of the original activity) in the presence of 16% NaCl. These results indicate that this enzyme has a higher salt tolerance and thermal stability than bacterial glutaminases that have been reported so far. In a model reaction of Japanese soy sauce fermentation, glutaminase from *S. maltophilia* exhibited high ability in the production of glutamic acid compared with glutaminases from *Aspergillus oryzae*, *Escherichia coli*, *Pseudomonas citronellolis*, and *Micrococcus luteus*, indicating that this enzyme is suitable for application in Japanese soy sauce fermentation.

Keywords Salt-tolerant glutaminase · L-Glutamic acid · Umami · Soy sauce fermentation · *Stenotrophomonas*

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Introduction

Glutaminase catalyzes the hydrolytic degradation of L-glutamine to L-glutamic acid. The physiological roles of glutaminases, especially in organs and tissues of mammals such as the brain, kidney, neurons, and macrophage, have been studied [3, 7, 20, 24]. The enzymological properties of microbial glutaminases from *Escherichia coli* [2, 14], *Pseudomonas aeruginosa* [19], *Clostridium welchii* [4], *Bacillus* sp. [12, 17], *Micrococcus luteus* [13], *Saccharomyces cerevisiae* [18], *Cryptococcus albidus* [6], *Rhizobium etli* [1], and *Aspergillus* sp. [22, 23] have been reported. Glutamic acid, on the other hand, is known to be an important amino acid contributing not only to a pleasant taste, "Umami", but also to the nutritional properties of food. Therefore, glutaminase has been thought to be a useful additive to improve the quality of food in terms of both flavor and nutrition. In Japan, in particular, most studies on microbial glutaminases have been performed from the standpoint of applications for Japanese soy sauce fermentation, which occurs under a high concentration of NaCl (14–18%). We have studied glutaminase from the viewpoint of food science. We screened for microorganisms producing salt-tolerant glutaminase with the aim of applying such an enzyme to the food industry, especially to Japanese soy sauce fermentation. The bacterial strain NYW-81 was found to exhibit the highest glutaminase activity among

bacteria isolated from food and soil samples. Although this bacterium could not grow under high temperature and high NaCl concentration, the glutaminase enzyme exhibited rather high salt tolerance in cell-free extracts. Glutaminase from strain NYW-81 has been considered to be useful in Japanese soy sauce fermentation. In this paper, we describe the classification of the isolated bacterium, strain NYW-81, and the characterization of the enzyme. Application of the enzyme to Japanese soy sauce fermentation process is also discussed.

Materials and methods

Bacterium and chemicals

The glutamine medium (G-medium) used for the isolation of glutaminase-producing microorganisms contained 1% glutamine as a sole carbon and nitrogen source, 0.1% K_2HPO_4, 0.1% KH_2PO_4, 0.01% $MgSO_4 \cdot 7H_2O$, 0.1% NaCl and 0.05% yeast extract (pH 7.0). A small amount of food or soil sample was suspended in sterilized water and spread on G-medium agar plates. After incubation at 30°C for 1–2 days, colonies grown on the plate were isolated on G-medium slants. G-medium inoculated with each isolate was incubated at 30°C for 1–2 days with shaking, following which cells were harvested and treated by sonication or with cetyltrimethylammonium bromide. The glutaminase activity of each isolate was measured according to the assay method described below. Strain NYW-81 was selected from 161 isolates grown on G-medium. Biochemical characterization of the isolate was performed according to *Bergey's Manual of Systematic Bacteriology* [9]. Chemicals were commercial products of reagent grade from Wako Pure Chemical Industries, Japan, unless otherwise stated.

16S rDNA sequence analysis

The 16S rDNA sequence of strain NYW-81 was determined by directly sequencing a PCR product of the 16S rDNA gene. The gene was amplified using *ExTaq* DNA polymerase (Takara Shuzo, Kyoto, Japan), primers 20f (5′-TGTAAATCGGCCAGTAGAGTTTGATCCTGGCTC-3′) and 1510r (5′-CAGGAAACAGCTATGACCGGCTACCTTGTTACGACT-3′), and chromosomal DNA of strain NYW-81. DNA sequence similarity searches were performed using the BLAST program of GenBank (http://www.ncbi.nlm.nih.gov).

