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Extracellular Maltase of *Bacillus brevis*†

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Bacillus brevis NRRL B-4389 produced extracellular maltase (α-glucosidase; EC 3.2.1.20) only in the presence of short α-1,4-glucosidic polymers, such as maltose and maltotriose. An optimum medium was developed; it contained 2.5% maltose, 0.5% nonfat dry milk, 0.4% yeast extract, and 0.01% $CaCl_2$. The enzyme was produced extracellularly during the logarithmic phase of growth; no cell-bound activity was detected at any time. Partial purification of the maltase was accomplished by using diethylaminoethyl cellulose batch adsorption, ammonium sulfate precipitation, and Sephadex G-200 gel filtration. Maltase, isomaltase (oligo-1,6-glucosidase), and glucosyltransferase activities were purified 20.0-, 19.1-, and 11.5-fold, respectively. Some properties of the partially purified maltase were determined: optimum pH, 6.5; optimum temperature, 48 to 50°C; pH stability range, 5.0 to 7.0; temperature stability range, 0 to 50°C; isoelectric point, pH 5.2; and molecular weight, 52,000. The relative rates of hydrolysis of maltose (G_2), maltotriose (G_3), G_4, methyl-α-D-maltoside, G_{40}, dextrin, and isomaltose were 100, 22, 12, 10, 10, 8, and 5%, respectively; the K_m on maltose was 5.8 mM; D-glucose, *p*-nitrophenyl-α-D-glucoside, and tris(hydroxymethyl)aminomethane were competitive inhibitors; transglucosylase activity of the enzyme on maltose resulted in the synthesis of isomaltose, isomaltotroise, and larger oligosaccharides.

Maltases (α-glucosidases; EC 3.2.1.20) presently play an important role in the industrial production of glucose syrups used by the food industry. To the present, only two extracellular maltases from bacteria have been characterized in detail (9, 29). It was the purpose of this study to isolate a bacterium that produced extracellular maltase, to maximize enzyme production, and to purify and characterize the maltase.

MATERIALS AND METHODS

Isolation and mutagenesis. Over 100 isolates capable of producing extracellular maltase were obtained from soil samples by using the screening procedure of Wang et al. (30). An isolate that produced the most maltase in shake culture was identified as *Bacillus brevis* (S. J. McWethy, Ph.D. thesis, Iowa State University, Ames, 1977). The isolate was subjected repeatedly to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis (1). Conditions for mutagenesis were: 100 μg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine per ml, pH 6.0 (0.05 M tris(hydroxymethyl)-aminomethane [Tris]-maleate buffer), and 20 min of exposure at 35°C. Mutants were screened for extracellular maltase production (30), and a three-step mutant that produced elevated levels of maltase was selected for further study. The mutant has been deposited in the culture collection at the Northern Regional Research Center (Agricultural Research Service, U.S. Department of Agriculture, Peoria, Ill.) and has been designated as B-4389.

Maltase production and purification. Fernbach flasks, each containing 1,100 ml of 2.5% maltose (autoclaved separately as a 10% solution), 0.5% nonfat dry milk, 0.4% yeast extract, and 0.01% $CaCl_2$ (pH 6.3), were used for enzyme production. Details of medium development and enzyme production can be found elsewhere (McWethy, Ph.D. thesis). Each flask was inoculated with 10 ml of a logarithmic culture of *B. brevis* B-4389 grown in the same medium. After incubation on a platform shaker for 15 h at 30°C, the cells were removed at 4°C by using a Sharples centrifuge (Sharples Corp., Philadelphia, Pa.).

Maltase induction in *B. brevis* B-4389 was examined by using 125-ml flasks, each containing 50 ml of 0.5% nonfat dry milk, 0.4% yeast extract, and 0.01% $CaCl_2$; test flasks were inoculated with 0.5 ml of a logarithmic culture of B-4389. After 4 h at 30°C on a platform shaker (mid-logarithmic phase), various carbohydrates were added. At 8 h (early stationary phase), 4 h after carbohydrate addition, cells were removed by centrifugation, and the supernatants were assayed for maltase activity. Cell-bound activity was determined by washing pelleted cells with 0.05 M sodium β-glycerophosphate buffer, pH 6.5, and resuspending them in the same buffer before assays for maltase activity were conducted.

