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An enzymatically produced novel cyclic tetrasaccharide, *cyclo*-{→6)-α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→} (cyclic maltosyl-(1→6)-maltose), from starch

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Abstract—A bacterial strain M6, isolated from soil and identified as *Arthrobacter globiformis*, produced a novel nonreducing oligosaccharide. The nonreducing oligosaccharide was produced from starch using a culture supernatant of the strain as enzyme preparation. The oligosaccharide was purified as a crystal preparation after alkaline treatment and deionization of the reaction mixture. The structure of the oligosaccharide was determined by methylation analysis, mass spectrometry, and ^{1}H and ^{13}C NMR spectroscopy, and it was demonstrated that the oligosaccharide had a cyclic structure consisting of four glucose residues joined by alternate α-(1→4)- and α-(1→6)-linkages. The cyclic tetrasaccharide, *cyclo*-{→6)-α-D-Glc*p*(1→4)-α-D-Glc*p*(1→6)-α-D-Glc*p*(1→4)-α-D-Glc*p*(1→}, was found to be a novel oligosaccharide, and was tentatively called cyclic maltosyl-maltose (CMM). CMM was not hydrolyzed by various amylases, such as α-amylase, β-amylase, glucoamylase, isoamylase, pullulanase, maltogenic α-amylase, and α-glucosidase, but hydrolyzed by isomalto-dextranase to give rise to isomaltose. This is the first report of the cyclic tetrasaccharide, which has alternate α-(1→4)- and α-(1→6)-glucosidic linkages.

Keywords: Cyclic tetrasaccharide; Cyclic maltosyl-(1→6)-maltose; *Arthrobacter globiformis*

1. Introduction

Generally, nonreducing oligosaccharides are divided into two groups: linear and cyclic oligosaccharides. Trehalose (α-D-glucopyranosyl α-D-glucopyranoside) is a typical linear nonreducing oligosaccharide occurring in bacteria, yeasts, fungi, plants, and invertebrates. Due to its particular physical features, trehalose is able to stabilize proteins or fatty acids and function as a bioprotectant against various stresses of desiccation, heat, freezing, or osmotic shock.[1,2] A mass production of trehalose from starch has been developed using two bacterial enzymes, maltooligosyltrehalose synthase (EC 5.4.99.15) and maltooligosyltrehalose trehalohydrolase (EC 3.2.1.141),[3–5] and now this saccharide is used in the fields of food, cosmetic, and pharmaceutical industries. Cyclomaltohexaose (α-cyclodextrin), one of the most well-known cyclic oligosaccharides, is produced from linear α-(1→4)-glucans by the intramolecular transglycosylation reaction of a cyclomaltodextrin glucanotransferase (EC 2.4.1.19).[6] The cyclic oligosaccharide consists of six glucose units linked by α-(1→4)-bonds, and has a hydrophobic cavity in the center of the structure. Guest molecules with suitable sizes can be accommodated in the cavity, and the formation of the inclusion complex is applied for stabilizing labile materials,[7] masking odors,[8] and modifying viscosity.[9] Côté and co-workers first reported that the smallest cyclic oligosaccharide of D-glucose, *cyclo*-{→6)-α-D-Glc*p*(1→3)-α-D-Glc*p*(1→6)-α-D-Glc*p*(1→3)-α-D-Glc*p*(1→}, was produced from a dextran-like polysaccharide, alternan, by its degrading enzyme.[10–12] The cyclic

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oligosaccharide was called cyclic tetrasaccharide (CTS) or cycloalternan (CA). Recently, we discovered two novel enzymes, 6-α-glucosyltransferase and 3-α-isomaltosyltransferase, in *Bacillus globisporus*, and succeeded in a mass production of the cyclic tetrasaccharide from starch by a joint reaction of both enzymes.[13–15] Although the characteristics of CA are not yet clear in detail, potential applications of the saccharide for food, cosmetics, and medicines are anticipated because of its unique structure. Nonreducing oligosaccharides produced from starch are of great interest from the functional and industrial points of view.

In the course of our search for soil bacteria producing novel nonreducing oligosaccharides from starch, we obtained a bacterial strain M6 that produced a cyclic tetrasaccharide different from CA. In this study, we report on the isolation and the structure of the novel cyclic tetrasaccharide that is enzymatically produced from starch.