Cultivation of strain NYW-81

To determine culture conditions, strain NYW-81 was cultivated in a medium containing the carbon and nitrogen sources (0.3–1.0%) shown in Table 1, 0.1% K_2HPO_4, 0.1% KH_2PO_4, 0.01% $MgSO_4 \cdot 7H_2O$, 0.1% NaCl and 0.05% yeast extract, with shaking at 30°C for 24 h. For enzyme purification, the bacterium was grown in medium containing 1.0% glutamate as a sole carbon and nitrogen source, 0.1% K_2HPO_4, 0.1% KH_2PO_4, 0.01% $MgSO_4 \cdot 7H_2O$, 0.1% NaCl and 0.05% yeast extract, with shaking at 30°C for 42 h.

Koji culture and protease preparation

The Koji medium was composed of 30 g wheat bran moistened with 30 mL tap water in a 15-cm-diameter Petri dish and autoclaved at 120°C for 30 min. A spore suspension of *Aspergillus oryzae* JMC2228 was prepared by cultivation in a medium (pH 6.5) composed of 0.1% yeast extract, 1% malt extract and 2% agar at 28°C for 5 days. A 1 mL aliquot of this *A. oryzae* spore suspension was inoculated into the wheat bran and mixed well. Cultivation was carried out at 28°C for 72 h. At 24 h cultivation, the culture was mixed again for aeration. The wheat bran Koji was extracted with water, and ammonium sulfate was added to the extract to 90% saturation. The precipitated protein was collected by centrifugation at 8,000 *g* for 20 min at 4°C. The precipitate was dissolved in 10 mM potassium phosphate buffer (pH 7.0) and dialyzed against the same buffer. This preparation was used as a crude protease. One unit of protease activity was defined as the amount of enzyme catalyzing the formation of 1 μg trichloroacetic acid-soluble peptides from 2% casein solution per minute. Crystalline egg albumin was used as a standard for

Table 1 Effect of carbon and nitrogen sources on the production of glutaminase. Cells were grown in a 500-mL Sakaguchi flask containing 100-mL culture medium (see Materials and methods) on a reciprocal shaker at 30°C for 24 h. Concentration of carbon and nitrogen sources was 1.0% except casamino acids (0.3%) and yeast extract (0.3%). Experiments were performed in triplicate

Carbon source	Nitrogen source	Total activity (U)	Total protein (mg)	Specific activity (U/mg)
Glucose	$(NH_4)_2\ SO_4$	0.07	2.59	0.025
Glucose	Polypeptone	0.53	16.4	0.032
Glycerol	$(NH_4)_2\ SO_4$	1.08	10.9	0.099
Glycerol	Polypeptone	3.42	28.2	0.121
Glucose	L-Glutamate	3.04	16.9	0.282
L-Glutamate		8.53	22.2	0.381
Polypeptone		3.62	24.3	0.152
Polypeptone	Casamino acids	3.04	22.5	0.134
Polypeptone	Yeast extract	5.78	19.8	0.281

estimation of peptide concentration using the Lowry method [11].

Glutamic acid production in a model reaction of soy sauce fermentation

A model system (5 mL) consisted of 0.5 g soybean protein, 0.75 g NaCl, and the crude protease obtained from Koji culture (final NaCl concentration; 15%). Estimating the effect of glutaminase on glutamic acid production, a certain amount of glutaminase from microbial source was added to the model reaction mixture. The reaction was carried out at 30°C for 48 h.

Glutaminase and protein assays

Glutaminase activity was assayed by determining the concentration of L-glutamate formed with L-glutamate dehydrogenase (GLDH, beef liver). The standard reaction mixture contained 100 mM Tris–HCl buffer (pH 9.0), 30 mM L-glutamine, and the enzyme in a final volume of 1 mL. After being allowed to react for 10–20 min at 30°C, the reaction was terminated by boiling for 3 min. From 50 to 100 μL of the first reaction mixture was transferred to an L-glutamate dehydrogenase reaction mixture containing 50 mM Tris–hydrazine buffer (pH 9.0), 1.5 mM NAD^+, 0.5 mM ADP, and five units of glutamate dehydrogenase in a total volume of 1 mL. The absorbance change at 340 nm was measured after incubating the mixture at 30°C for 60 min. One unit of glutaminase was defined as the amount of enzyme that catalyzes the formation of 1 μmol L-glutamate per minute. To examine substrate specificity, amino acid amidohydrolase activity for each substrate, L-glutamine, D-glutamine, L-asparagine, and D-asparagine, was assayed by determining the amount of ammonia released with GLDH. Protein concentration was measured using the Lowry method [11] with crystalline bovine serum albumin as the standard.