The method of maltase purification used diethylaminoethyl cellulose type 20 (Schleicher & Schuell Co., Keene, N.H.), added at a concentration of 5 g/liter to 6,040 ml of cold, cell-free broth (pH 5.9). After

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an 8-h adsorption period at 4°C, the diethylaminoethyl cellulose was collected by vacuum filtration and washed with five 100-ml portions of cold deionized water. Three successive 500-ml portions of 0.5 M NaCl (pH 6.5) were stirred at 4°C for 4 h with the cellulose to elute the maltase activity. Solid ammonium sulfate was then added to the pooled eluate (1,450 ml, 4°C) to achieve 85% saturation. The precipitate was collected by centrifugation at 16,000 × *g* for 30 min and then was dissolved in 0.05 M sodium β-glycerophosphate buffer, pH 6.5, yielding 145 ml of crude maltase. Portions (20 ml) of the resulting preparation were subjected to Sephadex G-200 gel filtration. A column (2.5 by 100 cm; Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) was packed at room temperature to a height of 76 cm with Sephadex G-200 (Pharmacia Fine Chemicals) and was equilibrated with 0.05 M sodium β-glycerophosphate buffer, pH 6.5, at 4°C. A flow rate of 14 ml/h was used, and 5-ml fractions were collected. Fractions containing the greatest maltase activities (see Results) were pooled and frozen at −20°C. The maltase preparations resulting from each gel filtration were pooled, yielding a final volume of 556 ml of partially purified maltase.

Analytical methods. Reaction mixtures used for maltase assays consisted of 1 ml of the appropriate enzyme dilution plus 9 ml of 5 mM maltose (Calbiochem, San Diego, Calif.) in 0.1 M Sorensen phosphate buffer, pH 6.5. Reaction mixtures were incubated at 50°C for 15 min, unless otherwise indicated, and reactions were stopped by heating the mixtures in a boiling water bath for 5 min. The amount of glucose produced was determined with PGO (peroxidase-glucose oxidase) reagent (Sigma Technical Bulletin no. 510, Sigma Chemical Co., St. Louis, Mo., 1969), dissolved in 0.5 M Tris-hydrochloride buffer, pH 7.0. Glucose assays consisted of 0.5 ml of sample and 5.0 ml of PGO reagent incubated for 30 min at 37°C. The colored reaction was stopped by the addition of 1 ml of 4 N sulfuric acid. One unit of maltase activity was defined as the amount of enzyme required to produce 1 μmol of glucose per ml per min from maltose under the conditions indicated.

Isomaltase (oligo-1,6-glucosidase; EC 3.2.1.10) activity was determined by using reaction mixtures containing 0.5 ml of enzyme dilution and 1.0 ml of 18 mM isomaltose (Calbiochem) in 0.1 M Sorensen phosphate buffer, pH 6.5. Reaction mixtures were incubated at 50°C for 60 min and were stopped by using 5-min exposures in a boiling water bath. One unit of isomaltase activity was defined as the amount of enzyme required to produce 1 μmol of glucose per ml per h from isomaltose under the conditions indicated.