2. Results

2.1. Screening of bacterial strains producing nonreducing oligosaccharides

About 1500 bacterial strains from soil were tested for production of nonreducing oligosaccharides from soluble starch. After glucoamylase digestion of the reaction mixtures, samples were treated with alkali to degrade reducing saccharides, and analyzed by thin-layer chromatography (TLC). As shown in Figure 1, a strain M6 produced two kinds of oligosaccharides (saccharide **1** and **2**), whose R_f values were 0.32 and 0.15, respectively, in addition to glucose and maltose (Lane 1). Saccharide **1** remained after alkaline treatment (Lane 3) and showed a different R_f value from those of other nonreducing oligosaccharides, such as trehalose, cyclomaltodextrins, and CA. These results indicated that saccharide **1** produced by the strain M6 might be a novel nonreducing oligosaccharide. Saccharide **2** was hydrolyzed by not only glucoamylase (Lane 2) but also by pullulanase (data not shown), indicating that saccharide **2** was a noncyclic sugar and had an α-(1→6)-glycosidic linkage in its structure. From the comparative analysis with authentic sugars by high-performance liquid chromatography (HPLC), we concluded that saccharide **2** was 6^2-*O*-α-maltosyl-maltose. The strain M6 was isolated from a soil sample in Okayama, Japan. Morphological, cultural, and physiological characterizations classified the strain into *Arthrobacter globiformis*, according to 'Bergey's Manual of Systematic Bacteriology'.[16]

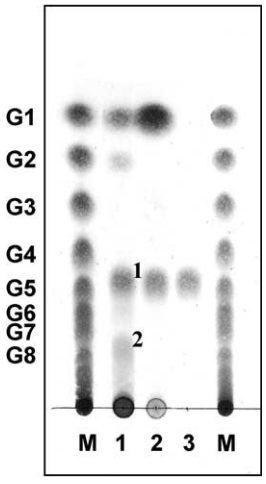

Figure 1. TLC analysis of the reaction products by *A. globiformis* M6. A reaction mixture containing 50 μL of 4% soluble starch in 50 mM acetate buffer (pH 6.0) and 50 μL of the culture broth from *A. globiformis* M6 was incubated at 40 °C for 24 h. Samples were spotted onto a TLC plate, developed twice, and then detected by 20% sulfuric acid. Lane M, maltooligosaccharides standard solution (G1, glucose; G2, maltose, and so on); Lane 1, after the reaction; Lane 2, after glucoamylase digestion; Lane 3, after alkaline treatment.

2.2. Isolation of saccharide 1

The preliminary experiment showed that the enzymatic activity catalyzing the formation of saccharide **1** from starch was present in the culture supernatant of *A. globiformis* M6. Therefore, the culture supernatant (1 L) was used as enzyme preparation and reacted with 20 g of soluble starch at 40 °C for 24 h. After glucoamylase treatment of the reaction mixture, saccharide **1** reached 23% of the total sugar content by HPLC (Fig. 2). The

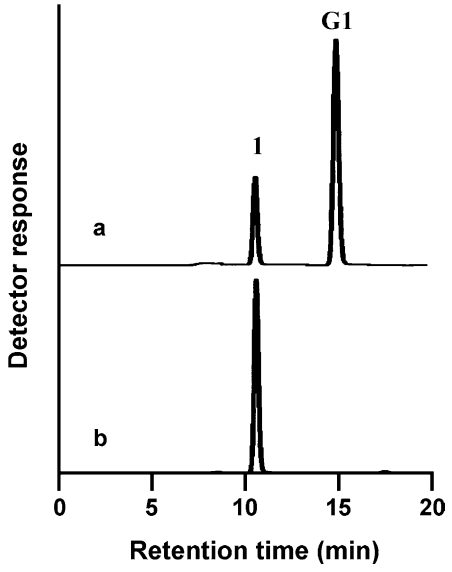

Figure 2. HPLC profiles of the reaction mixture and saccharide **1** isolated. A reaction mixture (2 L) containing 20 g of soluble starch and 1 L of the culture supernatant from *A. globiformis* M6 in 50 mM acetate buffer (pH 6.0) was incubated at 40 °C for 24 h. Samples were analyzed by HPLC using a Shodex SUGAR KS-801 column. (a) The reaction mixture after glucoamylase digestion; (b) the crystal preparation of saccharide **1**. G1, glucose.

content of saccharide **1** was more than 97% after alkaline treatment and deionization. During the concentration of the sugar solution by evaporation, crystals of saccharide **1** formed easily. The crystals were recovered by filtration in a yield of 4.0 g. The crystal preparation of saccharide **1** was shown to be homogenous on HPLC (Fig. 2).