Glutaminase purification

All purification steps were performed at 4–8°C unless otherwise stated. The buffer used was 10 mM Tris–HCl buffer (pH 8.0, buffer I). Harvested cells were washed twice with 10 mM buffer I and collected by centrifugation (14,000 *g*) at 4°C for 20 min. The washed cells were suspended in buffer I and disrupted by sonication at 4–8°C for an appropriate time. The cell debris was removed by centrifugation (14,000 *g*) at 4°C for 20 min. The cell-free extract was dialyzed against buffer I. The dialyzed enzyme solution was applied to a DEAE-Toyopearl column (TOSO, Japan; φ3×35 cm) equilibrated with buffer I. After washing the column with buffer I, the unabsorbed fractions with enzyme activity were collected and dialyzed against 10 mM sodium carbonate buffer (pH 10.0, buffer II). The dialyzed enzyme solution was applied to a DEAE-Toyopearl column (φ1.5×40 cm) equilibrated with buffer II. After washing the column with the same buffer, the enzyme was eluted with a linear salt gradient of 0–0.15 M NaCl in buffer II. The active fractions were collected and dialyzed against buffer I. Solid ammonium sulfate was added to the enzyme solution to 33% saturation and applied to the Butyl-Toyopearl column (φ1.5×40 cm) equilibrated with the buffer containing ammonium sulfate to 33% saturation. After washing the column with the same buffer, the enzyme was eluted with a linear salt gradient of 33–25% saturated ammonium sulfate in buffer I. The active fractions were collected and dialyzed against buffer I. The enzyme solution was loaded onto a hydroxyapatite (Sigma, St. Louis, MO) column (φ1×20 cm) and the enzyme eluted with a linear gradient of 5–100 mM potassium phosphate buffer (pH 7.0). The enzyme, concentrated by ultrafiltration (Amicon YM10), was applied to a Sephacryl S-200 (Amersham Pharmacia, Little Chalfont, UK) gel filtration column (φ1.5×150 cm) and run with buffer I supplemented with 100 mM NaCl. The active fractions were collected and dialyzed against buffer I. The enzyme preparation was concentrated by ultrafiltration and stored at 4°C.

Measurement of molecular weight

The molecular weight of the native enzyme was estimated by gel filtration with a TSK-GEL G2000SW for HPLC (7.5×600 mm) equilibrated with buffer I supplemented with 100 mM NaCl. The column was calibrated with albumin (66,000, bovine serum), carbonic anhydrase (29,000, bovine erythrocytes), cytochrome *c* (12,400, horse heart), and aprotinin (6,500, bovine lung) as molecular standards. The molecular weight of the denatured enzyme was estimated with SDS-PAGE in 12.5% (w/v) acrylamide gel using the Laemmli method [10]. Pre-stained protein markers (Nacalai tesque, Kyoto, Japan) were used as marker proteins.

N-terminal amino acid sequence analysis

The purified enzyme was resolved by SDS-PAGE, blotted onto a polyvinylidene difluoride membrane (Immobilon, Millipore, Japan), and stained with a Ponceau solution. The N-terminal amino acid sequence of the membrane-bound protein was determined with a Shimadzu PPSQ-21A protein sequencer.

Results

Characterization of the bacterial isolate

Strain NYW-81, an isolate exhibiting relatively high glutaminase activity, was an aerobic, Gram-negative

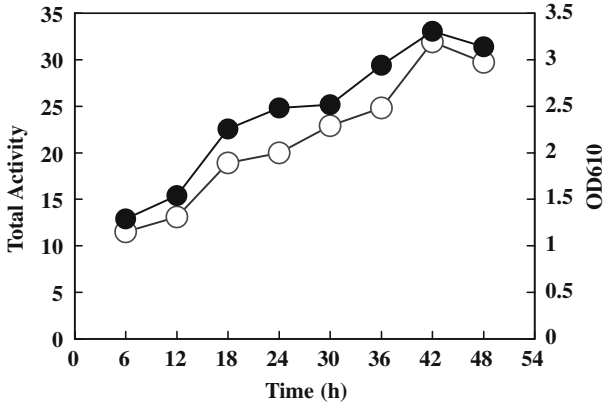

Fig. 1 Time-course of bacterial growth (*filled circles*) and glutaminase activity (*open circles*). Cultures were grown in 500-mL medium (2-L Sakaguchi flask) containing 1.0% glutamate as a carbon and nitrogen source on a reciprocal shaker at 30°C