Glucosyltransferase activity was assayed by using autoradiography. Reaction mixtures consisted of 50 μl of enzyme dilution, 50 μl of 62 mM maltose in 60 mM sodium β-glycerophosphate buffer (pH 6.5), and 100 μl of 60 mM [U-^{14}C]glucose (New England Nuclear Corp., Boston Mass; specific activity, 4.2 mCi/mmol). Reaction mixtures were incubated at 50°C; 15-μl samples were removed at various times and spotted onto Whatman no. 1 chromatography paper. After three ascents with 70% *n*-propanol (26), labeled products were detected by using 1- to 3-day exposures to X-ray film (Eastman Kodak Co., Rochester, N.Y.), cut out, and counted in a toluene cocktail (Permafluor; Packard Instrument Co., Inc., Downers Grove, Ill.) by using a Packard Tri-Carb liquid scintillation spectrometer. Glucosyltransferase activity was defined as the relative percentage of counts per minute transferred from [^{14}C]glucose oligosaccharides after 30 min of incubation of the reaction mixtures.

The method of Lowry et al. (19) was used to determine protein concentration. Bovine serum albumin (Sigma Chemical Co.) was the protein standard.

Methods used for isoelectric focusing have been described (20). The resulting gels were either stained with Coomassie brilliant blue ("Techniques for High Resolution Electrophoresis," Application Note 32 A, Ortec, Inc., Oak Ridge, Tenn.; 1973) or sliced into 2-mm sections, eluted in 0.5-ml volumes of buffer at 4°C, and assayed for enzyme activity.

Sephadex G-200 gel filtration was used for molecular weight determinations according to the manufacturer's instructions ("Calibration Instruction Manual for Protein Molecular Weight Determinations by Sephadex Gel Filtration," Pharmacia Fine Chemicals, 1969). A column (2.5 by 100 cm) was packed to a height of 76 cm with Sephadex G-200 and equilibrated with 0.05 M sodium β-glycerophosphate buffer, pH 6.5. A flow rate of 14 ml/h at 4°C was used. Molecular weight markers were aldolase (fructose-bisphosphate aldolase), chymotrypsinogen A, ovalbumin, and ribonuclease A (Calibration Kit; Pharmacia Fine Chemicals).

To examine products in reaction mixtures, pieces (30 by 40 cm) of Whatman no. 1 filter paper were spotted with samples 2.5 cm from the bottom edge and stapled into cylinders. The solvent was 70% *n*-propanol filled to a depth of 1 cm in cylindrical tanks (15 by 45 cm, Pyrex). Three ascents of approximately 30 cm were completed on each chromatogram at 35°C. After drying, the chromatograms were developed with a silver nitrate spray (26).

RESULTS

After mutagenesis and screening, one three-step mutant, B-4389, produced extracellular maltase (in a medium of 0.5% maltose, 0.5% peptonized milk, and 0.2% yeast extract) in a quantity of 1.8 μmol of glucose per ml per 30 min at 35°C. This was an increase of 2.3-fold over maltase production by the parent strain. Otherwise, B-4389 remained morphologically and physiologically identical to the parent *B. brevis* strain.

Maltose and maltotriose were equally efficient inducers of B-4389 maltase. Although only 1.15 U of maltase per ml was obtained in the presence of 2 mM maltose and 1.73 U/ml was obtained in the presence of 2 mM (0.1%) maltotriose, 1.75 U/ml was obtained when the maltose concentration was increased to 0.1%. The following carbohydrates (0.1%) were negative for enzyme induction: amylopectin, esculin, G_{40} (an α-1,4-glucosyl oligosaccharide of average degree of polymerization of 40; J. F. Robyt, Iowa State Uni-

versity, Ames), glucose, glycogen (shellfish), isomaltose, methyl-α-D-glucoside, *p*-nitrophenyl-α-D-glycoside, starch (soluble), and sucrose. In the absence of added carbohydrate, no extracellular maltase was detected.

When maltose (1.0%) was added to 4-h cultures (mid-logarithmic phase), extracellular maltase activity was first detected 60 to 75 min after maltose addition (data not shown). When maltose (1.0%) was added at 8 h postinoculation (early stationary phase), only small amounts of extracellular maltase were produced. The results of other experiments showed that extracellular maltase was produced primarily during the logarithmic phase of growth (data not shown); the level obtained during stationary phase remained stable through 27 h after inoculation. No cell-bound maltase activity could be detected at any time during the experimental periods.

