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Production and Properties of α-Glucosidase from *Lactobacillus acidophilus*

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Lactobacillus acidophilus IFO 3532 was found to produce only intracellular α-glucosidase (α-D-glucoside glucohydrolase; EC 3.2.1.20). Maximum enzyme production was obtained in a medium containing 2% maltose as inducer at 37°C and at an initial pH of 6.5. The enzyme was formed in the cytoplasm and accumulated as a large pool during the logarithmic growth phase. Enzyme production was strongly inhibited by 4 μM $CuSO_4$, 40 μM $CoCl_2$, and beef extract; $MnSO_4$ and the presence of proteose peptone and yeast extract in the medium greatly enhanced enzyme production. A 16.6-fold purification of α-glucosidase was achieved by $(NH_4)_2SO_4$ fractionation and DEAE-cellulose column chromatography. The enzyme showed high specificity for maltose. The K_m for α-*p*-nitrophenyl-β-D-glucopyranoside was 11.5 mM, and the V_{max} for α-*p*-nitrophenyl-β-D-glucopyranoside hydrolysis was 12.99 μmol/min per mg of protein. The optimal pH and temperature for enzyme activity were 5.0 and 37°C, respectively. The enzyme activity was inhibited by Hg^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+}, urea, rose bengal, and 2-iodoacetamide, whereas Mn^{2+}, Mg^{2+}, L-cysteine, L-histidine, Tris, and EDTA stimulated enzyme activity. Transglucosylase activity was present in the partially purified enzyme, and isomaltose was the only glucosyltransferase product. Amylase activity in the purified preparation was relatively weak, and no isomaltase activity was detected.

The lactic acid bacteria are composed of a diverse group of bacteria that are known for their fermentative activities. Large numbers of lactobacilli species are found to be present as part of the human intestinal flora (8, 22). It has been suggested that species of *Lactobacillus* may enhance resistance to common intestinal disorders by being able to maintain the normal intestinal microflora through the production of glycosidases which split off various sugars and thus supply energy for the growth of other intestinal flora (20, 21).

The enzyme α-glucosidase (α-D-glucoside glucohydrolase; EC 3.2.1.20) has been reported to occur in animals (11), plants (14), molds, yeasts, and fungi (3, 23, 26, 31). Although a number of studies on bacterial α-glucosidases have been carried out (1, 2, 10, 17, 24, 25, 28, 30), there is a paucity of information on the α-glucosidase from *Lactobacillus* sp. (12). This paper describes the results of a study on the production and properties of the α-glucosidase from *Lactobacillus acidophilus* IFO 3532.

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MATERIALS AND METHODS

Organism and medium. *L. acidophilus* IFO 3532 was obtained from the Institute of Fermentation, Osaka, Japan. Unless otherwise stated, the cultures were maintained and grown in MRS medium (5) which contained: glucose, 20 g; Lab-lemco (Difco Laboratories, Detroit, Mich.), 10 g; yeast extract (Difco), 5 g; proteose peptone (Difco), 10 g; KH_2PO_4, 2 g; Na_3-acetate, 5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $MnSO_4 \cdot 4H_2O$, 0.037 g; Tween 80, 1 ml; and $(NH_4)_3$-citrate, 2 g in 1,000 ml of distilled water. The pH of the medium was adjusted to 6.5 with 0.1 N HCl before autoclaving. A Millipore filter-sterilized stock solution of glucose was used.

Effect of carbohydrates on α-glucosidase production. Overnight cultures of *L. acidophilus* IFO 3532 grown in MRS medium at 37°C were harvested by centrifugation (Sorvall model SS-1) at 4,000 × *g* for 20 min. After three washes with 0.05 M potassium phosphate buffer (pH 6.5), the cell pellet was resuspended in the same buffer and used to inoculate a series of MRS media containing only one of the following carbohydrates: glucose, lactose, maltose, cellobiose, or melibiose at a final concentration of 2% in combination with various pH values. Production of α-glucosidase in these cultures was assayed.

Determination of optimal pH and temperature for α-glucosidase activity. The optimum pH and temperature for enzyme activity were determined by adding a 0.5-ml enzyme extract in 0.05 M phosphate buffer (pH 7.0) to 2 ml each of the following buffers: 0.1 M citrate-

phosphate (pH 5 to 7), 0.1 M phosphate (pH 7 to 8) and 0.1 M Tris-hydrochloride (pH 8 to 9). Then 0.5 ml of 5 mM α-*p*-nitrophenyl-β-D-glucopyranoside (α-PNPG) in deionized water was added. Incubation was carried out at temperatures between 20 and 45°C, and the enzyme was assayed.