2.3. Structure of saccharide 1

APCI-MS of saccharide **1** showed an $[M+Na]^+$ ion peak with an *m*/*z* ratio of 671, for an apparent mass of 648. The value was consistent with that of maltotetraose anhydride, indicating that saccharide **1** consisted of four glucose residues linked circularly, as in CA. Methylation analysis gave equimolar quantities of 2,3,4-tri-*O*-methyl per-*O*-acetylated glucitol and 2,3,6-tri-*O*-methyl per-*O*-acetylated glucitol (Table 1). Saccharide **1** was not hydrolyzed by α-amylase, β-amylase, glucoamylase, isoamylase, pullulanase, maltogenic α-amylase, and α-glucosidase. However, treatment with isomalto-dextranase resulted in its complete conversion to isomaltose (data not shown). These results showed that the structure of saccharide **1** is *cyclo*-{→6)-α-D-Glc*p*(1→4)-α-D-Glc*p*(1→6)-α-D-Glc*p*(1→4)-α-D-Glc*p*(1→}. To confirm this structure, NMR spectroscopy measurements were carried out. The ^{13}C NMR spectrum contained only

Table 1. Methylation analysis of saccharide **1**

Methylation products	Ratio
2,3,4-Tri-*O*-methyl 1,5,6-*O*-acetyl glucitol	1.00
2,3,6-Tri-*O*-methyl 1,4,5-*O*-acetyl glucitol	0.97

Relative molar ratios of methylated alditol acetates. Data are normalized relative to the 2,3,4-tri-*O*-methyl derivative.

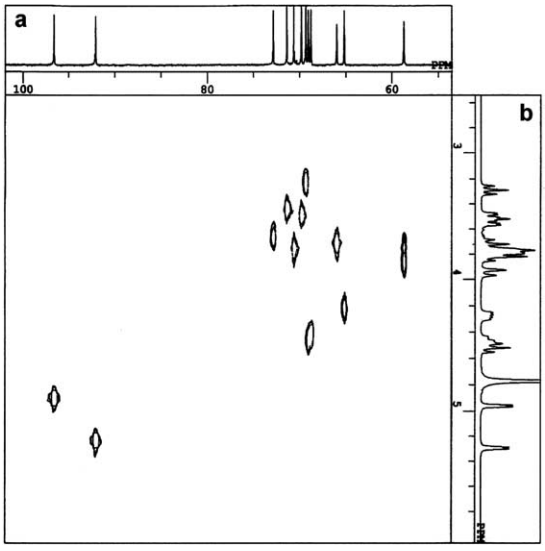

Figure 3. ^1H–^{13}C COSY spectrum of saccharide **1**. (a) ^{13}C NMR; (b) ^1H NMR.

Table 2. Chemical shifts and coupling constants for saccharide **1**

Carbon number	Chemical shifts (ppm)		Coupling constants (Hz)
	δ_C	δ_H	
6-O-Glycosylated residues			
1	95.9	5.26	$J_{1,2}$ 3.8
2	73.6	3.51	$J_{2,3}$ 9.9
3	74.4	3.78	$J_{3,4}$ 9.9
4	73.1	3.26	$J_{4,5}$ 9.6
5	72.5	4.43	
6	69.8	3.73, 3.73	
4-O-Glycosylated residues			
1′	100.4	4.93	$J_{1',2'}$ 3.3
2′	75.1	3.46	$J_{2',3'}$ 9.9
3′	72.8	4.48	$J_{3',4'}$ 9.7
4′	76.6	3.68	$J_{4',5'}$ 10.1
5′	69.0	4.24	
6′	62.5	3.73, 3.90	

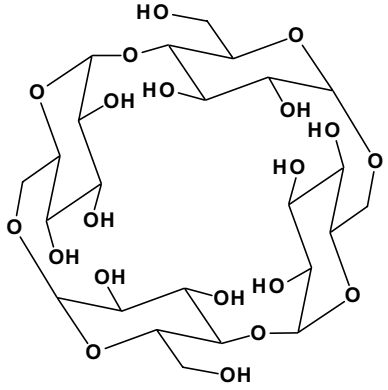

Figure 4. Structure of saccharide **1**. The drawing is intended to show linkage information only, and no particular conformation is implied.