A representative elution profile from a Sephadex G-200 gel filtration is shown in Fig. 1. A single peak of maltase activity was observed that directly corresponded to one of isomaltase activity. Fractions corresponding to 55 through 130 ml after a 145-ml void volume were pooled and designated as purified maltase. The results of purification of extracellular maltase from *B. brevis* B-4389 are presented in Table 1. Maltase, isomaltase, and glucosyltransferase activities were monitored throughout purification; the enzyme activities were purified 20.0-, 19.1-, and 11.5-fold, respectively. The overall yield of maltase activity was 73%.

When the partially purified enzyme preparation was subjected to isoelectric focusing, a single band of maltase activity was detected (data not shown). The enzyme activity had an isoelectric point of pH 5.2 and correlated directly to a major band of protein when stained. Isomaltase activity also was associated with this major protein band. Two minor protein bands also were detected by the staining method, showing that the purified maltase was not a homogeneous preparation.

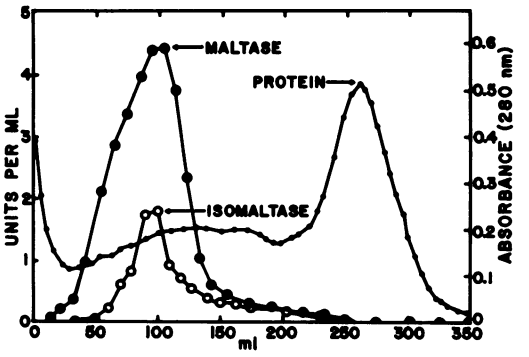

FIG. 1. *Elution pattern from a Sephadex G-200 column of the extracellular maltase from B. brevis B-4389. Presented is an elution profile from a column (2.5 by 76 cm) of Sephadex G-200 immediately after the passage of the void volume (145 ml) at a flow rate of 14 ml/h.*

The partially purified maltase was active at pH values of 6.0 to 7.2, with an optimum at pH 6.4 to 6.9 (data not shown). The optimum temperature was 48 to 50°C (Fig. 2). Greater than 95% of the enzyme activity was recovered after 3 h at 40°C between pH values of 5.0 and 7.0; 92, 12, and 0% recoveries were observed after exposures at pH values of 7.5, 8.0, and 8.5, respectively. The extracellular maltase was stable to temperatures of 50°C or lower at pH 6.5 (Fig. 3); 72 and 0% of activity were recovered after exposures at 55 and 60°C, respectively.

The molecular weight of the purified enzyme, as determined by Sephadex G-200 gel filtration, was 52,000 (Fig. 4).

The hydrolysis of maltose by B-4389 maltase was not phosphorolytic, because initial reaction velocities were equivalent in Sorensen citrate, McIlvain citrate–phosphate, Sorensen phosphate, and sodium β-glycerophosphate buffers at the same pH (6.5) and concentration (0.1 M).

The results of experiments conducted to determine the subtrate specificity of B-4389 maltase are presented in Table 2. Substrates containing α-1,4-glucosidic bonds were most rapidly hydrolyzed. For these substrates, generally, as the degree of polymerization increased, hydrolytic activity decreased. Methyl-α-D-maltoside was hydrolyzed at 1/10 the rate of maltose, and a small amount of activity was detected on the α-1,6-disaccharide, isomaltose. Only a slight amount of activity was detected on soluble starch. The following carbohydrates (1 mg/ml) were not hydrolyzed by the B-4389 enzyme: amylopectin, amylose (potato), cellobiose, dextran, gentiobiose, glycogen (oyster), lactose, melezitose, melibiose, methyl-α-D-glucoside, methyl-β-D-glucoside, *p*-nitrophenyl-α-D-glucoside, *p*-nitrophenyl-β-D-glucoside, phenyl-α-D-glucoside, phenyl-β-D-glucoside, D-raffinose, α- and β-Schardinger dextrins, sucrose, trehalose, and D-turanose.