Bacterial dry weight was determined from a standard curve which correlated readings of the Klett-Summerson photoelectric colorimeter fitted with a no. 66 filter to weight of cells dried at 110°C for 18 h.

Effects of inorganic salts and complex organic nutrients on α-glucosidase production. Various concentrations of certain inorganic salts were added individually to a modified MRS medium which was free of magnesium and manganese. An attempt was also made to eliminate the complex organic nutrients, one at a time, in the MRS medium to evaluate their individual effect on α-glucosidase production. All enzyme assays were carried out with late log growth phase cultures.

Preparation of crude extract and α-glucosidase assay. At various intervals during cultivation, 50 ml of culture was taken and centrifuged at 27,000 × *g* for 20 min in a refrigerated centrifuge (Sorvall Superspeed RC2-B), followed by three washes of the cell pellets with potassium phosphate buffer (pH 6.5). The supernatant, together with the washing, was assayed for extracellular α-glucosidase activity. The cell pellets were resuspended in cold potassium phosphate buffer (pH 6.5) and sonicated (Sonication Cell Disruptor, model W-200-R) at 0°C for 5 min. After centrifugation at 27,000 × *g* for 20 min, the supernatant was assayed for intracellular α-glucosidase. The cell debris collected was washed three times with the same buffer and resuspended in the buffer to determine the α-glucosidase activity associated with the cell debris fraction. α-Glucosidase activity was measured by the release of *p*-nitrophenol from α-PNPG, purchased from Sigma Chemical Co. (St. Louis, Mo.). A 2-ml portion of the substrate solution (α-PNPG) at 5 mM in 0.05 M potassium citrate buffer (pH 5.0) was mixed with 0.5 ml of the crude extract, and the mixture was incubated at 37°C for 20 min. A solution of 2.5 ml of cold sodium carbonate (0.5 M) was then added to the mixture to stop the reaction. The yellow *p*-nitrophenol released was read at 400 nm in a spectrophotometer. A unit of enzyme activity was equivalent to 1 μmol of *p*-nitrophenol liberated from α-PNPG per min. The specific activity of α-glucosidase was expressed as micromoles of *p*-nitrophenol liberated from α-PNPG per milligram of protein per minute. Protein contents of the fractions were determined by the Lowry spectrophotometric method (13).

Partial purification of α-glucosidase. Unless otherwise indicated, all procedures were carried out at 4°C. The cells were suspended in 50 ml of 0.05 M potassium phosphate buffer (pH 6.5) containing 1 mM mercaptoethanol and sonicated at 0°C. Centrifugation was done at 27,000 × *g* for 20 min. The protein in the supernatant was precipitated by the addition of $(NH_4)_2SO_4$ to 50% saturation (4°C); after 30 min the precipitate was removed by centrifugation and discarded. The supernatant was then brought to 100% saturation by the addition of ammonium sulfate. After standing overnight, the precipitate was collected by centrifugation at 27,000 × *g* for 20 min and redissolved in 0.05 M potassium phosphate buffer (pH 6.5) containing 1 mM mercaptoethanol. The solution was dialyzed overnight against 0.02 M potassium phosphate buffer (pH 6.5) containing 1 mM mercaptoethanol. The enzyme solution was then purified by DEAE-cellulose column chromatography. DEAE-cellulose was packed into a column (4 by 22 cm) and equilibrated with 0.05 M potassium phosphate buffer (pH 6.5) containing 1 mM mercaptoethanol. Elution was carried out with a linear gradient of increasing concentrations of sodium chloride (from 0 to 0.5 M) in the same buffer. The fractions containing α-glucosidase activity were collected and concentrated by ammonium sulfate precipitation (100% saturation), and the solution was allowed to stand for 30 min. The precipitate was then collected by centrifugation and dissolved in 0.05 M potassium phosphate buffer (pH 6.5) containing 1 mM mercaptoethanol. The solution was again dialyzed overnight against 0.02 M phosphate buffer (pH 6.5) containing 1 mM mercaptoethanol.

The K_m and V_{max} for α-PNPG were determined from a Lineweaver-Burk plot using α-PNPG concentrations of between 1 and 10 mM.

