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Production of Maltopentaose and Biochemical Characterization of Maltopentaose-Forming Amylase

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Abstract *Bacillus* sp. AIR-5, a strain from soil, produced an extracellular maltopentaose-forming amylase from amylose and soluble starch. This bacterium produced 8.9 g/l of maltopentaose from 40 g/l of soluble starch in a batch fermentation and the maltopentaose made up 90% of the maltooligosaccharides produced (from maltose to maltoheptaose). The culture supernatant was concentrated using a 30 K molecular weight cut-off membrane and purified by DEAE-Cellulose and Sephadex G-150 column chromatographies. The purified protein showed one band on a native-PAGE and its molecular mass was estimated as 250 kDa. The 250-kDa protein was composed of tetramers of a 63-kDa protein. The isoelectric point of the purified protein was pH 6.9, and the optimum temperature for the enzyme activity was 45°C. The enzyme was quickly inactivated above 55°C, and showed a maximum activity at pH 8.5 and over 90% stability between a pH of 6 to 10. The putative N-terminal amino acid sequence of AIR-5 amylase, ATINNGTLMQYFEWYVPNDG, showed a 96% sequence similarity with that of BLA, a general liquefying amylase.

Key words: Amylase, maltopentaose production, purification, fermentation

α-Amylase [α-(1→4)-D-glucan glucanohydrolase, E.C 3. 2. 1. 1] is an endo-acting enzyme that hydrolyzes starch by cleaving α-1,4-glucosidic linkages at random [6, 9]. It is also one of the most important commercial enzymes, with wide applications in starch-processing, brewing, alcohol production, textiles, and other industries [25, 38]. Numerous α-amylases originating from eubacteria, fungi, plants, and animals have been characterized and their genes cloned [31, 41]. Several reports have also been published on the production of maltooligosaccharides (G_2, G_3, G_4, G_5, and so on) from starch [27, 28, 30, 33]. G_2 (maltose) from starch has been widely studied using plant β-amylases [29]. As for G_3-G_6, G_3 (maltotriose)-forming amylases have been discovered in *Streptococcus bovis* [30] and *Bacillus subtilis* [31], G_4 (maltotetraose)-forming amylase in *Pseudomonas stutzeri* [27], G_5 (maltopentaose)-forming amylases in *B. licheniformis* [28] and *B. cereus* NY-14 [42], and G_6 (maltohexaose)-forming amylases in *Bacillus circulans* [32] and *Bacillus.* sp. US 100 [20]. Maltooligosaccharides (G_3-G_6) are used as reagents in research and clinical applications for the determination of serum amylase activity. In particular, maltopentaose is used as a substrate for the accurate determination of amylase concentrations in body fluids such as serum or urine [35]. Furthermore, because of the low sweetness, good solubility, and low viscosity of the solutions, maltopentaose can be used as a carbohydrate source in liquid alimentation for infants, elderly people, or recovering patients [4, 16, 14, 15]. Yet the price of pure maltopentaose is high because of the difficult purification process involved [43]. Maltopentaose has been produced by the hydrolysis of starch or amylose utilizing various amylases. However, all these processes have disadvantages in that the amylases must be purified and the hydrolyzates contain large amounts of maltooligosaccharides other than maltopentaose. Recently, the current authors isolated a bacterium, *Bacillus* sp. AIR-5, which can digest soluble starch and produce maltopentaose effectively in a culture supernatant with a high concentration (over 90%) of maltopentaose and low concentrations of maltotetraose

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(G4) and maltohexaose (G6). This work reports on the purification and biochemical characterization of the maltopentaose-forming amylase from *Bacillus* sp. AIR-5.

MATERIALS AND METHODS

Organism and Culture Conditions

The *Bacillus* sp. AIR-5 was isolated from soil samples in Gwang-Ju, South Korea. The optimum temperature and pH for the growth of this organism were 28°C and pH 7.0, respectively. The culture was maintained in an MPS medium composed of 2% (w/v) soluble starch (Yakuri Pure Chemical, Osaka, Japan), 0.3% (w/v) tryptone (Difco Laboratories, MI, U.S.A.), 0.5% (w/v) NaCl, 0.5% (w/v) K_2HPO_4, 0.001% (w/v) $MgSO_4 \cdot 7H_2O$, 1 mM $CaCl_2$, and a 1.5% (w/v) agar. The medium pH was adjusted to 7.0 prior to sterilization.