bacterium that could grow at 30°C, but not at 40°C. The biochemical characteristics of the bacterium were as follows: oxidase, +; catalase, +; reduced nitrate, +; O/F test, oxidation; urease, −; starch hydrolysis, −. Strain NYW-81 could grow on D-glucose and D-fructose as carbon sources, but not on D-mannose, D-xylose, lactose, mannitol, sucrose, or xylitol. A DNA fragment amplified from the genomic DNA of strain NYW-81 with a set of primers corresponding to 16S rDNA consensus sequences was directly sequenced. The nucleotide sequence determined (~1,300 bp) was analyzed by BLAST, and strain NYW-81 was classified as *Stenotrophomonas maltophilia* (identity: 98%, GenBank accession number: AJ293471). From these results, we designated strain NYW-81 as *S. maltophilia* NYW-81. The strain has been deposited with The American Type Culture Collection (ATCC) and NITE Biological Resource center (NBRC).

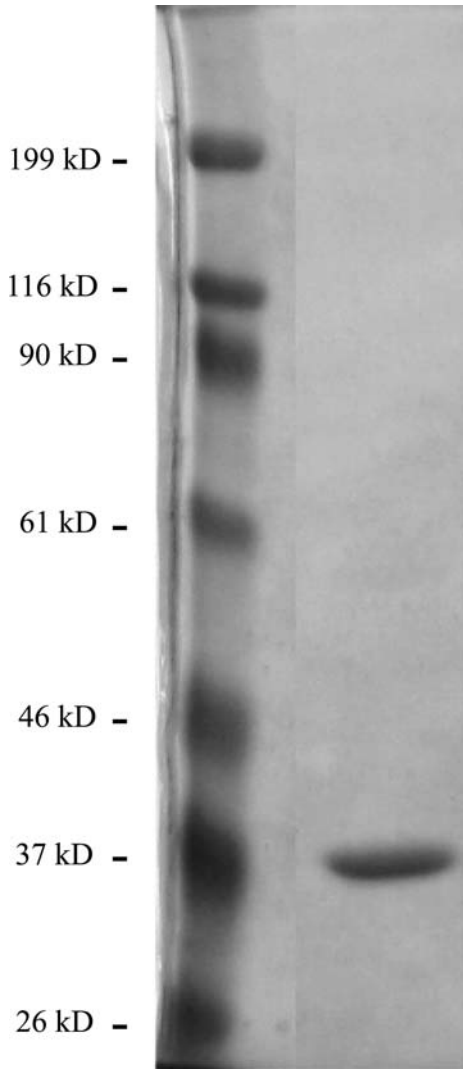

Fig. 2 SDS-PAGE of purified glutaminase from *Stenotrophomonas maltophilia*. Lanes: *1* Molecular markers, *2* purified glutaminase

Effects of carbon and nitrogen sources on glutaminase production

The effects of various carbon and nitrogen sources on the production of glutaminase were tested (Table 1). While a medium containing polypeptone provided relatively high growth and glutaminase activity, glucose had a tendency to decrease both growth and enzyme activity. The highest activity was obtained when glutamate was used as carbon and nitrogen sources. Cell growth of strain NYW-81, which was concurrent with glutaminase production, reached a maximum at 42 h at 30°C in a medium containing glutamate as carbon and nitrogen sources (Fig. 1).

Table 2 Purification of glutaminase

	Total protein (mg)	Specific activity (U/mg)	Total activity (U)	Yield (%)	Purification (fold)
Cell-free extract	1466	1.3	1,900	100	1
DEAE-Toyopearl (first)	182	8.5	1,540	81	6.5
DEAE-Toyopearl (second)	30	31	920	48	24
Butyl-Toyopearl	6.3	91	570	30	70
Hydroxylapatite	1.5	140	210	11	108
Sephacryl S-200	0.5	325	162	8.5	250

Purification of glutaminase

The enzyme was purified to homogeneity about 250-fold with a yield from the crude extract of 8.5% (Table 2). SDS-PAGE analysis revealed a subunit molecular mass of 36 kDa (Fig. 2). The molecular mass of the native enzyme determined by gel filtration was 41 kDa, indicating that the enzyme is a monomer.