D-Glucose and *p*-nitrophenyl-α-D-glucoside inhibited maltase activity (0.3 U/ml) 50 and 25%, respectively, at 10 mM concentrations; both inhibitions were competitive (Fig. 5), with K_i values of 5.85 and 12.6 mM, respectively. δ-Gluconolactone caused only a 15% inhibition at a concentration of 50 mM. The following carbohydrates (5 and 10 mM) had little or no effect on enzyme activity (0.3 U/ml) under the test conditions: L-arabinose, erythritol, D-fructose, D-galactose, D-gluconate, D-glucosamine hydrochlo-

TABLE 1. *Parameters observed during the purification of extracellular maltase of B. brevis B-4389*

Purification step	Volume (ml)	Protein (mg/ml)	Maltase			Isomaltase			Glucosyltransferase		
			U/ml	Sp act[a]	Fold	U/ml	Sp act	Fold	U/ml	Sp act	Fold
Cell-free broth	6,040	2.92	3.89	1.33	1	0.33	0.11	1	94	33	1
Crude preparation	145	16.7	119	7.1	5.3	8.0	0.48	4.4	2,700	160	4.8
Purified preparation	556	1.16	30.8	26.6	20.0	2.4	2.1	19.1	440	380	11.5

[a] Units per milligram of protein.

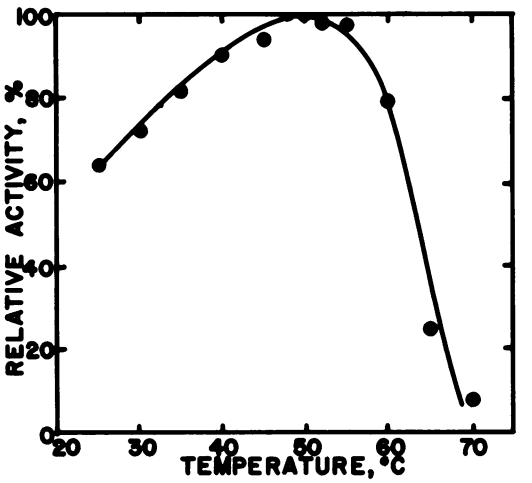

FIG. 2. *Temperature-activity profile of the partially purified extracellular maltase (0.3 U/ml) from B. brevis B-4389. Digests were incubated for 15 min in 0.1 M phosphate buffer, pH 6.5. The enzyme activity at 48°C was taken as 100%.*

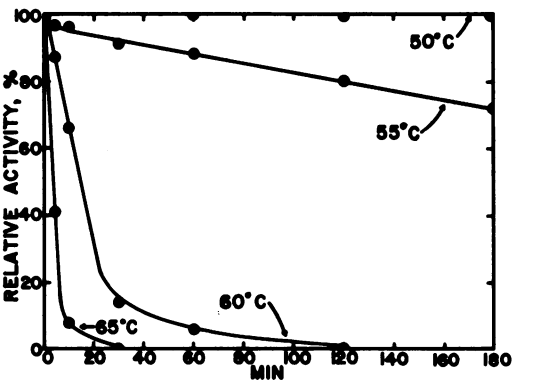

FIG. 3. *Temperature stability of the purified maltase (0.3 U/ml) from B. brevis B-4389. The maltase preparation was diluted in 0.1 M phosphate buffer, pH 6.5, and held at various temperatures. Residual activities were measured at various times; digests were incubated at 50°C for 15 min in 0.1 M phosphate buffer, pH 6.5.*

ride, glycerol, D-lactose, maltitol, mannitol, D-mannose, melezitose, D-melibiose, methyl-α-D-galactoside, methyl-α-D-glucoside, 1-o-methyl-β-D-glucoside, methyl-α-D-mannoside, methyl-α-D-xyloside, o-nitrophenyl-β-D-galactoside, p-nitrophenyl-β-D-glucoside, phenyl-α-D-glucoside, phenyl-β-D-glucoside, raffinose, D-ribose, α- and β-Schardinger dextrins (5 mM), D-sorbitol, sucrose, D-trehalose, turanose, and D-xylose.