Effects of cations on activity of partially purified α-glucosidase. The metal ions $BaCl_2 \cdot 2H_2O$, $MnCl_2 \cdot 4H_2O$, $MgCl_2 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, $CoCl_2 \cdot 6H_2O$, $CaCl_2 \cdot 2H_2O$, $NiSO_4 \cdot 7H_2O$, KCl, $HgCl_2$, $ZnCl_2$, and $Pb(CH_3COO)_2$ were tested for their effects on partially purified α-glucosidase activity.

Inhibition studies. The effects of urea and organic compounds on the activity of partially purified α-glucosidase were investigated by methods described elsewhere (7, 9, 31). The following compounds were used: 1 mM L-cysteine, 10 mM L-histidine, 10 mM and 100 mM Tris-hydrochloride, 10 mM and 50 mM EDTA, 10 mM rose bengal, and 10 mM 2-iodoacetamide.

Transglucosylase, isomaltase, and glucoamylase activities of partially purified α-glucosidase. A mixture of 2.5 ml of 10% glucose or 10% maltose, 0.5 ml of 0.05 M potassium citrate buffer (pH 5.0), and 2 ml of enzyme solution was incubated at 37°C. A parallel incubation was carried out with a mixture containing 2.5 ml of 10% glucose and 2.5 ml of the same buffer. At intervals, a portion of the reaction mixture was removed and heated in a boiling water bath for 3 min. The samples were spotted on filter paper (Whatman no. 1) and developed by multiple ascending techniques. The solvent system was butanol-pyridine-water (6:4:3). The spots were detected with the silver-dip method (15, 29). For determining isomaltase activity, a mixture of 2.5 ml of 10% isomaltose, 0.5 ml of 0.05 M potassium citrate buffer (pH 5.0), 1.5 ml of enzyme solution, and 0.5 ml of distilled water was incubated at 37°C for 24 h. Portions of the reaction mixture were taken out and developed on paper chromatography as described above. Glucoamylase activity was detected in a reaction mixture which contained 2 ml of soluble starch (0.5%) in 0.05 M potassium citrate buffer (pH 5.0) and 1 ml of enzyme solution. After incubation at 37°C for 30 and 60 min, a 0.2-ml portion of the mixture was taken out and added to 5 ml of 0.167 mM I_2-KI solution. The optical density of this solution was measured at 700 nm with a spectrophotometer.

RESULTS

Effects of carbohydrates, initial pH, and growth temperature on α-glucosidase production.

TABLE 1. Effects of carbohydrates and initial pH of culture medium on α-glucosidase production in *L. acidophilus* IFO 3532[a]

Carbohydrate	pH		Intracellular α-glucosidase (U/mg of cells)	Carbohydrate	pH		Intracellular α-glucosidase (U/mg of cells)
	Initial	Final			Initial	Final	
Glucose	5.0	4.2	18.0		7.0	5.8	14.0
	5.5	4.5	10.0		7.5	6.0	20.0
	6.0	4.8	26.4		8.0	6.3	8.0
	6.5	5.0	18.0		9.0	7.1	0.1
	7.0	5.2	19.0	Lactose	5.0	4.0	23.0
	7.5	5.8	9.0		5.5	4.3	37.0
	8.0	6.2	22.0		6.0	4.8	25.0
	9.0	7.3	0.2		6.5	5.1	23.0
Maltose	5.0	4.3	76.0		7.0	5.6	69.6
	5.5	4.6	113.0		7.5	5.9	74.2
	6.0	4.9	148.0		8.0	6.1	90.0
	6.5	5.1	203.0		9.0	6.8	2.0
	7.0	5.3	150.0	Cellobiose	5.0	4.1	28.0
	7.5	5.8	130.0		5.5	4.4	72.6
	8.0	6.1	72.0		6.0	4.9	22.6
	9.0	7.2	0.1		6.5	5.1	68.6
Melibiose	5.0	4.5	11.0		7.0	5.7	122.8
	5.5	4.9	20.0		7.5	6.0	41.0
	6.0	5.1	16.6		8.0	6.6	26.0
	6.5	5.3	23.0		9.0	7.0	2.4

[a] Cultures were grown for 26 h at 37°C before assay.

It was found that *L. acidophilus* IFO 3532 produced only intracellular α-glucosidase in MRS medium containing 2% carbohydrates as inducers (Table 1). Maximal intracellular enzyme production was obtained with maltose at an initial pH of 6.5, and enzyme production was reduced at other tested pH values. Extremely low levels of enzyme were detected at pH 9.0 in all cases. The optimal temperature for enzyme production was 37°C (Fig. 1).

