Biotechnology and Bioprocess Engineering **2003**, 8: 106-111 Special Issue: A Tribute to Dr. Sun Ho Park ©KSBB

Transglycosylation Reaction and Raw Starch Hydrolysis by Novel Carbohydrolase from *Lipomyces starkeyi*

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Abstract A novel carbohydrolase, which is a DXAMase, containing both dextranase and amylase equivalent activities, was purified from *Lipomyces starkeyi* KSM22. The purified DXAMase was also found to hydrolyze cellobiose, gentiobiose, trehalose and melezitose, while disproportionation reactions were exhibited with various di- and tri-saccharides, such as maltose, isomaltose, gentiobiose, kojibiose, sophorose, panose, maltotriose, and isomaltotriose with various kinds of oligosaccharides produced as acceptor reaction products. Furthermore, the purified DXAMase hydrolyzed raw waxy rice starch and produced maltodextrin to the extent of 50% as a glucose equivalent.

Keywords: carbohydrolase, *Lipomyces starkeyi*, disproportionation, oligosaccharide, raw starch

INTRODUCTION

Dextranase (EC 3.2.1.11) is an enzyme that selectively hydrolyzes the α-(1→6) glucosidic bond contained in dextran into small carbohydrate molecules. Commercial dextranase is produced by fermentation with either *Penicillium* sp. or *Chaetomium* sp. However, since these fungi also produce various antibiotics and toxic metabolites in addition to dextranase, this creats the difficulties in obtaining FDA approval. Except for a few bacterial dextranases, microbial dextranases generally are inducible [1,2]. Kim and Day reported on the isolation of a derepressed and partial constitutive mutant, *L. starkeyi* ATCC 74054, for both dextranase and amylase and described the characterization of its enzyme [3]. Since then, *L. starkeyi* ATCC 74054 has been used for mixed culture fermentation in cooperation with *Leuconostoc mesenteroides* for the production of small-size dextran using sucrose and/or starch [3-5]. Plus, *L. starkeyi* ATCC 74054 has been further mutated, resulting in a hyper dextranase producing constitutive mutant, *L. starkeyi* KSM 22 [6]. A novel carbohydrolase (DXAMase) showing mixed activities of both dextranase and amylase has been produced using soluble starch, where only one activity band was exhibited with non-denaturing SDS-PAGE for both dextranase and amylase and both were always co-purified [6]. DXAMase is able to inhibit or prevent insoluble glycan formation and partially remove pre-formed plaque [6], while *L. starkeyi* has been used in food-related applications and is not known to produce antibiotics or toxic metabolites [7]. Reaction in which glucosyl groups are transferred from one saccharide donor to identical or similar saccharide acceptors is known as disproportionation reactions (e.g., 2 maltose → D-glucose + maltotriose) [7]. There have already been several reports about the disproportionation reactions of enzymes. The amylosucrase from *Neisseria polysaccharea* catalyzes the disproportionation reaction of a maltooligosaccharide donor and transfers the glucosyl unit to the non-reducing end of another maltooligosaccharide acceptor [8]. Meanwhile, cycloisomaltooligosaccharide glucanotransferase (CITase) produces several linear isomaltooligosaccharides from isomaltopentaose based on a disproportionation reaction [9].

Starch is a major food component and also used as a raw material for many industrial products. The application of starch as a raw material usually requires the prior disruption of its inert granule structure, which involves additional processing steps. The hydrolysis of raw starch produces various benefits derived at different levels of the processing and food utilization chain, including direct improvements in the industrial utilization

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of starch and the regulation of hydrolysis to prevent post-harvest losses or to provide novel nutritional benefits [10].

Accordingly, the current describes the transglycosylation and disproportionation reactions of DXAMase with various saccharides in relation to the synthesis of various structure oligosaccharides and the digestion of raw starch granules by DXAMase.

MATERIALS AND METHODS

Organism and Growth Condition

The *L. starkeyi* KSM22 was maintained on a slant of an LW medium containing 1% (w/v) soluble starch and 0.02% (w/v) 2-deoxy-D-glucose. The LW medium consisted of 0.3% (w/v) yeast extract, 0.3% (w/v) KH_2PO_4, and 1% (v/v) mineral solution [2% (w/v) $MgSO_4 \cdot 7H_2O$, 0.1% (w/v) NaCl, 0.1% (w/v) $FeSO_4 \cdot 7H_2O$, 0.1% (w/v) $MnSO_4 \cdot H_2O$, 0.13% (w/v) $CaCl_2 \cdot 2H_2O$]. All chemicals were reagent grade.