Tris inhibited *B. brevis* maltase activity 23 and 35% at concentrations of 5 and 10 mM, respectively. Tris was a competitive inhibitor (Fig. 5), with a calculated K_i of 14.5 mM. The sulfhydryl-binding reagents p-chloromercuribenzoic acid (0.05 mM), N-ethylmaleimide (0.05 mM), iodoacetamide (0.01 mM), and iodoacetic acid (0.01 mM); the reducing agent L-cysteine (1.0 mM); the amine L-histidine (10 mM); and the chelating agent ethylenediaminetetraacetic acid (10 mM) had no substantial effects on maltase activity.

To study the effects of cations on the maltase, a preparation of B-4389 maltase was dialyzed against 0.1 M borate buffer, pH 6.5, for 24 h at 4°C. Cations were then added at 1 and 5 mM levels to digests containing 0.3 U of enzyme per ml and were incubated at 50°C for 15 min in 0.1 M borate buffer, pH 6.5. Glucose was assayed in the presence of 5 mM ethylenediaminetetraacetic acid. The addition of $CaCl_2$ increased activity by 12% over that of the control (no cation addition); Mg^{2+}, Sr^{2+}, Li^{2+}, Na^{+}, and Ba^{2+} were without effect; Co^{2+}, Ni^{2+}, Hg^{2+}, Cd^{2+}, Pb^{2+}, Fe^{3+}, Al^{3+}, Cu^{2+}, and Zn^{2+} were strong or complete inhibitors.

The values for K_m and V_{max} were determined by using a method of linear regression analysis (8) of Lineweaver-Burk (18) and Hanes (13) equations. The Lineweaver-Burk plot is shown in Fig. 5. V_{max} for maltose hydrolysis was calculated as 0.21 μmol of glucose per ml per min, and K_m was calculated as 5.8 mM.

When digests of maltose (0.5%) and B-4389 maltase (3.1 U/ml) were examined by using paper chromatography (McWethy, Ph.D. thesis), maltose was hydrolyzed and proportionate increases in glucose concentrations were noted. Transferase activity was indicated by the appearance of oligosaccharides in the reaction mix-

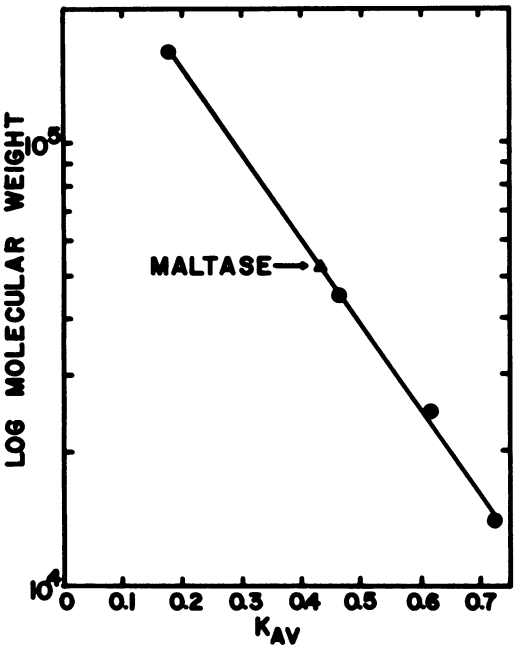

FIG. 4. *Molecular weight determination of the extracellular maltase from B. brevis B-4389 by using Sephadex G-200 gel filtration.* K_{av} *was defined as* $(V_e - V_o)/(V_t - V_o)$, *where* V_e = *elution volume for the protein,* V_o = *void volume (145 ml), and* V_t = *total bed volume (380 ml). Molecular weight of the standard proteins were: aldolase, 158,000; ovalbumin, 45,000; chymotrypsinogen A, 25,000; and ribonuclease A, 13,700.*