Changes in α-glucosidase levels during cultivation. The appearance of α-glucosidase during different phases of growth was studied in MRS medium containing 2% maltose with an initial pH of 6.5 at 37°C. A low level of intracellular α-glucosidase formation appeared at the very beginning of cultivation, and the level increased quickly during the logarithmic phase of growth (Fig. 2). A maximal level of intracellular α-glucosidase was observed at the 26th h of cultivation, at which the highest peak of growth was also attained. A rapid decline of the enzyme activity was detected within 2 h after the maximal level had been achieved, and subsequently the enzyme level decreased in a linear fashion. No extracellular α-glucosidase was detected during the whole cultivation period. A low level of α-glucosidase associated with the cell debris was observed only after 16 h of cultivation, and the level increased steadily until the 26th h of cultivation. Again, rapid decline of the enzyme associated with the cell debris occurred within 2 h after the maximal level had been reached. The cultivation time for maximal accumulation of cell debris-associated enzyme coincided with that of the intracellular enzyme (Fig. 2).

Effects of inorganic salts on α-glucosidase production. Lower levels of α-glucosidase were detected for cultures grown in MRS medium without magnesium and manganese than the control culture, which contained both compo-

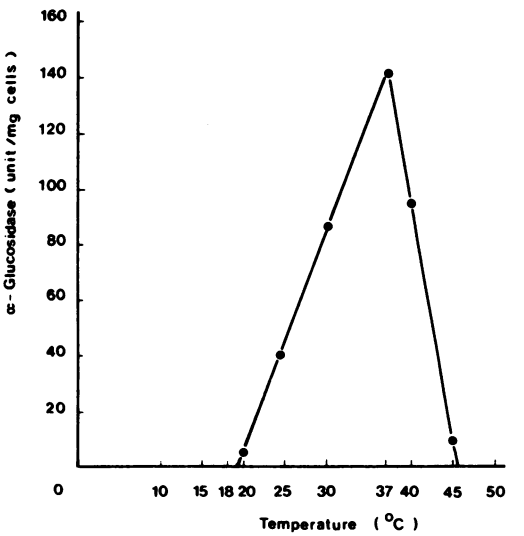

FIG. 1. Effect of growth temperature on α-glucosidase production by *L. acidophilus* IFO 3532 grown for 26 h in MRS medium containing 2% maltose at pH 6.5.

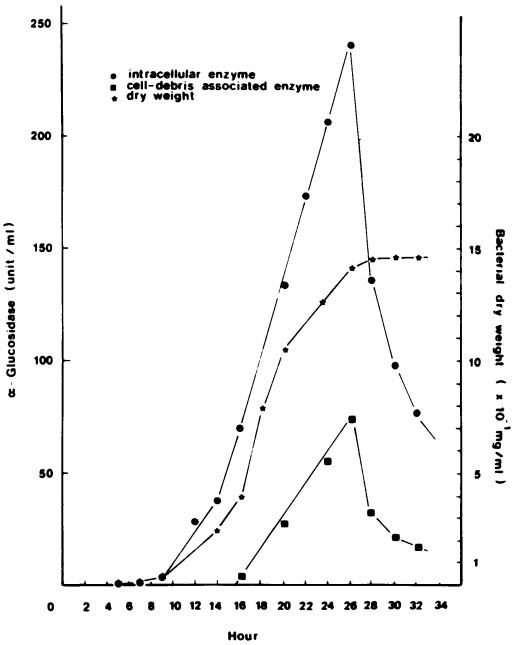

FIG. 2. Changes in α-glucosidase levels during cultivation of *L. acidophilus* IFO 3532 in MRS medium containing 2% maltose at pH 6.5 with a growth temperature of 37°C.

nents (Table 2). The addition of 200 μM $MnSO_4$ greatly enhanced enzyme production, and enzyme production was strongly inhibited by the presence of 4 μM $CuSO_4$ and 40 μM $CoCl_2$.