N-terminal amino acid sequence

The amino acid sequence of the first 22 amino acid residues of glutaminase from *S. maltophilia* strain NYW-81 was determined to be KEAETQQKLANV-VILATGGTIA.

Effects of pH and temperature

The enzyme was found to be most active at pH 9.0 but had relative activities from 60 to 80% between pH 5 and 10 (Fig. 3a). The enzyme was most stable at pH 8.0 and relatively stable in a basic pH range (Fig. 3b). The optimum temperature was about 60°C and stable up to 40°C (Fig. 4a,b). The enzyme retained 50% activity even after treatment at 70°C for 10 min, but over 30 min of incubation at 70°C resulted in complete loss of enzyme activity.

Substrate specificity

The enzyme hydrolyzed L-glutamine to L-glutamic acid as a best substrate. It also catalyzed the hydrolysis of D-

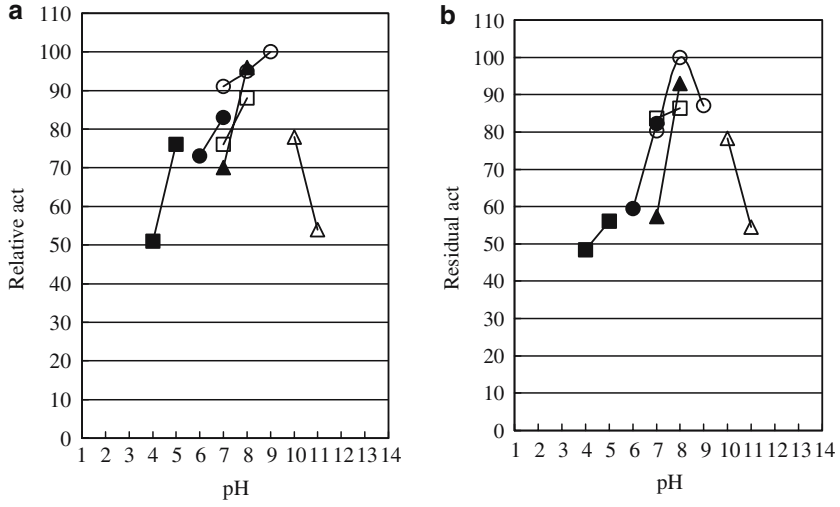

Fig. 3 Effect of pH on **a** activity and **b** stability of purified glutaminase. Activity was assayed at various pH values as described above using the following buffers: acetate buffer (pH 4–5; *filled squares*), bis–Tris buffer (pH 6–7; *filled circles*), HEPES buffer (pH 7–8; *filled triangles*), potassium phosphate buffer (pH 7–8; *open squares*), Tris–HCl (pH 7–9; *open circles*), and carbonate buffer (pH 10–11; *open triangles*). For pH stability, the purified enzyme was incubated at the pH indicated at 50°C for 10 min and residual activity was measured. Relative activity is expressed as a

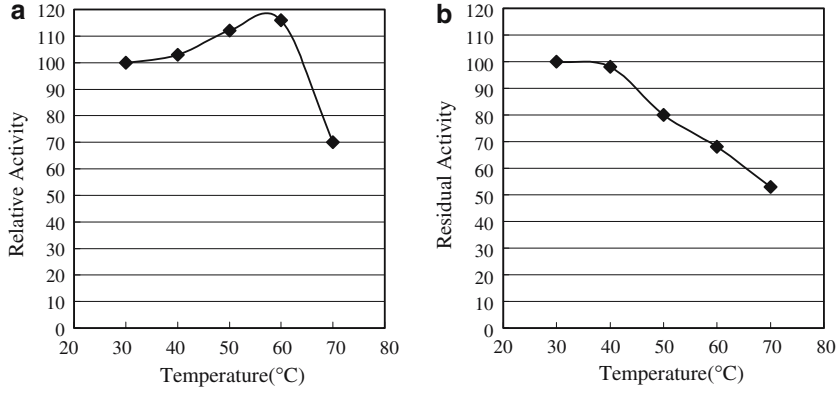

Fig. 4 Effect of temperature on **a** activity and **b** stability of purified glutaminase. Activity was assayed at various temperatures under standard reaction conditions. For thermal stability, the purified enzyme was incubated at the temperatures indicated as described above for 10 min, and residual activity was measured under standard conditions. Relative activity is expressed as a percentage