Enzyme Activity and Protein Assay

The enzyme activity was determined based on the rate of increase in a reducing sugar concentration in a reaction digest using the copper-bicinchoninate method with glucose as the standard [11]. One unit (IU) of enzyme activity was defined as the amount of enzyme that liberated the equivalent of 1 μmole of glucose in one minute under the described condition [6]. The dextranase preparation was incubated with 2% (w/v) dextran T-2000 (Sigma Chemical Co., USA) in a 50 mM citrate-phosphate buffer (pH 5.5) at 37°C for 10-30 min. The dextranase equivalent activity was used to describe the DXAMase activity, except as indicated, because the dextranase equivalent activity is proportional to that of the amylase equivalent activity. The protein was determined according to the Bradford method [12] and using a spectrophotometer at 280 nm. Bovine serum albumin (Sigma Chemical Co., USA) was used as the standard protein.

DXAMase Production and Purification

For DXAMase production, the *L. starkeyi* KSM22 was cultured using an LW medium containing 1% soluble starch in a 10-L jar fermenter (Hanil R&D Co., Korea). The culture supernatant was separated from the cells with a 100 K cut off hollow-fiber (Saehan, Korea), concentrated from 8.3 L to 830 mL using a 30 K cut off hollow-fiber (Millipore, USA) and further concentrated with 70% ammonium sulfate (Sigma Chemical Co, USA) to 60 mL. The protein concentration and enzyme activities were monitored throughout the course of the purification. A DEAE-Sepharose column (2.5 cm × 25 cm) was prepared and equilibrated with a 20 mM potassium phosphate buffer (pH 6.4). An ammonium sulfate concentrate (1.5 mL – 20 mg protein/mL) was applied to the column, which was then eluted with a linear NaCl gradient (0 – 2.0 M) in the potassium phosphate buffer. The active fractions were pooled and concentrated by lyophilization. The dextranase fractions from the DEAE-Sepharose column were size fractionated by Gel Permeation Chromatography (GPC) using a BIO-RAD A-0.5 m column (70 cm × 2.6 cm) prepared and equilibrated with a 50 mM citrate phosphate buffer (pH 5.5). 3 mL of the DEAE-Sepharose concentrate fraction was applied to this column (4 mg protein/mL). The fractions exhibiting DXAMase activity were pooled.

Electrophoresis

The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using the method of Laemmli [13] on 70 × 80 mm slab gels (10% acrylamide). The dextranase equivalent activity of DXAMase on the gel was detected by the method of Ohnishi *et al.* [14] with some modification. The protein samples were separated by SDS-PAGE containing 1% blue dextran. After electrophoresis, the SDS was removed by washing the gel with 20% of 2-propanol in 50 mM Tris-HCl (pH 8.0) for 1 h, then with a 50 mM Tris-HCl (pH 8.0) buffer containing 5 mM 2-mercaptoethanol for 1 h at room temperature with gentle shaking. The dextranase equiva-lent activity was visible as a white band on a blue background. The molecular weights of the proteins in the sample were estimated using a broad range of molecular mass standards (kDa; myosin 200, β-galactosidase 116, phosphorylase b 97.4, serum albumin 66.2, ovalbumin 45, carbonic anhydrase 31, trypsin inhibitor 21.5, lysozyme 14.4, aprotinin 6.4; Bio-Rad, USA). The proteins were stained with Coomassie Brilliant Blue R (Sigma Chemical Co., USA).