TABLE 2. *Substrate specificity of partially purified extracellular maltase from B. brevis B-4389*

Substrate (1 mg/ml)	Relative hydrolysis rate (%)[a]
Maltose	100
Maltotriose	22
Maltotetraose	12
Methyl-α-D-maltoside	10
G_{40}	10
Dextrin	8
Isomaltose	5
Soluble starch	<1

[a] Reaction mixtures were incubated at 50°C for 20 min in 0.1 M phosphate buffer, pH 6.5; the enzyme concentration was 0.3 U/ml.

tures. Two glucosyltransferase products, isomaltose and isomaltotriose, were identified by comparison to standards. Neither maltotriose nor panose (4-α-isomaltosyl-D-glucose) was present in the B-4389 maltase reaction mixture. From reaction mixtures containing only D-glucose (0.5%) and B-4389 maltase (3.1 U/ml), no transferase products were detected by paper chromatography through 3 h of incubation at 50°C (data not shown). However, when maltose (0.5%) was present, [^{14}C]glucose (in 0.5% glucose) was incorporated into various transferase products (data not shown).

By using paper chromatographic analyses of isomaltose (0.5%)-maltase (3.1 U/ml) digests, isomaltase activity was verified by the relative increase in glucose concentration with time (data not shown). Various transferase products also were observed; as in maltose digests, neither α-1,4-oligosaccharides nor panose was observed.

DISCUSSION

Data were presented supporting the conclusion that extracellular maltase was subject to induction in *B. brevis*. Maltase induction has been demonstrated in a number of bacterial species (3, 5, 10, 16, 25, 27; L.-H. Wang, Ph.D. thesis, Iowa State University, Ames, 1975). Induction in *B. brevis* was specific; the enzyme was produced only in the presence of small α-1,4-glucosidic polymers. This was similar to a *Pseudomonas fluorescens* induction system (10) that was initiated only in the presence of maltose or maltotriose. *B. brevis* maltase induction was clearly different from those of other *Bacillus* species that have been examined in detail (5, 25; Wang, Ph.D. thesis).

The pattern of extracellular maltase production by *B. brevis* B-4389 was similar to that of *B. subtilis* (29). For both strains, maltase appeared in the medium at the early logarithmic phase and maltase levels increased approximately parallel to cell growth. Unlike the mal-

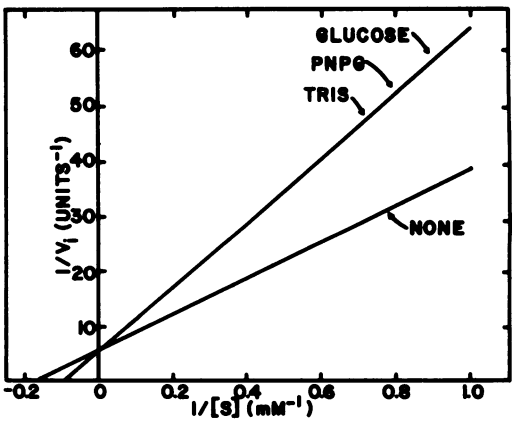

FIG. 5. *Lineweaver-Burk plots of the extracellular maltase (0.3 U/ml) from B. brevis B-4389. Reaction mixtures were incubated at 50°C for 15 min in 0.1 M phosphate buffer, pH 6.5; inhibitor concentrations were 5 mM for D-glucose, and 10 mM for p-nitrophenyl-α-D-glucoside (PNPG) and Tris.*

tase from a thermophilic *Bacillus* sp. (28), B-4389 maltase was not cell-bound and production was not associated with cell lysis (data not shown).