TABLE 2. Effects of inorganic salts on α-glucosidase production in *L. acidophilus* IFO 3532

Medium	α-Glucosidase (U/mg of cells)
MRS	186.6
M1[a]	117.0
M1 + 40 μM $MnSO_4 \cdot 4H_2O$	140.0
M1 + 200 μM $MnSO_4 \cdot 4H_2O$	326.2
M1 + 2 μM $CoCl_2 \cdot 6H_2O$	109.0
M1 + 4 μM $CoCl_2 \cdot 6H_2O$	83.0
M1 + 40 μM $CoCl_2 \cdot 6H_2O$	36.0
M1 + 5 μM $MnCl_2 \cdot 4H_2O$	140.0
M1 + 10 μM $MgCl_2$	134.0
M1 + 4 μM $NiSO_2 \cdot 7H_2O$	171.2
M1 + 8 μM $MgSO_4 \cdot 7H_2O$	117.0
M1 + 40 μM $MgSO_4 \cdot 7H_2O$	134.0
M1 + 200 μM $MgSO_4 \cdot 7H_2O$	195.6
M1 + 20 μM NaCl	175.0
M1 + 8 μM $CaCl_2 \cdot 2H_2O$	116.0
M1 + 8 μM $ZnCl_2$	99.0
M1 + 4 μM $BaCl_2 \cdot 2H_2O$	154.0
M1 + 8 μM $FeSO_4$	144.0
M1 + 4 μM $CuSO_4 \cdot 5H_2O$	55.4

[a] M1, Magnesium- and manganese-free MRS medium.

Reduced levels of α-glucosidase formation were observed with the addition of all other inorganic salts tested.

Effects of complex organic nutrients on α-glucosidase production. Table 3 shows the effects of complex organic nutrients on enzyme production. The highest level of enzyme was obtained when both proteose peptone and yeast extract were added to the medium. Enzyme production was higher with proteose peptone alone than with yeast extract alone in the medium. The original MRS medium containing all the organic nutrients produced a lower level of enzyme. The beef extract exhibited a strong inhibitory action on α-glucosidase production whenever it was present alone or together with proteose peptone or yeast extract in the medium.

Optimal pH and temperature for α-glucosidase activity. Experiments for evaluating pH effect on specific enzyme activity were carried out in MRS medium containing 2% maltose at 37°C. The optimal pH for enzyme activity was 5.0 (Fig. 3), and the optimal temperature for enzyme activity was 37°C (Fig. 4).

Effects of cations on α-glucosidase activity. The addition of Mn^{2+} and Mg^{2+} to the reaction mixture enhanced α-glucosidase activity (Table 4). The addition of Mn^{2+} increased the enzyme activity by 67.4% over that of the control (cation free), whereas a slight increase (3.5%) in enzyme activity was observed with the addition of Mg^{2+}. The presence of K^+, Ba^{2+}, and Pb^{2+} did not affect the enzyme activity significantly. On the other hand, Ca^{2+} and Co^{2+} were found to slightly inhibit enzyme activity, whereas Hg^{2+}, Cu^{2+}, Ni^{2+}, and Zn^{2+} were strong enzyme inhibitors. The adverse effect was particularly noted for Hg^{2+}, where only 2.8% of α-glucosidase activity remained (Table 4).

Effects of urea and organic compounds on α-glucosidase activity. Urea (4.5 M) was found to inhibit α-glucosidase activity (Table 5). A rapid inactivation of enzyme activity was observed

TABLE 3. Effect of complex organic nutrients on α-glucosidase production in *L. acidophilus* IFO 3532[a]

Addition of organic nutrient to complex organic nutrient-free MRS medium	α-Glucosidase (U/mg of cells)
Yeast extract + beef extract + proteose peptone	82.0
Yeast extract	122.0
Proteose peptone	154.0
Beef extract	37.8
Yeast extract + beef extract	64.5
Yeast extract + proteose peptone	160.0
Proteose peptone + beef extract	68.7

[a] The concentration of each component was yeast extract, 0.5%; beef extract, 0.5%; and proteose peptone, 0.5%.

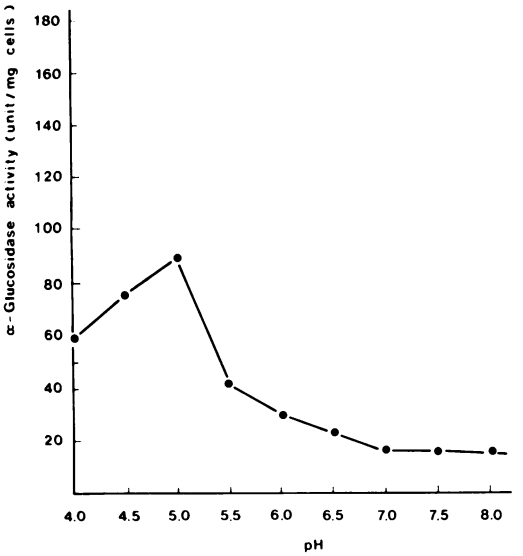

FIG. 3. pH profile of α-glucosidase activity of *L. acidophilus* IFO 3532 grown for 26 h in MRS medium at 37°C.