Disproportionation Reaction of DXAMase

The disproportionation reaction was performed by incubating the purified DXAMase (dextranase equivalent activity -5.2 unit/mL) and various disaccharides or trisaccharides [1% - (w/v)] in a citrate phosphate buffer (50 mM, pH 5.5) for 30 min at 37°C. The reaction products were analyzed by thin layer chromatography (TLC), using a Whatman K5F plate (Whatman Inc, New Jersey) with two to three ascents of 2:5:1.5 (v/v/v) nitromethane/1-propanol/water. The carbohydrates were visualized by dipping the plates into 5% (v/v) H_2SO_4 in methanol containing 0.3% (w/v) *N*-1-naphthylethylene-diamine, followed by drying and heating for 10 min at 121°C [15]. The quantity of each carbohydrate on the TLC plates was analyzed using the NIH image program with the maltooligosaccharide series (d.p. 1 – 7, Sigma Chemical. Co., USA) and isomaltooligosaccharide series (d.p. 1 – 7, Sigma Chemical. Co., USA) as the standard materials [16].

Digestion of Raw Starch Granules by DXAMase

A reaction mixture containing 50 mg of each raw starch granule (sweet corn starch, barely starch, sweet

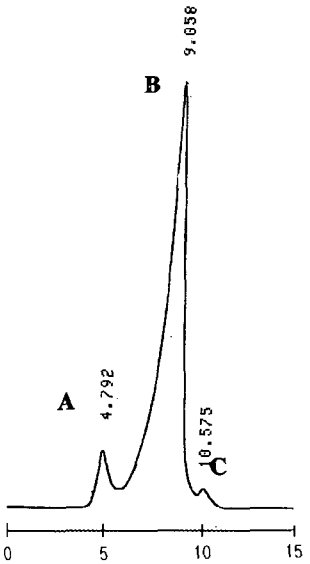

Fig. 1. Determination of molecular weight by GPC. GPC elution profile of purified DXAMase. The fraction peaks A, B and C were estimated as 640.9 kDa, 196.4 kDa, and 128.8 kDa, respectively.

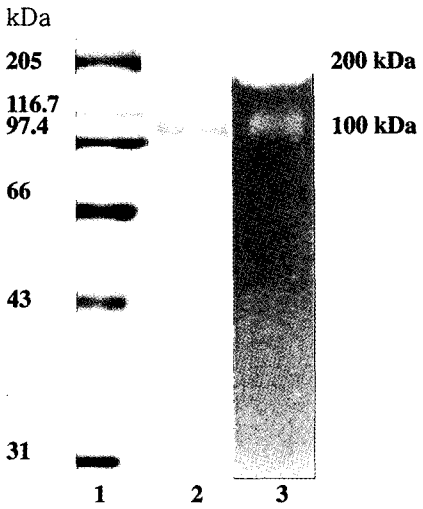

Fig. 2. SDS-PAGE and activity staining of purified DXAMase. Lane 1, Protein molecular weight marker (205, 116, 97.4, 66, 45, 31 kDa); lane 2, SDS-PAGE of purified DXAMase; lane 3, Activity staining of purified DXAMase.

potato starch, waxy maize starch, waxy rice starch) in 4 mL of a 50 mM citrate-phosphate buffer (pH 5.5) and 1 mL of DXAMase (amylase equivalent activity – 1.2 U/mL) was incubated at 37°C for 5 days. The degree of the raw starch hydrolyzate was measured using the copper-bicinchoninate method with glucose as the standard material [11] and the final product analyzed by TLC [6].

Scanning Electron Microscopy of Starch Granules

Scanning electron micrographs were obtained for the native starch granules and starch granules reacted with DXAMase (amylase equivalent activity – 1.2 U/mL) for 120 h. The samples were dehydrated in successive 5 min steps with 50, 60, 70, 95% and absolute ethanol. Each sample was coated with gold/palladium (3/2) in a hummer sputter coater (20 mm, 4 min). The micrograph used was a JEOL JPC-1100 SEM (JEOL, Tokyo, Japan).