Purification of Amylase

The *Bacillus* sp. AIR-5 amylase was produced in a fermentor (BL-10 l, Hanil R&D Co., Korea). The aeration rate, temperature, and stirring rate were 1.3 vvm, 28°C, and 200 rpm, respectively. After the removal of cells by centrifugation (6,000 ×g, 15 min) at 4°C, the supernatant was concentrated from 7.8-l to 800 ml using a 30 K molecular weight cut-off membrane (Millipore, Inc., MA, U.S.A.). The crude enzyme (0.05 mg protein/ml) was loaded onto a DEAE-Cellulose (Sigma, MO, U.S.A.) column (20 cm× 2 cm) previously equilibrated with a 20 mM Tris-HCl buffer (pH 8.5) and then eluted with Tris-HCl (pH 8.5) containing 0.5 M NaCl. The fractions exhibiting amylase activity were pooled and concentrated using a 30 K molecular weight cut-off stacked membrane (Amicon, MA, U.S.A.) under a continuous N_2 gas flow. The Bradford method was used for the protein assay [2]. To determine the maltopentaose-forming activity, each fraction was reacted with 1% (w/v) soluble starch at 37°C for 30 min, and the sample then was analyzed by thin-layer chromatography, as described previously [1, 11, 12, 13].

Gel Electrophoresis and Activity Staining

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on 8% cross-linked polyacrylamide gels in a Tris-glycine buffer (pH 8.8), as described by Laemmli [18]. The protein fractions were denatured by heat treatment for 5 min at 100°C in the presence of SDS (0.05%) and 2-mercaptoethanol (1%, v/v). Coomassie Brilliant Blue R-250 was used for staining the protein. Nondenaturing gel electrophoresis (native-PAGE) in the absence of SDS and 2-mercaptoethanol was performed using a 10% gel. The molecular weights of the proteins in the sample were estimated by SDS-PAGE [8% (w/v) acrylamide gel] and native-PAGE [10% (w/v)] and compared with a broad range of molecular mass standards (Bio-Rad, Hercules, U.S.A.), including myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase b (97.4 kDa), serum albumin (66.2 kDa), carbonic anhydrase (31 kDa), trypsin inhibitor (21.5 kDa), lysozyme (14.4 kDa), and aprotinin (6.5 kDa). After the gel electrophoresis, activity staining was performed by incubating the gel in a 1% (w/v) soluble starch solution (pH 8.5) at 37°C overnight, followed by incubation of the gel in an iodine solution [1 g of I_2 and 10 g of KI in 100 ml of 50% (v/v) methanol] for 1 min, followed by washing the gel with deionized water [7]. A protein band with amylase activity exhibited a clear zone with a dark blue background. The isoelectricfocusing of the protein was performed with a Mini IEF Cell [26] gel electrofocusing system (Model Number I111, Bio-Rad) with pI calibration standards: phycocyanin (3 bands, pI 4.45, 4.65, and 4.75), β-lactoglobulin B (pI 5.1), bovine carbonic anhydrase (pI 6.0), human carbonic anhydrase (pI 6.5), equine myoglobin (2 bands, pI 6.8 and 7.0), human hemoglobin A (pI 7.1), human hemoglobin C (pI 7.5), lentil lectin (3 bands, pI 7.8, 8.0, and 8.2), and cytochrome *c* (pI 9.6).

Assay of Amylase Activity

One-half ml of 1% (w/v) gelatinized soluble starch (preincubated at 37°C), 0.4 ml of 20 mM Tris-HCl buffer (pH 8.5), and 0.1 ml of the enzyme solution were mixed and incubated at 37°C. The activity was determined by the copper-bicinchoninate reducing-value method using 1% (w/v) soluble starch [5]. One unit was defined as the amount of enzyme which liberated 1 μmole maltose equivalent using 1% (w/v) soluble starch as the enzyme substrate.