Table 3 Substrate specificity of glutaminase. The assay mixture for amidohydrolase activity contained 100 mM Tris–HCl buffer (pH 9.0), 30 mM amino acid (L-Gln, D-Gln, L-Asn or D-Asn), and enzyme in a final volume of 1.0 mL. After a 10-min reaction at 30°C, the reaction was stopped by boiling for 3 min and centrifuged. An aliquot (20 μL) of the reaction mixture supernatant was added to the mixture for ammonia determination containing 100 mM Tris–HCl buffer (pH 8.0), 10 mM 2-oxoglutarate, 0.24 mM NADH, and 5 U L-glutamate dehydrogenase (GLDH) in a total volume of 1.0 mL. The absorbance at 340 nm was measured after incubating the mixture for 90 min at 30°C. γ-Glutamyl-*p*-nitroanilide was inert

Substrate	Relative activity (%)
L-Glutamine	100
D-Glutamine	86
L-Asparagine	73
D-Asparagine	68

Table 4 Effect of various reagents on enzyme activity[a]

Compound	Relative activity (%)	
	0.1 mM	1 mM
None	100	100
$CoCl_2$	84	86
$MnCl_2$	87	78
$MgCl_2$	88	80
$CuCl_2$	83	80
$ZnCl_2$	100	93
$NiCl_2$	99	99
KCl	102	125
NaCl	101	100
EDTA	–[b]	90
DTT	88	–[b]

[a]L-Glutamine was used as a substrate
[b]Not available

glutamine as well as D-asparagine and L-asparagine as good substrates. The enzyme was most active on L-glutamine (100%), followed by D-glutamine (86%), L-asparagine (73%), and D-asparagine (68%) (Table 3).

Effects of metal ions and other reagents on glutaminase activity

The effect of metal ions on enzyme activity was examined (Table 4). The enzyme was partially (12–22%) inhibited by Co^{2+}, Mn^{2+}, Mg^{2+}, and Cu^{2+} at concentrations of 0.1–1.0 mM. Zn^{2+}, Ni^{2+}, and Na^{+} ions had no negative effects on enzyme activity. K^{+} activated the enzyme 1.25-fold at a concentration of 1 mM.

Effect of NaCl on glutaminase activity

The effect of NaCl on enzyme activity was investigated. Glutaminase activity decreased gradually with an increase in NaCl concentration but, even in the presence of 16% NaCl, the enzyme still retained 86% of its activity in the absence of NaCl (Fig. 5).

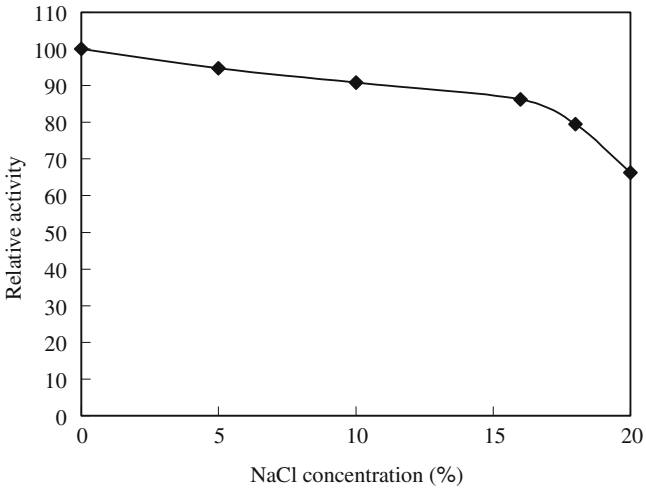

Fig. 5 Effect of NaCl concentration on glutaminase activity. Activity was assayed in the standard reaction mixture supplemented with NaCl at various concentrations (0–20%). Relative activity is expressed as a percentage of the activity in the absence of NaCl

Effect of glutaminase supplementation on glutamic acid production in a model reaction of soy sauce fermentation