RESULTS AND DISCUSSION

Relative Molecular Weight Determinations of Native DXAMase

The purified DXAMase after size-exclusion GPC exhibited a major DXAMase peak (fraction B) corresponding to a molecular weight of about 196.4 kDa, indicating that the native DXAMase was a dimeric enzyme of two 100 kDa proteins containing both dextranase and amylase equivalent activities (Fig. 1). Fraction A (retention time at 4.792 min), assigned as a 640.9 kDa protein did not show any hydrolase activities. The SDS-PAGE results and activity staining of the purified DXAMase also supported that the DXAMase existed in a dimeric form (Fig. 2). DXAMase (4.85 U/μmol) was shown as a major band of approximately 100 kDa (lane 2 in Fig. 2). However, with non-denaturing SDS-PAGE (10%), the major dextranase equivalent activity of the DXAMase (9.0 U/μmol) was shown as a 200 kDa band, while the minor activity as a 100 kDa band (10%) (lane 3 in Fig. 2), thereby confirming the dimeric form of the DXAMase A dimeric form for an active enzyme is not rare. The maltogenic amylase of *Bacillus substilis* SUH4-2 isolated from Korean soil exists in a monomer-dimer equilibrium with a molar ratio of 3:2 in a 50 mM KH_2PO_4-NaOH buffer (pH 7.0) [17] and the amylase from *Pisum sativum* L. has a molecular weight of 11 kDa and contains polypeptides with a molecular weight of 6 kDa, suggesting that the protein molecules are dimers [18].

Disproportionation Reaction

The mechanism of a maltooligosaccharide disproportionation reaction has already been explained by Albenne *et al.* [8]. First, this disproportionation reaction leads to the formation of G_{n-1} and G_{n+1} products from a G_n substrate. Second, multi reactions involving all products can occur when they are accumulated in sufficient concentration in the enzyme reaction digest, resulting in the synthesis of new maltooligosaccharides, such as G_{n-2} and G_{n+2}. Table 1 shows the disproportionation reaction results of the DXAMase with various disaccharides [maltose, isomaltose, and kojibiose] and trisaccharides [panose, maltotriose, and isomaltotriose]. During the disproportionation reaction the D-glucopyranose of the di- and tri-saccharides was first hydrolyzed, then this D-glucopyranose was linked to other di-and tri-saccharides by an α-(1→6) linkage. The maltose disproportionation

Table 1. Disproportionation reaction products of DXAMase

Substrate (substrate left)*	Disproportionation reaction products				
	d.p.2	d.p.3	d.p.4	d.p.5	Oligosaccharides larger than d.p. 5
Maltose (39.9%)*	Isomaltose (20.3%)**	Panose (5.4%), Isomaltotriose (11.3%)	6^2-α-D-isomaltosyl maltose (3.9%)		19.2%
Isomaltose (44.9%)*		Isomaltotriose (24.7%)	Isomaltotetraose (5.1%)		25.3%
Kojibiose (14.3%)*	Isomaltose (13.6%)	6^2-α-D-glucopyranosyl kojibiose (14.3%), Isomaltotriose (1.1%)	6^2-α-D-isomaltosyl kojibiose (1.3%)		55.4%
Sophorose (67.2%)*		6^2-α-D-glucopyranosyl sophorose (11.3%)			21.5%
Cellobiose (65.4%)*		6^2-α-D-glucopyranosyl cellobiose (12.7%)			21.9%
Gentiobiose (80.9%)*		6^2-α-D-glucopyranosyl gentiobiose (8.5%)			10.6%
Panose (11.0%)*	Isomaltose (29.3%)		6^2-α-D- isomaltosyl maltose (3.1%)		56.6%
Maltotriose (26.3%)*	Maltose (22.8%)	Panose (5.0%)	6^2-α-D-glucopyranosyl maltotriose (5.0%), 6^2-α-D-isomaltosyl maltose (8.6%)	6^2-α-D-isomaltosyl maltotriose (6.6%), maltopentaose (4.3%)	21.4%
Isomaltotriose (22.9%)*	Isomaltose (31.7%)		Isomaltoteraose (9.7%)		35.7%

*Amount of each substrate remaining in the reaction digest after the disproportionation reaction.

** The relative amounts of disproportionation reaction products were calculated based on considering of the total amount of carbohydrates in the reaction digest as 100%.