End Product Analysis

The hydrolysis products were analyzed by two ascents on a Whatman K5F thin layer chromatography plate (Whatman, England) using the solvent mixture of either 2/5/1.5 (v/v/v) or 2/5/2 (v/v/v) of nitromethane/1-propanol/water, followed by dipping the plate into 0.3% (w/v) N-(1-naphthyl)-ethylenediamine and 5% (w/v) sulfuric acid in methanol. The plate was baked at 120°C for 10 min. The NIH image program was used to quantify the amount of carbohydrate after the TLC using standard carbohydrates [1, 11, 12, 13].

Enzyme Characterization

The optimum pH for the AIR-5 amylase acitivity was determined from the reaction rate over a pH range of 4–10. The buffers used were: Na-acetate buffer (pH 4–6), Na-phosphate buffer (pH 6–7.5), Tris-HCl buffer (pH 8–10), and glycine-HCl (pH 9–10). The pH optimum was determined at 37°C for a 1-h reaction. To measure the pH stability of the AIR-5 amylase, 0.1 ml of the enzyme solution was mixed with 0.9 ml of 20 mM buffer solution with various pHs (pH 4–10) and incubated for 1 h at 37°C. Then, 0.5 ml of 1% (w/v) soluble starch, 0.4 ml of 20 mM

Tris-HCl buffer (pH 8.5), and 0.1 ml of the treated enzyme solution were mixed and incubated for 1 h. The optimum reaction temperature was determined by incubating 0.1 ml of the enzyme solution, 0.5 ml of 1% (w/v) soluble starch, and 0.4 ml of 20 mM Tris-HCl buffer (pH 8.5) for 1 h at various temperatures. To determine the thermostability, 0.1 ml of the enzyme solution (5 U/ml) was incubated with 0.4 ml of 20 mM Tris-HCl buffer (pH 8.5) at various temperatures for 1 h, then 0.5 ml of 1% (w/v) soluble starch solution was added. The reducing sugar formed was determined after 1 h at 37°C. To determine the effect of calcium ion on the thermostability, the enzyme was incubated for 1 h at various temperatures in the presence and absence of 1 mM calcium chloride, and the residual activity was then determined.

Effects of Various Metal Ions and Inhibitors on Enzyme Acitivties

To determine the effects of various metals and inhibitors, 0.1 ml of the enzyme solution (3.2 U/ml) was incubated with 0.4 ml of 20 mM Tris-HCl (pH 8.5) containing various metal ions or inhibitors and 0.5 ml of 1% (w/v) soluble starch was then added and reacted for 1 h at 37°C.

Relative Activities of Enzyme on Various Substrates

A reaction mixture consisting of 0.5 ml of 1% (w/v) substrate solution, 0.4 ml of 20 mM Tris-HCl (pH 8.5) buffer solution, and 0.1 ml of the enzyme solution (3.2 U/ml) was incubated for 1 h at 37°C. At given time intervals, 100 µl of each sample was withdrawn and mixed with 20 µl of 0.1 M NaOH to inactivate the enzyme. The reducing sugar formed was determined using the copper-bicinchoninate reducing value method.

Kinetics of Enzyme Inhibition

The enzyme, substrate, and inhibitor were prepared in 20 mM Tris-HCl buffer (pH 8.5). The inhibitor (0.1 ml) was preincubated with the substrate at 37°C for 5 min. A reaction mixture consisting of 0.5 ml of 1% (w/v) substrate, 0.4 ml of 20 mM Tris-HCl (pH 8.5) buffer, and 0.1 ml of the enzyme (3.2 U/ml) were incubated for 1 h at 37°C. Every 5 min, 100 µl of the sample was collected. To stop the reaction, each aliquot was mixed with 0.1 M NaOH. The AIR-5 amylase activity was measured using the copper-bicinchoninate reducing-value method with a micro plate reader.