TABLE 4. Effects of cations on α-glucosidase activity of *L. acidophilus* IFO 3532[a]

Cation	α-Glucosidase (% activity)
Control[b]	100
$BaCl_2 \cdot 2H_2O$	97.7
$MnCl_2 \cdot 4H_2O$	167.4
$MgCl_2 \cdot 6H_2O$	103.5
$CuCl_2 \cdot 2H_2O$	73.3
KCl	94.0
$CaCl_2 \cdot 2H_2O$	83.7
$HgCl_2$	2.8
$CoCl_2 \cdot 6H_2O$	82.6
$NiSO_4 \cdot 7H_2O$	68.6
$Pb(CH_3COO)_2$	97.7
$ZnCl_2$	60.9

[a] The reaction mixture contained 0.02 ml of partially purified enzyme, 0.02 ml of metal ion solution (0.1 M) in deionized water, and 0.36 ml of 0.05 M potassium phosphate buffer (pH 5.0). Incubation was at 37°C for 30 min. Then 2 ml of 5 mM α-PNPG at pH 5.0 was added, and incubation was continued for another 15 min. An equal volume of 0.5 M cold sodium carbonate was added to stop the reaction, and enzyme activity was assayed.

[b] Original α-glucosidase activity.

after the enzyme was exposed to urea for only a few minutes. A 40% inhibition was achieved when the enzyme was treated with urea for 22 min.

The activity of α-glucosidase was enhanced by the presence of L-cysteine, L-histidine, Tris, and EDTA, whereas rose bengal and 2-iodoacetamide exhibited inhibitory action on enzyme activity (Table 5).

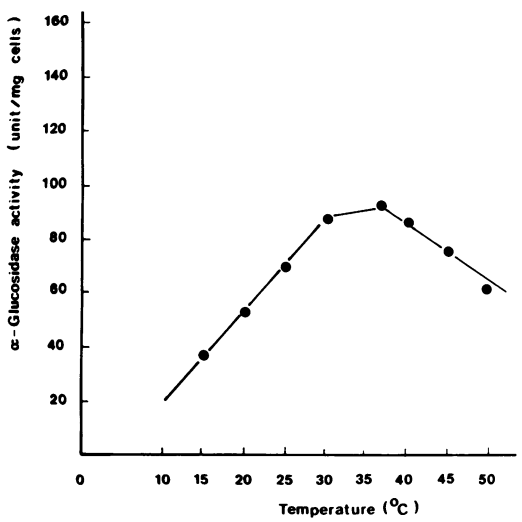

FIG. 4. Temperature profile of α-glucosidase activity of *L. acidophilus* IFO 3532 grown for 26 h in MRS medium at pH 5.0.

Localization of intracellular α-glucosidase. A preliminary experiment indicated that no extracellular α-glucosidase was formed in strain IFO 3532. The intracellular α-glucosidase resided mainly in the cytoplasmic fraction (Fig. 2). Another experiment was subsequently carried out by first removing the unbroken cells from the homogenate after sonication and assaying the clear extract (cytoplasmic fraction) and the cell fragments for α-glucosidase activity. It was again confirmed that the intracellular α-glucosidase resided mainly in the cytoplasmic fraction, which had 302 U of α-glucosidase activity per

TABLE 5. Effects of organic compounds on α-glucosidase activity of *L. acidophilus* IFO 3532[a]

Organic compound	α-Glucosidase (% activity)
Control[b]	100
L-Cysteine (1 mM)	115.8
L-Histidine (10 mM)	115.8
Tris (100 mM)	162.3
(10 mM)	144.7
EDTA (50 mM)	118.1
(10 mM)	108.2
Rose bengal (10 mM)	54.6
2-Iodoacetamide (10 mM)	57.8
Urea (4.5 M)	60.0

[a] The reaction mixture contained 0.04 ml of partially purified enzyme, 0.2 ml of potassium phosphate buffer (pH 5.0), 0.04 ml of organic compound, and 0.14 ml of distilled water. Incubation was at 37°C for 15 min.

[b] Original α-glucosidase activity.