reaction products were isomaltose (20.3%), panose (6^2-α-D-glucopyranosyl maltose) (5.4%), isomaltotriose (11.3%), and 6^2-α-D-isomaltosyl maltose (3.9%). Panose and isomaltotriose were formed by transferring the glucose with an α-(1→6) linkage into maltose and isomaltose, respectively. 6^2-α-D-isomaltosyl maltose was produced by an α-(1→6) isomaltose linkage with maltose. The isomaltose disproportionation reaction products were isomaltotriose (24.7%) and isomaltotetraose (5.1%); the glucose from isomaltose was linked by α-(1→6) to isomaltose, resulting in isomaltotriose. The kojibiose disproportionation reaction products were isomaltose (13.6%), 6^2-α-D-glucopyranosyl kojibiose (14.3%), isomaltotriose (1.1%) and 6^2-α-D-isomaltosyl kojibiose (1.3%). This isomaltose and isomaltotriose were synthesized by linking their glucose with the glucose from the kojibiose hydrolysis. The released glucose was then transferred to other kojibiose, resulting in 6^2-α-D-glucopyranosyl kojibiose and subsequently 6^2-α-D-isomaltosyl kojibiose. The common disproportionation reaction product of maltose and kojibiose was isomaltose. Interestingly, disaccharides containing a beta glucosidic linkage such as sophorose, cellobiose, and gentiobiose, were also used as disproportionation reaction substrates or acceptors, yet the glucose taken off was linked by α-(1→6) to other sophorse, cellobiose, or gentiobiose. 6^2-α-D-isomaltosyl maltose (3.1%) was formed by the transfer of glucose to α-(1→6) linked panose. Isomaltose (29.3%) was formed by the hydrolysis of glucose from panose. In the case of maltotriose, many kinds of disproportionation reaction products were formed; maltose (22.8%), panose (5.0%), 6^2-α-D-glucopyranosyl maltotriose (5.0%), 6^2-α-D-isomaltosyl maltose (8.6%), 6^2-α-D-isomaltosyl maltotriose (6.6%), and maltopentaose (4.3%). Panose and 6^2-α-D-glucopyranosyl maltotriose were formed by the transfer of the glucose residue into maltose and maltotriose by α-(1→6) linkages. 6^2-α-D-isomaltosyl maltose and 6^2-α-D-isomaltosyl maltotriose were formed by the transfer of the isomaltosyl residue into maltose and maltotriose, respectively. The isomaltotriose disproportionation reaction products were mainly isomaltose (31.7%) and isomaltoteraose (9.7%).

Among the disaccharides and trisaccharides, kojibiose and panose were the best substrates for disproportionation: each formed 85.7% and 89.0% of the total disproportionation reaction products, respectively. Among the disproportionation reactions with beta linkage compounds, cellobiose [β-(1→4)] was the most effective substrate, 34.6% of total product, followed by sophorose [32.8%; β-(1→2) linkage] and gentiobiose [19.1%; β-

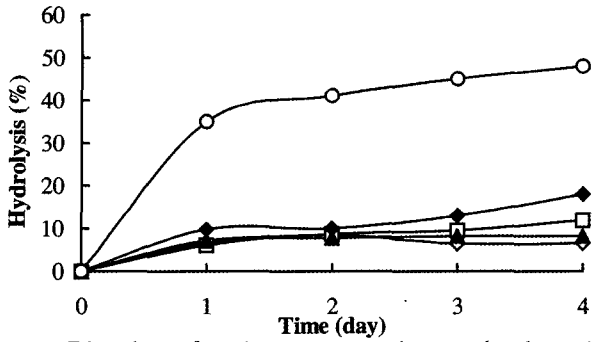

Fig. 3. Digestion of various raw starch granules by using DXAMase. Sweet corn starch (◇), barley starch (□), sweet potato starch (▲), waxy maize starch (◆), and waxy rice starch (○).

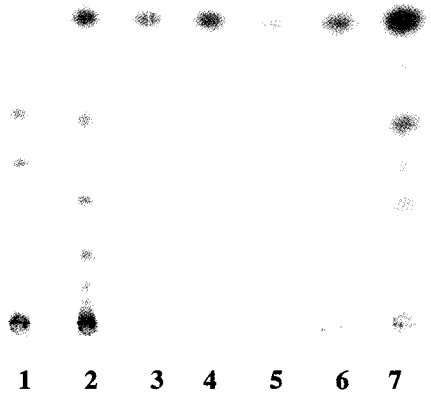

Fig. 4. Hydrolysis products of raw starch granules with DXAMase. Lane 1, maltooligosaccharides standards; lane 2, isomaltooligosaccharides standards; lane 3, hydrolyzate of sweet corn starch; lane 4, hydrolyzate of barley starch; lane 5, hydrolyzate of sweet potato starch; lane 6, hydrolyzate of waxy maize starch; lane 7, hydrolyzate of waxy rice starch.