N-Terminal Amino Acid Sequencing

The purified enzyme was subjected to SDS-PAGE. The electrotransfer of the proteins to a polyvinylidene difluoride (PVDF) membrane was carried out as described previously [24]. The analysis of the N-terminal sequence was carried out using an automated Edman degradation protein sequencer (Model 477A, Applied Biosystems, CA, U.S.A.). The N-terminal amino acid sequence was determined using an on-line phenylthiohydantoin analyzer (Model 120A, Applied Biosystems, CA, U.S.A.).

RESULTS AND DISCUSSION

Maltopentaose Production by *Bacillus* sp. AIR-5

The isolated strain was able to produce a high concentration of maltopentaose in the fermentor when a MPS medium was used. The optimum conditions for maltopentaose production were observed at an aeration rate of 1.3 vvm, stirring rate of 200 rpm, pH of 7.0, and 4% of soluble starch. As shown in Fig. 1, starch was digested by *Bacillus* sp. AIR-5 during growth, and various oligosaccharides were produced for up to 24 h of cultivation. Thereafter, the initially produced high molecular weight oligosaccharides [bigger than maltohexaose (G6), ▽] were further hydrolyzed into maltopentaose (G5, O in the Fig. 1), and maltose (G2, ●), maltotriose (G3, ■), and any low dp (degree of polymerization) carbohydrates (from glucose to maltotriose) were further consumed by *Bacillus* sp. AIR-5. The maximum concentration of maltopentaose (11 g/l) was produced after 30 h of cultivation, and there were also high concentrations of maltohexaose and some maltotetraose. At the end of the fermentation (after 48 h of cultivation), 8.9 g/l maltopentaose (22.4% of theoretical yield) was obtained from the soluble starch (40 g/l), which was 90% of the total oligosaccharides (from maltose to maltoheptaose) produced in the culture broth. Yosighi *et al.*

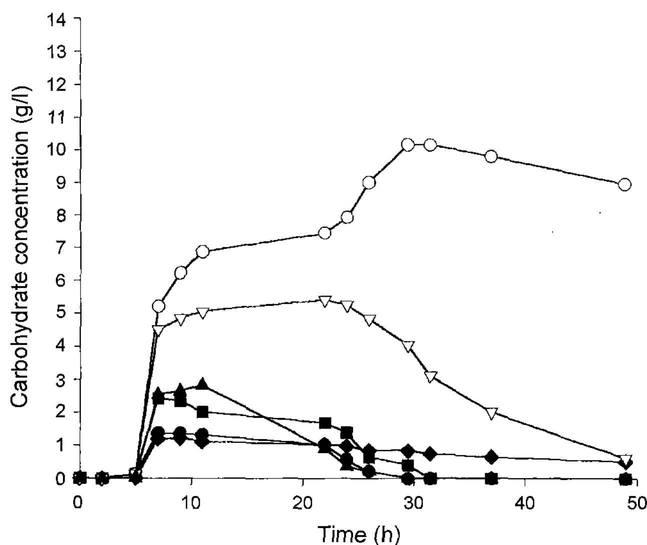

Fig. 1. Time course of maltopentaose production by *Bacillus* sp. AIR-5.

Cultivation was for 48 h at 200 rpm, 1.33 vvm, and 28°C in the fermenter. The concentration of each product was measured after TLC using the NIH image analysis program [1]. -●- maltose (G2), -■- maltotriose (G3), -◆- maltotetraose (G4), -○- maltopentaose (G5), -▽- maltohexaose (G6), -▲- maltoheptaose (G7).

Table 1. Purification summary of AIR-5 amylase.

Step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Yield (%)	Purification fold
Culture supernatant	24736	84.2	294	100	-
Concentration*	11184	32.1	348	45.2	1.2
DEAE-cellulose	9504	4.0	2370	38.4	8.1
Sephadex G-150	4751	1.2	4131	19.2	14.2

Concentration* by 30 K molecular weight cut-off membrane filtration (Millipore, MA, U.S.A.).

reported that, under optimum conditions, the maltopentaose produced by *Bacillus cereus* NY-14 made up 29% to 47% of the residual sugars [43]. Thus, the maltopentaose in the culture of *Bacillus* sp. AIR-5 had a higher purity and concentration than those in any other reports.