12 signals (Fig. 3a) in agreement with the stereochemically symmetrical structure of the oligosaccharide in solution. No NMR peaks attributable to a reducing-end *C*-1 were detected. Similarly, the ^1H NMR spectrum showed only two anomeric proton signals, of equal intensity (Fig. 3b). ^1H–^{13}C COSY revealed the α-configurations of glucose residues in saccharide **1** that was confirmed by the *C*-1 signals at 95.9 ppm {δ ^1H, 5.26 (d, $J_{1,2}$ 3.8 Hz)} and 100.4 ppm {δ ^1H, 4.93 (d, $J_{1',2'}$ 3.3 Hz)}. These NMR data are summarized in Table 2. Thus, we determined the structure of saccharide **1** (Fig. 4), a novel cyclic tetrasaccharide tentatively named cyclic maltosyl-(1→6)-maltose (CMM).

2.4. Some properties of CMM

The water content of CMM crystals was 12.8%. TG/DTA showed that a gravimetric decrease of 12.14% occurred in a temperature range of 60–95 °C, suggesting that the CMM crystals were pentahydrate crystals (data not shown). The melting point of the crystals could not

Table 3. Solubility of cyclic tetrasaccharides in water at various temperatures

Temperature (°C)	Solubility (mmol/100 mL of H_2O)	
	CMM	CA
10	10.4	67.1
20	10.9	71.1
30	12.0	80.1
40	13.6	90.4
50	16.1	114.5
60	19.2	143.8
70	24.3	173.9
80	32.1	231.9

be determined because they decomposed without melting above 275 °C. The value for its optical rotation was +207.9° at 20 °C. The reducing value of CMM was negligible; therefore, CMM caused no Maillard reaction with glycine or peptone. When aqueous solutions of CMM (4%) were heated at 100 °C for 24 h, 84% and 22% of the total CMM were decomposed at pH 2 and 3, respectively. However, CMM remained unchanged in a pH range of 4–10. Moreover, heat treatment of 4% solution in 33 mM phosphate buffer (pH 7.0) at 120 °C for 90 min resulted in no degradation of CMM. The sweetness of CMM was about 18% of that of sucrose. Table 3 summarizes the solubility of CMM in water at various temperatures, in comparison with CA. The solubility of CMM was about seven times lower than that of CA.

3. Discussion

Recently, we have reported that a cyclic tetrasaccharide, consisting of four glucose residues joined by alternate α-(1→3)- and α-(1→6)-linkages, was produced from starch by two glycosyltransferases.[13,15] Although we named the sugar cyclic tetrasaccharide (CTS) at that time, it should be called cycloalternan (CA) as per Kim et al.,[17] because another cyclic tetrasaccharide has been found as described in this paper. Structural analyses revealed that saccharide **1** is a novel cyclic tetrasaccharide, *cyclo*-{→6)-α-D-Glc*p*(1→4)-α-D-Glc*p*(1→6)-α-D-Glc*p*(1→4)-α-D-Glc*p*(1→}. It should be noted that saccharide **2** (see Fig. 1), identified as 6^2-*O*-α-maltosyl-maltose, represents the open-chain tetrasaccharide formed by hydrolysis of one of the α-(1→6)-linkages of saccharide **1**. Therefore, we propose the name of cyclic maltosyl-(1→6)-maltose (CMM) for this novel cyclic tetrasaccharide.