During purification, maltase and isomaltase were purified 20.0- and 19.1-fold, respectively (Table 1), suggesting that the two activities were intrinsic to the enzyme from B-4389. However, glucosyltransferase activity was purified only 11.5-fold (Table 1), considerably less than the values for maltase and isomaltase purification. This apparent anomaly may be explained on the basis of relative inaccuracy of the transferase assay. It is reasonable to conclude, therefore, that the data from Table 1 support the supposition that the *B. brevis* B-4389 enzyme was intrinsically capable of glucosyltransferase activity as well as maltase and isomaltase activities. A similar situation was described and a similar conclusion was drawn for the purification of rat liver α-glucosidase (14).

Other data supporting the idea that both maltase and isomaltase activities were intrinsic capabilities of the B-4389 enzyme were: (i) during gel filtration (Fig. 1), isomaltase and maltase activities were eluted in parallel, and (ii) both activities corresponded to the same major protein band resulting from isoelectric focusing.

The pH optimum (6.5), temperature optimum (48 to 50°C), and pH stability range (5.0 to 7.0) of the partially purified enzyme from B-4389 were similar to those of other bacterial maltases examined in detail (2, 10, 17, 29, 33). The molecular weight of B-4389 extracellular maltase, as determined by gel filtration, was 52,000 (Fig. 4). This value is higher than those for other *Bacillus* maltases previously determined: 12,000 for *B. cereus* (33) and 33,000 for *B. subtilis* (29). B-4389 maltase was competitively inhibited by D-glucose, *p*-nitrophenyl-α-D-glucoside, and Tris (Fig. 5). Whereas many reports have been published documenting competitive inhibition of maltase activities by glucose and Tris, only one has mentioned inhibition by *p*-nitrophenyl-α-D-glucoside (12). The results of sulfhydryl effectors indicated that B-4389 maltase was not a sulfhydryl enzyme. Also, because the chelating agent, ethylenediaminetetraacetic acid, did not inhibit the enzyme activity, it seemed that the extracellular maltase was not a metalloprotein or that the metal ion was very tightly bound.

Results of substrate specificity tests (Table 2) revealed that the enzyme from *B. brevis* B-4389 was specific for α-1,4- and α-1,6-glucosidic bonds. On α-1,4-glucosidic polymers, the rates of hydrolysis for the enzyme decreased as the degree of polymerization increased from 2 (maltose) to 40 (G_{40}). The specificity of the maltase was similar to that of an extracellular maltase from *B. subtilis* (29). When compared with maltases from other bacterial sources (2, 3, 9, 11, 17, 33), B-4389 maltase possessed a relatively high degree of specificity. Under the classification criteria of Chiba et al. (6), the enzyme from *B. brevis* would be classified as a "true" maltase because: (i) it possessed a high degree of specificity to maltose, (ii) it had no activity on aryl-α-D-glucosides, and (iii) it did not contain amyloglucosidase (exo-1,4-α-glucosidase) activity.

In addition to maltase and isomaltase activities, the enzyme from *B. brevis* B-4389 contained glucosyltransferase activity; glucosyltransferase activity has been documented for many other bacterial α-glucosidases (2, 15, 17, 21, 29, 31, 33). Late reaction (24-h) products from B-4389 maltase digests included glucose, isomaltose, and isomaltotriose. Because only α-1,6-oligosaccharides were detected as transferase products, the conclusion was that the enzyme was capable only of glucosyl transfer via α-1,6-glucosidic bonds. The action pattern of B-4389 maltase seems distinct from those of microbial α-glucosidases that were able to synthesize α-1,4-oligosaccharides by glucosyltransferase activity (2, 7, 32–34). Also, unlike many enzymes previously examined (4, 7, 22–24), the enzyme from *B. brevis* was not able to synthesize the trisaccharide panose.

The enzyme required the presence of a primer substrate, such as maltose, to exhibit glucosyltransferase activity. This conclusion is based on two observations: (i) free [^{14}C]glucose was transferred to maltose by the *B. brevis* enzyme, and (ii) no transferase products resulted from reaction mixtures containing only glucose and B-4389 maltase.

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