Purification of Enzyme

A maltopentaose-forming amylase was purified and named as AIR-5 amylase. The purified AIR-5 amylase was obtained by DEAE-cellulose ion-exchange and Sephadex G-150 column chromatographies with a yield of 19.2%, as summarized in Table 1. The purification fold was about 14 (4,131 U/mg protein). The molecular mass of the purified protein was estimated to be approximately 250 kDa on the native-PAGE (10%). The band of 250 kDa on the native-PAGE was composed of tetramers of a 63-kDa subunit (Fig. 2). Other reported maltopentaose-forming amylases are different in size and reported as monomers: 22.5 kDa from *Bacillus licheniformis* [28], 55 kDa from *Bacillus cereus* [42], and 72.5 kDa from *Pseudomonas* sp. KO 8940 [17]. The isoelectric point of the 250-kDa protein was estimated to be 6.9.

Effects of pH and Temperature on Activity and Stability

The optimum temperature was observed to be around 45°C. At 37°C, the enzyme exhibited over 93% of the maximum activity. The optimum pH of the AIR-5 amylase was observed between 8–8.5. Since over 90% of its original activity was maintained within a pH range of 6–10, AIR-5 amylase would appear to be an alkalophilic amylase (Fig. 3). The maltopentaose-forming amylases from *Bacillus* have optimum pHs between 5.0 and 7.5 [21, 22], and an acidic amylase from *Bacillus acidocaldarius* has an optimum pH of 3.5 [3]. This alkalophilic characteristic of the enzyme can be used as an ingredient of laundary detergent [40]. As shown in Table 2, the enzyme was completely inactivated at 55°C after 1 h in a reaction buffer without Ca^{2+}. Interestingly, in a reaction buffer with 3 mM Ca^{2+} at 55°C, the AIR-5 amylase maintained 52% of its original activity for 1 h. As such, Ca^{2+} would also seem to be an activator. With the addition of 6 mM Ca^{2+}, the relative activity of AIR-5 amylase was increased up to 160% of that without Ca^{2+} (data not shown).

Effects of Metal Ions on Amylase Activity

The addition of metal ions increased the relative activity of AIR-5 amylase in the order of 1 mM Ca^{2+}, Ba^{2+}, Mg^{2+},

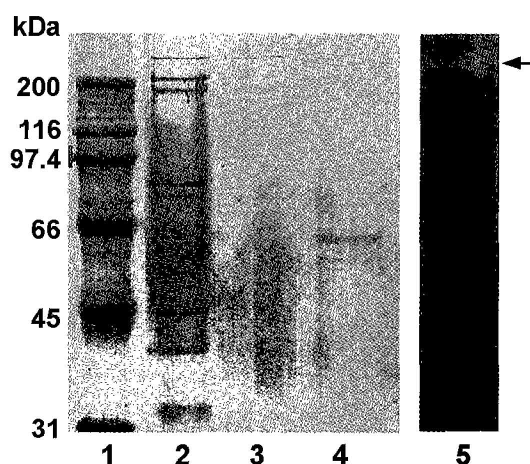

Fig. 2. Native- and SDS-PAGE of AIR-5 amylase.
Lane 1, size marker (kDa); lane 2, culture supernatant; lane 3, native-PAGE of purified enzyme; lane 4, denatured SDS-PAGE of purified enzyme; lane 5, active staining of purified enzyme.