Both of the cyclic tetrasaccharides have heterogeneous linkages in their structure. However, they are different in that CMM contains two maltosyl residues linked by α-(1→6)-linkages, while CA contains two nigerosyl moieties linked by α-(1→6)-linkages. Bradbrook et al. reported that CA has a plate-like shape with a shallow depression on one side in crystalline form.[18] Computer modeling using Chem-3D and MM2 indicated that the least strained conformation of CMM may be shaped like a bowl with a deeper depression on one side. Figure 4 illustrates the symmetric structure of CMM, on the basis of the ^{13}C NMR spectroscopy that exhibited only 12 signals. However, conformational modeling indicated that the least strained conformation of CMM may be asymmetric, possessing α-(1→6)-linkages with the glycoside-bond torsion angle ϕ (O-5–C-1–O-6–C-6) of 46.8° and 154.4°. The X-ray crystal structure analysis of CMM will give us more detailed information on its conformation.

The characteristics of CMM are not yet clear in detail, because the cyclic tetrasaccharide has been just found as described in this paper. However, potential applications of the saccharide for food, cosmetics, and pharmaceuticals are anticipated owing to its unique structure. In the course of the structural determination of CMM, we found that the saccharide was resistant to various amylases. Moreover, the digestibility of CMM in vitro examined by the method of Okada et al.[19] showed that human salivary or porcine pancreatic α-amylases, artificial gastric juice, and rat intestinal acetone powder could not hydrolyze CMM at all (data not shown). These results suggest that CMM may be utilized as a dietary fiber.

Among the numerous enzymes tested, isomalto-dextranase was the only one that could hydrolyze CMM. Isomalto-dextranase is the enzyme that acts on dextran to produce isomaltose in an exo-fashion. Later studies showed that the enzyme also possessed an isopullulanase-type activity on pullulan; however, the mode of action seemed to be an endo-fashion. In addition, it was reported that this enzyme could hydrolyze CA to yield isomaltose via an intermediate open tetrasaccharide, Glcα(1→6)Glcα(1→3)Glcα(1→6)Glc.[10] CMM now can be added to the list of substrates for this enzyme. If the CMM-producing bacterium, *A. globiformis* M6, produces the isomalto-dextranase activity, the enzyme may play a role in the metabolism of CMM.

We have already purified the enzyme from *A. globiformis* M6 involved in the formation of CMM from starch. The characterization of the enzyme and the mechanism of CMM synthesis will be described in the near future.

4. Experimental

4.1. Enzymes

Glucoamylase (EC 3.2.1.3) from *Rhizopus* sp. was purchased from Seikagaku Corp. (Tokyo, Japan). Isomalto-dextranase (EC 3.2.1.94) from *A. globiformis* T6 was prepared according to the method of Okada et al.[20] α-Amylase (EC 3.2.1.1), β-amylase (EC

3.2.1.2), isoamylase (EC 3.2.1.68), pullulanase (EC 3.2.1.41), maltogenic α-amylase (EC 3.2.1.133), and α-glucosidase (EC 3.2.1.20) used were of commercial grade.

4.2. Saccharides

Partially hydrolyzed starch, Pinedex #4 (dextrose equivalent 19 ± 2), and soluble starch were purchased from Matsutani Chemical Industry (Itami, Japan) and Katayama Chemical Industries (Osaka, Japan), respectively. 6^2-*O*-α-Maltosyl-maltose was prepared from maltose by the condensation reaction of pullulanase.[21] CA was prepared from starch with 6-α-glucosyltransferase and 3-α-isomaltosyltransferase as previously described.[14]

4.3. Screening of bacteria

Bacterial strains isolated from soil were incubated with reciprocal shaking at 27 °C for 5 days in a liquid medium containing 1.5% Pinedex #4, 0.5% polypeptone, 0.1% yeast extract, 0.1% K_2HPO_4, 0.06% $NaH_2PO_4 \cdot 2H_2O$, 0.05% $MgSO_4 \cdot 7H_2O$, and 0.3% $CaCO_3$ (pH 6.8). Reaction mixtures containing 0.1 mL of the culture broth and 0.1 mL of 4% (w/v) soluble starch in 50 mM acetate buffer (pH 6.0) were incubated at 40 °C for 24 h, and then boiled for 10 min to stop the reaction. Glucoamylase (4 units) was added into the reaction mixture and incubated at 40 °C for 16 h. After alkaline treatment (pH 12) at 100 °C for 1 h, nonreducing sugars in the reaction mixture were detected by TLC.