(1→6) linkage]. When compared to the disproportionation reaction of the glucansucrase from *S. mutans* 6715, which capable of forming β-(1→3) linked products as well as β-(1→6) linked products, the DXAMase reaction did not synthesize any β-(1→3) linked products [8].

Reaction of Amylase Equivalent Activity of DXAMase with Raw Starch Granules

The percent conversion of starch to small saccharides or maltodextrin differed for the different kinds of starches (Fig. 3). Porcine pancreatic alpha amylase (PPA) and *Bacillus amyloliquefaciens* alpha amylase (BAA) have been found to react with starch granules from maize, amylomaize-7 and potato, and then produced maltodextrin [19].

In the current study, waxy rice raw starch was the most susceptible to the DXAMase and being converted into maltodextrin(Fig. 4), where the hydrolyzates were composed of 47.0% glucose, 9.5% maltose, 20.7% maltotriose, 7.7% maltotetraose, 9.1% maltopentose and 6.0% other maltodextrin. Sweet raw corn starch was hydrolyzed to 77.6% glucose, 4.7% maltose, 12.6% maltotriose 1.0% maltotetraose, 1.7% maltopentose and 2.4% maltohexaose. Barley raw starch was hydrolyzed to 74.2% glucose, 5.6% maltose, 14.6% maltotriose, 1.5% maltotetraose, 2.3% maltopentose and 1.8% maltohexaose. Sweet potato raw starch only released glucose as a hydrolysis product. Maize raw starch was hydrolyzed to 69.5% glucose, 8.6% maltose, 13.3%maltotriose, 2.2% maltotetraose, 3.9% maltopentose and 2.5% maltohexaose. Since the dextranase equivalent activity of DXAMase can effectively cleave the α-(1→6)-D-glucopyranosyl linkages and waxy rice raw starch is composed mostly of amylopectin with only 2% amylose[20], it was mostly hydrolyzed comparing to other starches. The morphologies of the waxy rice raw starch granules before and after treatment with the DXAMase were examined by scanning electron microscopy. The SEM photographs of the native waxy rice raw starches (Fig. 5 A) revealed angular and irregular shapes, while the partially degraded porous waxy maize raw starch was shown as significant shell structures with deep holes (Fig. 5 B).

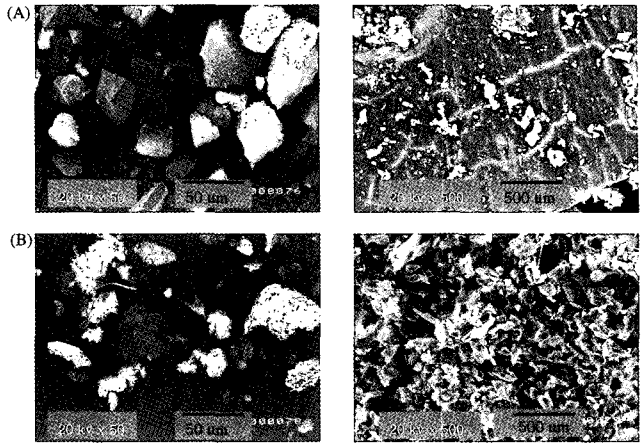

Fig. 5. Scanning electron microscopy photographs of native waxy rice starch granules (A) and DXAMase treated waxy rice starch granules (B).

Acknowledgements This work was supported in part by Korea Science and Engineering Foundation with a grant (1999-21100-002-2). The experiment at PLS was support by MOST and POSCO.

REFERENCES

[1] Barrett, J. F., T. A. Barrett, and R. Curtiss (1987) Purification and partial characterization of the multicomponent dextranase complex of *Streptococcus sorbrinus* and the cloning of the dextranase gene. *Infect. Immun.* 55: 729-802.