The effect of microbial glutaminases on the production of glutamic acid in a model reaction of soy sauce fermentation was investigated. The addition of glutaminase from *S. maltophilia* to the model reaction mixture resulted in higher production of glutamic acid than if glutaminase was not supplied (Fig. 6). The effect of glutaminase from *S. maltophilia* on glutamic acid production was compared with those of other glutaminases from microbial sources. As shown in Table 5, the

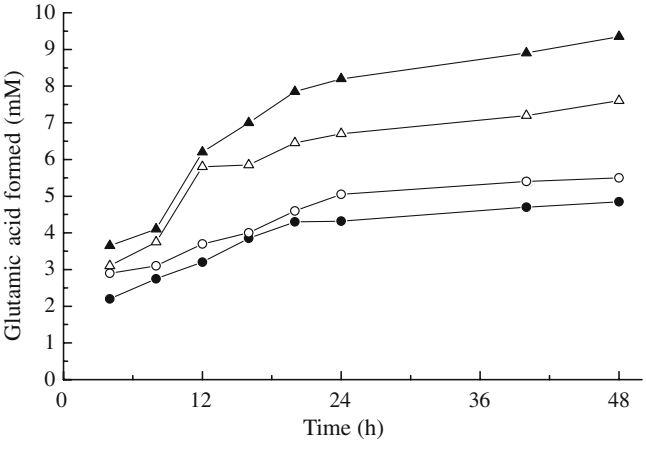

Fig. 6 Effect of glutaminase from *S. maltophilia* on glutamic acid production. The reaction was performed in a model reaction of soy sauce fermentation according to the procedure described in Materials and methods. Crude protease (200 U) including 0.02 U glutaminase from *A. oryzae* were used. *Filled triangles* 1.8 U *S. maltophilia* glutaminase, *open triangles* 0.4 U, *filled circle* no glutaminase, *open circles* no glutaminase without NaCl

Table 5 Effects of microbial glutaminases on glutamic acid production. Model reactions of soy sauce fermentation were performed in reaction mixtures composed of 0.5 g soybean protein, 0.75 g NaCl, 300 U crude protease including 0.03 U glutaminase from *Aspergillus oryzae*, and 0.5 U glutaminase of each microbial strain except for "No addition". After 48 h, the concentration of glutamic acid in the supernatant of each reaction mixture was measured using L-glutamate dehydrogenase (GLDH). *Escherichia coli* glutaminase was purchased from Sigma and the other glutaminases were prepared using column chromatography. Experiments were performed in triplicate

Strain of glutaminase	Glutamic acid concentration (mM)	
	pH 5	pH 6
No addition	4.09	4.75
Stenotrophomonas maltophilia	6.51	8.55
A. oryzae	4.85	5.69
E. coli	4.07	4.71
Micrococcus luteus	4.46	6.21
Pseudomonas citronellolis	5.14	6.29

addition of glutaminase from *S. maltophilia* to a model reaction mixture of soy sauce fermentation was clearly effective at both pH 5 and 6. The concentration of glutamic acid in a model reaction supplemented with glutaminase from *S. maltophilia* was 8.55 mM at 30°C for a 48 h-incubation at pH 6, and 3.80 mM higher than that achieved (4.75 mM) with no addition of glutaminase. The effectiveness of the addition of glutaminase from *S. maltophilia* on glutamic acid production was four times higher than that of *A. oryzae* [22], the Koji mold, which is commonly used for soy sauce fermentation process. Moreover, glutaminase from *S. maltophilia* was 2.6 times more effective for glutamic acid production than high salt-tolerant glutaminase from *M. luteus* K-3 [13].

Discussion

Glutaminase from *S. maltophilia* strain NYW-81 is a high salt-tolerant enzyme that had 86% relative activity in the presence of 16% NaCl, a concentration corresponding to that of the soy sauce fermentation process. The salt tolerance of the enzyme was somewhat lower than that of the enzymes from *M. luteus* [13] and *Bacillus subtilis* [17] (100 and 94% activity, respectively, in the presence of 16% NaCl). Glutaminase from *S. maltophilia* NYW-81 is also a thermo-stable enzyme, retaining 50% residual activity even after heat treatment at 70°C for 10 min. The glutaminases from *M. luteus* and *B. subtilis*, which are considered to be applicable to food chemistry, lost all activity after heat-treatment at 70°C for 10 min. Although the NYW-81 enzyme has pH and temperature optima of 9 and 60°C, respectively, at pH 5 and 6 the activity is 70% of that at pH 9, and 80% at 30°C the enzyme is as active as at 60°C. This indicates that the enzyme works adequately under the pH and temperature conditions in which soy sauce fermentation is carried out.