4.4. Isolation of saccharide 1

A reaction mixture (2 L) containing 20 g of soluble starch and 1 L of the culture supernatant of strain M6 (see results) in 50 mM acetate buffer (pH 6.0) was incubated at 40 °C for 24 h. After the reaction was stopped by boiling for 10 min, glucoamylase (5000 U) was added into the reaction mixture and incubated at 40 °C for 16 h. The mixture was treated with alkaline (pH 12) at 100 °C for 1 h and then desalted passing it through ion-exchange resins [100 mL of Diaion SK1B (Mitsubishi Chemical Co., Tokyo, Japan), 200 mL of Diaion WA30 (Mitsubishi Chemical), and 100 mL of Amberlite IRA411S (Organo Co., Tokyo, Japan)]. During the concentration of the eluate by evaporation at 50 °C, crystals formed. The crystals were recovered on a filter, washed with small amount of water, and dried in vacuo.

4.5. Mass spectrometry

MS analysis was performed using an LCQ advantage ion-trap mass analyzer (Thermo Electron Co., Kanagawa, Japan) fitted with an atmospheric pressure chemical ionization (APCI) interface. Sugar samples (approximately 10 ppm in 50% MeOH containing 0.1% HOAc) were infused to the mass spectrometer using a pressurized inlet device. The mass spectrometer was operated in the positive-ion mode; the spray and capillary voltages were set to 5 kV and 20 V, respectively; the capillary temperature was carried out at 350 °C.

4.6. Methylation analysis

A sample (2 mg) was methylated according to the method of Ciucanu and Kerek[22] and the products were isolated by partition between $CHCl_3$ and water. Then, the methylated sample was hydrolyzed in acid, reduced with $NaBH_4$, and acetylated as described by Hakomori.[23] The resulting partially methylated alditol acetates were separated by gas chromatography (GC-14B; Shimadzu Co., Kyoto, Japan) on a silica gel capillary column (DB-5; 0.25 mm × 30 m; J&W Scientific Co., Folsom, CA, USA), with a temperature program of 130 °C for 2 min followed by 5 °C/min to 250 °C.

4.7. NMR spectroscopy

NMR spectra data were recorded for 5% solution in D_2O at 27 °C on a JNM-AL300 spectrometer equipped with 5-mm dual probe (300.4 MHz for ^1H, 75.45 MHz for ^{13}C; JEOL, Tokyo, Japan). Chemical shifts were expressed in ppm downfield from the signal of 3-(trimethylsilyl)-1-propane-sulfonic acid sodium salt (TPS), which was used as an internal standard.

4.8. Some properties of saccharide 1

The total sugar content was measured by the anthrone–sulfuric acid method and the reducing sugar was measured by the Somogyi–Nelson method.[24,25] Optical rotation was determined at 20 °C on sample dissolved in distilled water (Horiba model SEPA-300; Horiba Ltd., Kyoto, Japan). Thermal analysis was performed with an SSC/5200H thermoanalytical system (TG/DTA220U; Seiko Instruments Inc., Chiba, Japan), with samples in aluminum pans under a constant nitrogen flow of 50 mL/min. The heating rate was 10 °C/min. Solubility in water was examined at various temperatures. Excess of saccharide was added to a glass vessel containing distilled water (10 mL). After the mixture was kept at 20–80 °C for 24 h with stirring, the solid content of the filtrate was measured.

4.9. Other analytical methods

TLC was performed on a Kieselgel 60 plate (E. Merck, Darmstadt, Germany) developed twice with a solvent of 6:4:1 BuOH–pyridine–water. Sugar spots were detected by spraying 20% sulfuric acid in MeOH solution and

incubating the plates at 110 °C for 10 min. HPLC analysis for sugar compositions was performed with a model LC-10A liquid chromatograph (Shimadzu) equipped with a Shodex SUGAR KS-801 column (8 × 300 mm; Showa Denko Co., Tokyo, Japan) at 60 °C. Elution was isocratic with water at a flow rate of 0.5 mL/min. Elution of oligosaccharide was monitored with a model RID-10A refractive index detector (Shimadzu).