[2] Koenig, D. and D. F. Day (1988) The purification and characterization of a dextranase from *Lipomyces starkeyi*. *Eur. J. Biochem.* 183: 161-167.
[3] Kim, D. and D. F. Day (1995) Isolation of a dextranase constitutive mutant of *Lipomyces starkeyi* and its use for the production of clinical size dextran. *Lett. Appl. Microbiol.* 20: 268-270.
[4] Kim, D., H. C. Seo, and D. F. Day (1996) Dextran production by *Leuconostoc mesenteroides* in the presence of a dextranase producing yeast *Lipomyces starkeyi*. *Biotechnol. Tech.* 10: 227-232.
[5] Kim, D. and D. F. Day (1994) A new process for the production of clinical dextran by mixed-culture fermentation of *Lipomyces starkeyi* and *Leuconostoc mesenteroides*. *Enzyme Microb. Technol.* 16: 844-848.
[6] Ryu, S. J., D. Kim, H. J. Ryu, S. Chiba, A. Kimura, and D. F. Day (2000) Purification and partial characterization of a novel glucanhydrolase from *Lipomyces starkeyi* KSM 22 and its use for inhibition of insoluble formation. *Biosci. Biotechnol. Biochem.* 64: 223-228.
[7] Phaff, H. J. and C. P. Kortzaman (1984) Genus 14. *Lipomyces*, pp. 252-262. In R. Kreger-van. (ed.), *The Yeast: A Taxonomic Study*. Elsevier, Amsterdam, The Netherlands.
[8] Binder, T. P., G. L. Cote, and J. F. Robyt (1983) Disproportionation reactions catalyzed by *Leuconostoc mesenteroides* and *Streptococcus* glucansucrases. *Carbohydr. Res.* 124: 275-286.
[9] Albenne, C., LK. Skov, O. Mirza, M. Gajhede, G. Potocki-Véronèse, P. Monsan, and M. Remaud-Simound (2002) Maltooligoscaccharide disproportionation reaction: an intrinstic property of amylosucrase from *Neisseria polysaccaharea*. *FEBS Lett.* 527: 67-70.
[10] Oguma, T., K. Tobe, and M. Kobayashi (1994) Purification and properties of a novel enzyme from *Bacillus* spp. T-3040, which catalyzes the conversion of dextran to cyclic isomaltooligosccharides. *FEBS Lett.* 345: 135-138.
[11] Fox. J. D. and J. F. Robyt (1991) Miniaturization of three carbohydrate analyses using a microsample plate reader. *Anal. Bichem.* 195: 93-96.
[12] Bradford, M. M (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248-254.
[13] Laemmli, U. K (1970) Cleavage of Structural Proteins During the assembly of the head of bacteriophage T4. *Nature* 227: 680-685.
[14] Ohnishi, Y., S. Kubo, Ono. Yoshiaki, M. Nozaki, Y. Gonda, H. Okano, T. Matsuya, A. Matsushiro, and T. Morita (1995) Cloning and sequencing of the gene coding for dextranase from *Streptococcus salivaeius*. *Gene* 156: 93-96.
[15] Mukerjea, H., Kim, D., and J. F. Robyt (1996) Simplified and improved methylation analysis of saccharide, using a modified procedure and thin-layer chromatography. *Carbohydr. Res.* 292: 11-20.
[16] Tanirseven, A. and J. F. Robyt (1993) Interpretation of dextransucrase inhibition at high sucrose concentration. *Carbohydr. Res.* 245: 97-104.
[17] Cho, H. Y., Y. W. Kim, T. J. Kim, H. S. Lee, D. Y. Kim, J. W. Kim, Y. W. Lee, S. B. Lee, and K. H. Park (2000) Molecular characterization of a dimeric intracellular maltogenic amylase of *Bacillus* SUH4-2. *Biochim. Biophys. Acta* 1478: 333-340.
[18] Gatehouse, J. A., J. Gilroy, M. S. Hoque, and R. R. Croy (1985) Purification, properties and amino acid sequence of a low-Mr abundant seed protein from pea (*Pisum sativum* L.). *Biochem. J.* 225: 239-247.
[19] Yook, C. and J. F. Robyt (2002) Reactions of alpha amylases with starch granules in a aqueous suspension giving products in solution and in a minimum amount of water giving products inside the granule. *Carbohydr. Res.* 337: 1113-1117.
[20] Koenig, D. W. and D. F. Day (1989) Induction of *Lipomyces starkeyi* dextranase. *Appl. Environ. Microbiol.* 23: 2079-2081.

[Received December 6, 2002; accepted March 19, 2003]