TABLE 6. Partial purification of α-glucosidase of *L. acidophilus* IFO 3532

Purification procedure	Enzyme activity (U)	Protein (μg/ml)	Sp act (μmol/mg per min)	Recovery (%)	Purification (fold)
Crude extract	985.0	750	87.6	100	
Ammonium sulfate fractionation	925.0	117.9	523.0	94	5.97
DEAE-cellulose column chromatography	272.0	12.5	1,450.7	28	16.6

mg of cells, whereas only 46 U/mg of cells resided in the cell debris.

Purification and enzyme kinetics. The enzyme was extracted and partially purified by ammonium sulfate fractionation and DEAE-cellulose column chromatography. Results indicated that the enzyme achieved a 16.6-fold purification (Table 6). Both the enzyme activity and the protein content eluted from DEAE-cellulose column chromatography showed a single sharp peak (Fig. 5). The partially purified enzyme showed maximal absorption at 280 nm.

Typical Michaelis-Menten kinetics were observed when the rate of enzyme activity was plotted against substrate (α-PNPG) concentrations. The K_m for α-PNPG was 11.5 mM, and the V_{max} for α-PNPG hydrolysis was 12.99 μmol/min per mg of protein, as determined from the Lineweaver-Burk plot.

Transglucosylase, isomaltase, and glucoamylase activities of partially purified α-glucosidase. The presence of transferase activity was confirmed by the appearance of a compound, in addition to glucose, which was identified as isomaltose after maltose hydrolysis (Fig. 6). Neither maltotriose nor panose was present in the enzyme reaction mixtures. A reaction mixture containing only D-glucose and α-glucosidase did not show any transferase product. Paper chromatographic analysis of an isomaltose-enzyme digest did not reveal any isomaltase activity, as indicated by the absence of glucose formation (Fig. 6). Results also indicated that maltotriose was hydrolyzed into glucose and maltose by α-glucosidase (Fig. 6).

After the addition of soluble starch to the enzyme solution, a decline in optical density was observed which was proportional to increasing reaction time (the optical density dropped from 0.395 with an incubation time of 30 min to 0.378 with a 60-min incubation). Since this process differed from the rapid hydrolysis of soluble starch by the action of α-amylase, the slow hydrolyzing effect was thus considered as the weak glucoamylase activity of the partially purified α-glucosidase.

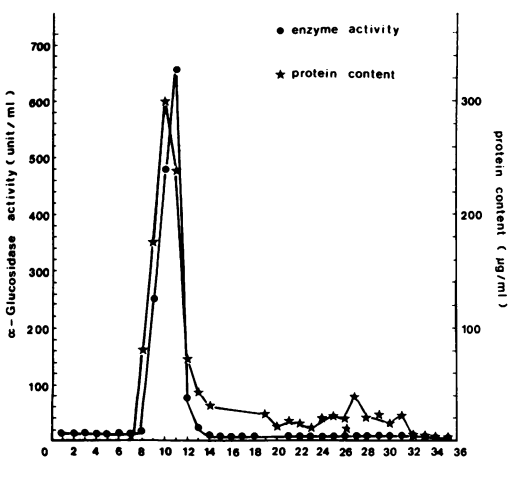

FIG. 5. Elution pattern of α-glucosidase and protein of *L. acidophilus* IFO 3532 from the DEAE-cellulose column. The column was equilibrated with 0.05 M phosphate buffer (pH 6.5) containing 1 mM mercaptoethanol. Elution was carried out at a flow rate of 25 ml/h with the same buffer used for equilibration, and 5-ml fractions were collected.

DISCUSSION

The significant difference in the pattern of α-glucosidase production between strain IFO 3532 and *L. acidophilus* NCTC 1723 is that strain NCTC 1723 produces both intracellular and extracellular α-glucosidase (12), whereas only intracellular α-glucosidase is produced by strain

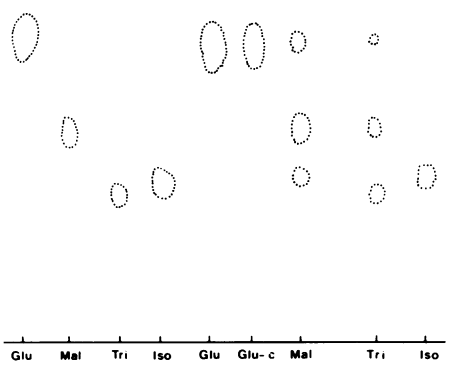

FIG. 6. Chromatographic analysis of partially purified *L. acidophilus* IFO 3532 α-glucosidase action on glucose, maltose, isomaltose, and maltotriose. Glu, Glucose; Glu-c, glucose control; Mal, maltose; Iso, isomaltose; Tri, maltotriose; (std), standard.