The glutaminase from *S. maltophilia* NYW-81 hydrolyzes L-asparagine, D-glutamine and D-asparagine in addition to L-glutamine. We performed a homology search with the N-terminal amino acid sequence of the enzyme using BLAST (Fig. 7). While no high homology with any glutaminases from other organisms was found (data not shown), the N-terminal sequence of the enzyme from *S. maltophilia* NYW-81 had a high homology with those of glutaminase–asparaginases and asparaginases from other bacteria, especially the glutaminase–asparaginase from *Pseudomonas* 7A (91% identity). Glutaminase–asparaginase, which is a glutaminase, hydrolyzes L- and D-asparagine as well as L- and D-glutamine. These results indicate that the enzyme from *S. maltophilia* NYW-81 can be classified as a glutaminase–asparaginase. A great deal of attention has been paid to various bacterial glutaminase–asparaginases from the clinical point of view because of their antitumor activities toward human acute lymphocytic leukemia. These enzymes from *Acinetobacter* sp. [8, 21] and *Pseudomonas* sp. [15, 16] have been extensively studied. However, there are no reports on glutaminase–asparaginase in applications in food chemistry. One of the most important factors determining the quality of soy sauce is the concentration of glutamic acid. In Japanese soy

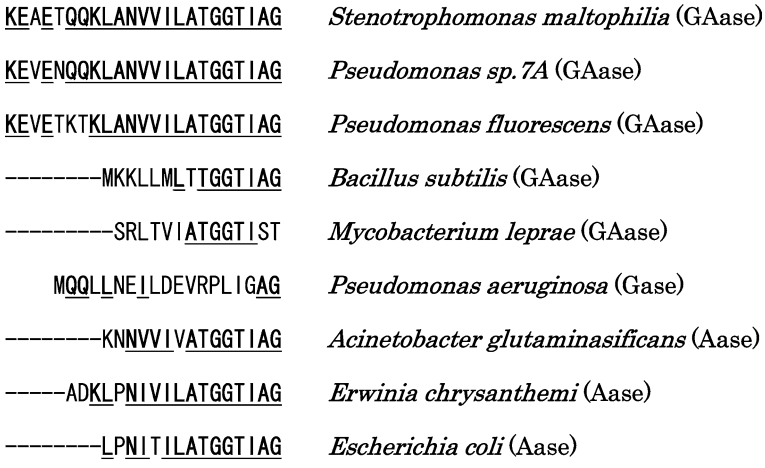

Fig. 7 N-Terminal amino acid sequence alignment of glutaminase from *S. maltophilia* with bacterial glutaminase–asparaginases (*GAase*), glutaminase (*Gase*), and asparaginases (*Aase*). Alignments are based on sequence analysis of the native proteins. Amino acid residues that are conserved at least among three genera or species are in *bold* and *underlined*

sauce fermentation, the main fermentation, in which degradation of soybean protein and formation of peptides and amino acids are observed, proceeds within the pH range 5–6. In this process, most of the L-glutamine is considered to be produced in the mash. Therefore, glutaminases are required to hydrolyze glutamine to glutamic acid effectively in this pH range. In a model reaction of soy sauce fermentation, glutaminase from *S. maltophilia* exhibited high effectiveness in the production of glutamic acid compared with other microbial glutaminases (Table 5). These results indicate that glutaminase from *S. maltophilia* has significant advantages over other microbial glutaminases in the production of glutamic acid in soy sauce fermentation processes. Recently, glutaminase from *Aspergillus sojae*, one of the Koji molds used in Japanese soy sauce fermentation, was purified to homogeneity and characterized. This enzyme exhibited both glutaminase and asparaginase activities [5]. Aspartic acid is also an important amino acid contributing to both flavor and nutrition that is used as a supplement in beverages and food. As well as the enzyme from *A. sojae*, glutaminase from *S. maltophilia* will be useful in the production of glutamic acid and aspartic acid in Japanese soy sauce fermentation. Molecular cloning of the glutaminase gene to allow construction of a high level expression plasmid for *E. coli* for further investigation into salt-tolerance at the molecular level, and for application in the food industry, is currently in progress.

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