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References

1. Arguelles, J. C. *Arch. Microbiol.* **2000**, *174*, 217–224.
2. Oku, K.; Watanabe, H.; Kubota, M.; Fukuda, S.; Kurimoto, M.; Tsujisaka, Y.; Komori, M.; Inoue, Y.; Sakurai, M. *J. Am. Chem. Soc.* **2003**, *125*, 12739–12748.
3. Tabuchi, A.; Mandai, T.; Shibuya, T.; Fukuda, S.; Sugimoto, T.; Kurimoto, M. *J. Appl. Glycosci.* **1995**, *42*, 401–406.
4. Mukai, K.; Tabuchi, A.; Nakada, T.; Shibuya, T.; Chaen, H.; Fukuda, S.; Kurimoto, M.; Tsujisaka, Y. *Starch/Stärke* **1997**, *49*, 26–30.
5. Chaen, H. *J. Appl. Glycosci.* **1997**, *44*, 115–120.
6. French, D. *Adv. Carbohydr. Chem.* **1957**, *12*, 189–260.
7. Loftsson, T.; Brewster, M. E. *J. Pharm. Sci.* **1996**, *85*, 1017–1025.
8. Goubet, I.; Dahout, C.; Semon, E.; Guichard, E.; Le Quere, J. L.; Voilley, A. *J. Agric. Food Chem.* **2001**, *49*, 5916–5922.
9. Tran, C. D.; De Paoli Lacerda, S. H. *Anal. Chem.* **2002**, *74*, 5337–5341.
10. Côté, G. L.; Biely, P. *Eur. J. Biochem.* **1994**, *226*, 641–648.
11. Misaki, A.; Torii, M.; Sawai, T.; Goldstein, I. J. *Carbohydr. Res.* **1980**, *84*, 273–285.
12. Biely, P.; Côté, G. L.; Burgess-Cassler, A. *Eur. J. Biochem.* **1994**, *226*, 633–639.
13. Nishimoto, T.; Aga, H.; Mukai, K.; Hashimoto, T.; Watanabe, H.; Kubota, M.; Fukuda, S.; Kurimoto, M.; Tsujisaka, Y. *Biosci. Biotechnol. Biochem.* **2002**, *66*, 1806–1818.
14. Aga, H.; Higashiyama, T.; Watanabe, H.; Sonoda, T.; Nishimoto, T.; Kubota, M.; Fukuda, S.; Kurimoto, M.; Tsujisaka, Y. *J. Biosci. Bioeng.* **2002**, *94*, 336–342.
15. Aga, H.; Nishimoto, T.; Kuniyoshi, M.; Maruta, K.; Yamashita, H.; Higashiyama, T.; Nakada, T.; Kubota, M.; Fukuda, S.; Kurimoto, M.; Tsujisaka, Y. *J. Biosci. Bioeng.* **2003**, *95*, 215–224.
16. Sneath, P. H. A.; Mair, N. S.; Sharpe, M. E.; Holt, J. G. In *Bergey's Manual of Systematic Bacteriology*; Williams & Wilkins: Baltimore, 1984; Vol. 2.
17. Kim, Y. K.; Kitaoka, M.; Hayashi, K.; Kim, C. H.; Côté, G. L. *Carbohydr. Res.* **2003**, *338*, 2213–2220.
18. Bradbrook, G. M.; Gessler, K.; Côté, G. L.; Momany, F.; Biery, P.; Bordet, P.; Pérez, S.; Imberty, A. *Carbohydr. Res.* **2000**, *329*, 655–665.
19. Okada, K.; Yoneyama, M.; Mandai, T.; Aga, H.; Sakai, S.; Ichikawa, T. *J. Jpn. Soc. Nutr. Food Sci.* **1990**, *43*, 23–29 (in Japanese).
20. Okada, G.; Takayanagi, T.; Sawai, T. *Agric. Biol. Chem.* **1988**, *52*, 495–501.
21. Sakano, Y.; Sano, M.; Kobayashi, T. *Agric. Biol. Chem.* **1985**, *49*, 3041–3043.
22. Ciucanu, I.; Kerek, F. *Carbohydr. Res.* **1984**, *131*, 209–217.
23. Hakomori, S. *J. Biochem. (Tokyo)* **1964**, *55*, 205–208.
24. Somogyi, S. *J. Biol. Chem.* **1952**, *195*, 19–23.
25. Nelson, N. *J. Biol. Chem.* **1944**, *153*, 375–380.