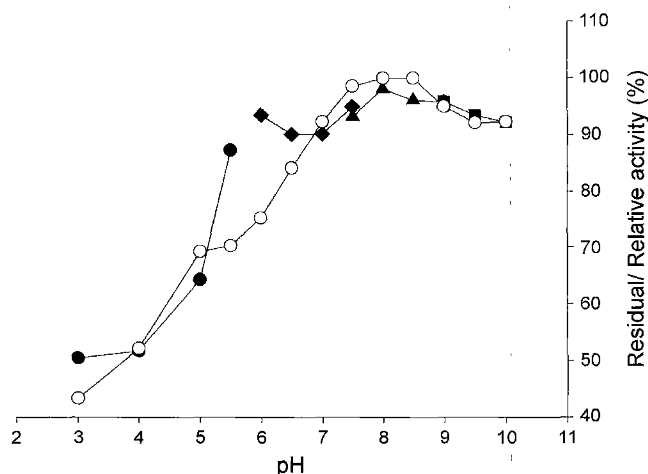

Fig. 3. Effect of pH on the activity and stability of AIR-5 amylase. Each value (open circle) shows the relative value of the maximum specific activity at pH 8.0. The closed circles indicate the pH stability of the enzyme. The AIR-5 amylase (5 U/ml) was preincubated in a 20 mM buffer (pH 8.5) at 37°C for 2 h, then samples were used to measure the residual activity under standard enzyme assay conditions. The buffers used (20 mM each) were: -●-, Na-acetate buffer; -▲-, Tri-HCl buffer; -◆-, Na-phosphate buffer; -■-, Glycine-NaOH buffer.

Table 2. Effects of metal ions and inhibitors on the enzyme activity.

Metal ions	Concentration (mM)	Residual activity (%)
None	1	100
$CaCl_2 \cdot 2H_2O$	1	122
$BaCl_2 \cdot 2H_2O$	1	120
$MgCl_2 \cdot 6H_2O$	1	117
$MnCl_2 \cdot 4H_2O$	1	110
RbCl	1	105
$FeSO_4 \cdot 7H_2O$	1	81
$CuCl_2 \cdot 2H_2O$	1	36
$ZnCl_2$	1	16
$HgCl_2$	1	9
EDTA*	5	49
2-mercaptoethanol	10	105
SDS	1 (mg/ml)	72

*Ethylenediaminetetraacetic acid.

Mn^{2+}, and Rb^{+}(Table 3). However, the enzyme was strongly inhibited by the same concentration of Hg^{2+}, Zn^{2+}, and Cu^{2+}. The addition of EDTA (5 mM) with Ca^{2+} inhibited the AIR-5 amylase activity by up to 51% of the original activity. Thus, Ca^{2+} would appear to act as a cofactor. It has been previously reported that the alkaline amylases of *Bacillus* sp. [8, 37] and *Bacillus* sp. A3-8 [23] are stable to EDTA treatment, while neutral amylases of *B. amyloliquefaciens*, Taka-amylase A, and liquefying amylase of *B. licheniformis* are sensitive to EDTA treatment. On the basis of the above results, AIR-5 amylase can be classified as a liquefying amylase [28, 34].

Table 3. Effect of Ca^{2+} on the thermostability of the AIR-5 amylase.

	Relative activity (%)	
Temperature (°C)	Without Ca^{2+}	With 3 mM Ca^{2+}
28	100	100
37	82	100
45	50	90
55	0	52

For the determination of thermostability of AIR-5 amylase, the enzyme (3.2 U/ml) was incubated at the indicated temperature with and without Ca^{2+} in 20 mM Tris-HCl buffer (pH 8.5) for 1 h.

Action Pattern and Substrate Specificity of AIR-5 Amylase

When AIR-5 amylase was reacted with soluble starch (1%, w/v), maltooligosaccharides with a high molecular weight (bigger than maltopentaose) were formed during the early stage of reaction, then these maltooligosaccharides were subsequently degraded to maltopentaose and small molecular weight saccharides. Finally, maltopentaose became preponderant (Fig. 4A). Figure 4B shows the hydrolysis product of AIR-5 amylase (3.2 U/ml) with 1% (w/v) maltooligosaccharide series (from maltose to maltooctaose) as the standard. AIR-5 amylase did not hydrolyze carbohydrates with size from G2 to G5, yet formed G5+G1 from G6, G5+G2 from G7, and G5+G3 from G8. When AIR-5 amylase (3.2 U/ml) was reacted with 1% (w/v) maltooligosaccharides (from G6 to G8) for 6 h, the remaining amounts of carbohydrates were 60% of G6, 25% of G7, and 0% of G8 initial concentration (Fig. 5). In