IFO 3532. Production of α-glucosidase by strain IFO 3532 is maximized when cells are grown at 37°C with 2% maltose as the inducer in MRS medium having an initial pH of 6.5. Lower levels of enzyme can be detected when cells are grown in medium with glucose, lactose, cellobiose, and melibiose as carbon sources. A similar finding has been reported in *Pseudomonas* sp. strain SB-15 (24), which is in contrast to the constitutive α-glucosidase reported in *Streptococcus pyogenes* (6), *Streptococcus mitis* 903 (17), and *Bacteroides fragilis* (2).

The pattern of changes of α-glucosidase levels during cultivation reveals that intracellular α-glucosidase formation appears at the beginning of the cultivation period, accumulates during the logarithmic phase of growth, and finally reaches a maximum at the 26th h of cultivation. In strain NCTC 1723, intracellular α-glucosidase formation appears only after 4 h of cultivation, and the level reaches maximum at about the 8th h of cultivation, which corresponds to the mid-logarithmic phase of the culture (12). The decline of intracellular α-glucosidase in strain NCTC 1723 after reaching the maximal level of production may be due to catabolic repression, since glucose is produced from the hydrolysis of maltose in the medium, which is not the case in strain IFO 3532. Strain IFO 3532, after 26 h of cultivation, shows a rapid decline in enzyme activity which is an indication of cessation of enzyme synthesis as well as enzyme destruction. The cessation of enzyme synthesis can probably be attributed to the decrease in RNA precursor pool or the amounts of substrate that are available for enzyme formation. However, factors leading to enzyme destruction in this case remain unclear at present.

Mn^{2+} is found to be required for maximizing α-glucosidase production. The highest level of enzyme production is obtained when both proteose peptone and yeast extract are added to the culture medium. It has been reported that proteose peptone with yeast extract supports the highest yield of α-glucosidase in *B. fragilis* (2). Both peptone and yeast extract have been shown to be more effective than meat extract in stimulating α-glucosidase production in *Bacillus* sp. strain KP 1006 and KP 1035 (25). That proteose peptone medium is superior to brain heart infusion broth for production of α-glucosidase was also reported for some oral streptococci (17).

Among the cations tested, Hg^{2+}, Cu^{2+}, Ni^{2+}, and Zn^{2+} are potent inhibitors of α-glucosidase activity. These cations also strongly inhibit α-glucosidase formation and activity in *Bacillus cereus* (30). The observation that EDTA does not inhibit α-glucosidase activity indicates that the enzyme is not a metalloprotein or that the metal ions are very tightly bound. The stimulatory effect on α-glucosidase by Tris can be attributed to the unionized form of the amine, since L-histidine also stimulates enzyme activity.

Urea may cause a molecular transition of enzyme structure (7) which might be responsible for its inhibitory effect on enzyme activity. The fact that rose bengal and 2-iodoacetamide inhibit α-glucosidase activity indicates that the α-glucosidase from strain IFO 3532 might contain both histidine and cysteine residues in the active center. This may be further supported by the enhancement of enzyme activity when L-cysteine is added to the reaction mixture during enzyme assay.

Results obtained from the present study indicate that under optimal conditions, the α-glucosidase of *L. acidophilus* IFO 3532 is more active than the α-glucosidase of *Pseudomonas fluorescens* (9) but less active than that of *B. fragilis* (2).

Transglucosylase activity of the partially purified α-glucosidase was detected. An intermediate substance identified as isomaltose was present as the glucosyltransferase product. Isomaltose has been confirmed as the main transglucosylation product from maltose digest by the action of α-glucosidase in *Aspergillus niger* (27), *Schizosaccharomyces pompe* (4), and *Bacillus brevis* (16). Based on the observation that no transferase product is detected in reaction mixtures containing only glucose and α-glucosidase, it is conceivable that the enzyme requires the presence of a primer substrate such as maltose to induce glucosyltransferase activity. On the other hand, the fact that α-1,6-linked isomaltose is detected as the transferase product leads to the conclusion that the enzyme is capable of glucosyl transfer via α-1,6-glucosidic bonds. Unlike many other enzymes previously examined (4, 18, 19), the α-glucosidase from *L. acidophilus* IFO 3532 is unable to synthesize the trisaccharide panose. The present study also indicates that α-glucosidase shows no isomaltase activity, and only relatively weak glucoamylase activity is found associated with the partially purified enzyme.

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