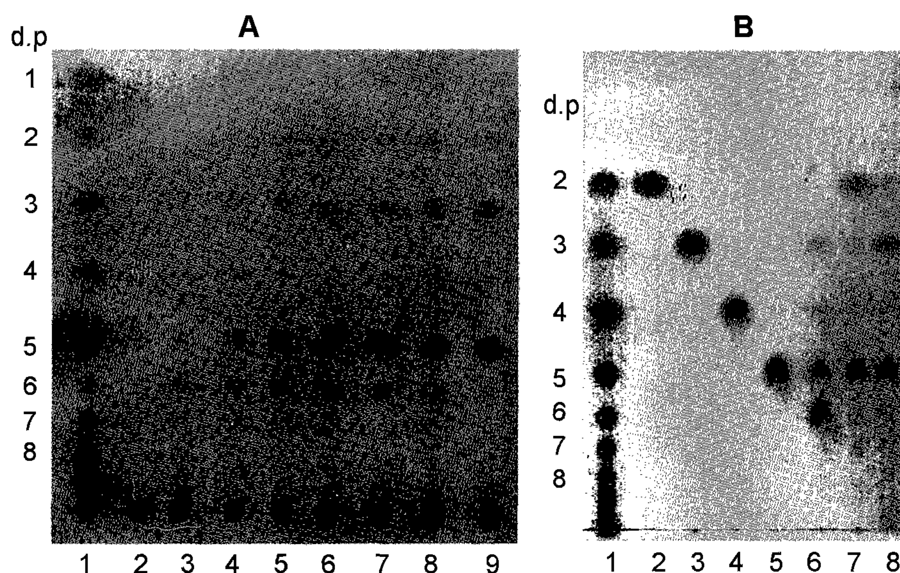

Fig. 4. Hydrolysis of soluble starch and maltooligosaccharides by AIR-5 amylase.
(A) Products from soluble starch (1% w/v). The reaction digest contained 1 U/ml of enzyme incubated at 37°C and pH 8.5. Samples were taken after 0, 15, 30, 45, 60, 120, 180, and 240 min (lanes 2 to 9, respectively). (B) Maltooligosaccharides ranging from G2 (maltose) to G8 (maltooctaose) (1% w/v), (lanes 2 to 8, respectively) were hydrolyzed by AIR-5 amylase (3.2 U/ml) after 60 min.

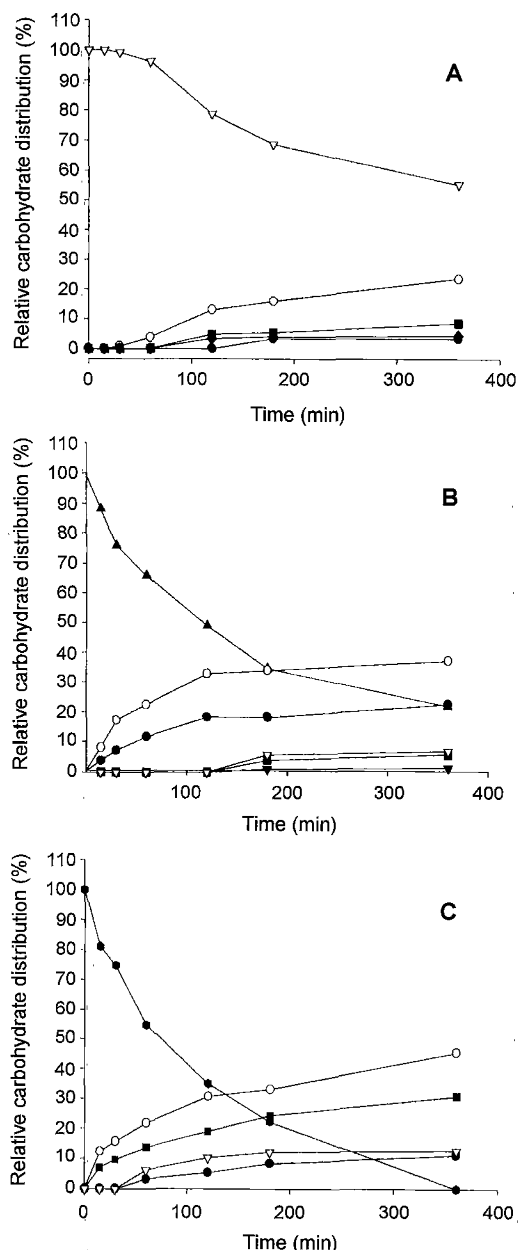

Fig. 5. Hydrolysis product of malto-hexaose, -heptaose, -octaose by AIR-5 amylase.
Hydrolysis products from maltohexaose (A), maltoheptaose (B), and maltooctaose (C) (1% w/v), respecitvely. The reaction (3.2 U/ml) was performed at 37°C in a 20 mM Tris-HCl buffer (pH 8.5). Samples were taken at the indicated intervals and 0.1 M NaOH was added to terminate the reaction. -◆- G1, -●- G2, -■- G3, -○- G5, -▽- G6, -▲- G7, -●- G8.

Table 4. Relative initial enzyme reaction rates for various substrates.

Substrate	Relative activity (%)
Soluble starch	100
Amylopectin	102
Amylose	106
Glycogen	83
Maltopentaose	0
Maltohexaose	20
Maltoheptaose	38
Maltooctaose	52

*The activity for soluble starch was assigned as 100%.

the case of the maltooctaose (G8) reaction digest, the maltopentaose portion of the hydrolysis product was 45% of the hydrolyzates. These results explain why AIR-5 produced maltopentaose as the main product in a soluble starch fermentation. AIR-5 amylase can also hydrolyze amylose, amylopectin, starch (soluble), glycogen, and maltooligosacharides, which are all composed of α-(1→4) and α-(1→6) glucosidic linkages. In particular, when AIR-5 amylase (3.2 U/ml) was reacted with 1% (w/v) glycogen at 37°C for 1 h, the percentages of each oligosaccharide formed were 7.4, 7.2, 1.9, 71, and 12.5% for G2, G3, G4, G5, and G6, respectively. However, the enzyme could not act on maltodextrins with sizes ranging from maltose to maltopentaose, dextran, pullulan, and α- and β-cyclodextrins (Table. 4). On the basis of its mode of action, AIR-5 amylase can be classified as an endo-type α-amylase.

N-Terminal Amino Acid Sequence Analysis

The N-terminal amino acid sequence was **ATINNGTLMQYFEWYVPNDG**. The N-terminal of AIR-5 amylase started with alanine rather than methionine. The signal peptide region may be cleaved by a signal peptidase while passing through the cell membrane [36]. When the N-terminal amino acid sequence of AIR-5 amylase was compared with liquefying α-amylases, AIR-5 amylase exhibited high sequence identity (96%, Fig. 6) to BLA (*B. licheniformis*), which is generally used in industrial processes for making oligosaccharides and maltopentaose [25]. Further molecular biological studies on AIR-5 amylase are in progress to understand the molecular basis of the enzyme, which may be of value in carbohydrate enzyme engineering.

AIR-5	-	A	T	I	N	N	G	T	L	M	Q	Y	F	E	W	Y	V	P	N	D	G	-
CPA	-	A	A	A	T	N	G	T	M	M	Q	Y	F	E	W	Y	V	P	D	N	G	-
BLA	-1	A	A	N	L	N	G	T	L	M	Q	Y	F	E	W	Y	M	P	N	D	G	19
BSA	1	A	A	D	F	N	G	T	M	M	Q	Y	F	E	W	Y	L	P	N	D	G	20
BAA	-3	T	S	A	V	N	G	T	L	M	Q	Y	F	E	W	Y	T	P	N	D	G	18
#707	2	H	N	G	T	N	G	T	M	M	Q	Y	F	E	W	Y	L	P	N	D	G	21
LAMY	2	H	N	G	T	N	G	T	M	M	Q	Y	F	E	W	H	L	P	N	D	G	21

Fig. 6. N-terminal amino acid sequence alignment of AIR-5 amylase with CPA, BLA, BSA, BAA, #707, or LAMY amylase.
CPA: *Cytophaga* sp. [4]; BLA: *Bacillus licheniformis* [19]; BSA: *Bacillus stearothermophilus* [22]; BAA: *Bacillus amyloliquefaciens* [34]; #707: *Bacillus* strain #707 [39]; LAMY: *Bacillus* sp. [10].

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