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Production of Cyclic Tetrasaccharide with 6-α-Glucosyltransferase and α-Isomaltosyltransferase

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Abstract: Cyclic tetrasaccharide (CTS; *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→}) is a cyclic oligosaccharide four D-glucosyl residues linked in an alternating α-1,3- and α-1,6-fashion. CTS has been reported for enzymatic synthesis from alternan, an α-(1→3)-α-(1→6)-D-glucan synthesized from sucrose by *Leuconostoc mesenteroides* NRRL B-1355 alternansucrase, by endo-glucanase (alternanase) from *Bacillus* sp. NRRL B-21195. While searching for nonreducing oligosaccharides that can be produced from α-1,4-glucan as the substrate, we screened bacteria isolated from soil in our own way, and obtained *Bacillus globisporus* C11, which produces CTS from starch. Two kinds of glycosyltransferase, 6-α-glucosyltransferase (6GT) and α-isomaltosyltransferase (IMT), were purified from the culture supernatant of this strain. It was found that CTS is produced by the sequential action of both enzymes. The genes for IMT (CtsY) and 6GT (CtsZ) were linked together on the chromosome, forming *ctsYZ*. Both of the gene products showed similarities to α-glucosidases belonging to glycoside hydrolase family 31 and conserved two aspartic acids corresponding to the putative catalytic residues of these enzymes. *CtsYZ* and four open reading frames upstream of *ctsYZ* form a gene cluster, *ctsUVWXYZ.* The reaction conditions for CTS synthesis were examined using 6GT and IMT from *B. globisporus* C11. The optimum reaction conditions to obtain CTS from Pinedex #100 (partial hydrolyzate of starch, 1.3%-hydrolysis) were the following: substrate concentration, 3%; pH, 6−7; temperature, 30°C; enzyme dosages, 1 U/g-dry solid 6GT, 10 U/g-dry solid IMT. In these optimum conditions the CTS yields reached 62% at the reaction time of 48 h. A mass-production method of highly purified CTS crystals at a reasonable cost was established, and the functions and characteristics of CTS were studied.

Key words: cyclic tetrasaccharide, *Bacillus globisporus,* 6-α-glucosyltransferase, α-isomaltosyltransferase

When saccharides are produced as foodstuffs in fairly large quantities, starch is a very fascinating source. Maltooligosaccharides such as glucose, maltose and maltotetraose are produced by hydrolysis of starch. Cyclodextrins (cyclic oligosaccharides composed of 6, 7, or 8 D-glucosyl residues linked by α-1,4-linkages),[1] isomaltooligosaccharides[2] and nigero-oligosaccharides[3] are also produced from starch by enzymatic transfer reactions. In additions, these saccharides can be converted into branched cyclodextrins, sorbitol and maltiotol chemically or enzymically. Starch is used as donor substrate to produce various glycosides such as glucosyl ascorbic acid,[4] and as a source for the production of many saccharide-related products. The reason seems to be that not only starch is inexpensive but also various starch-related enzymes are present in nature. Indeed, there are many oligosaccharides synthesized enzymically. However, nonreducing glucooligosaccharides synthesized from starch are very few in kind. Examples of such oligosaccharides are trehalose[5−11] and cyclodextrins. Cyclic tetrasaccharide (CTS; *cyclo*{→6)-α-D-Glu*p*-(1→3)-α-D-Glu*c*p-(1→6)-α-D-Glu*c*p-(1→3)-α-D-Glu*p*-(1→}) is also one of the nonreducing glucooligosaccharides.[12] A system of synthesizing this cyclic oligosaccharide from starch was recently found in *Bacillus globisporus* C11. A CTS-synthesizing mechanism controlled by two enzymes and the sequence of the genes encoding them were reported in succession.[13,14] These studies indicate that there is a good possibility of industrial production of CTS and a gene cluster related to the synthesis and transport of CTS is present in microorganisms. In this paper, we describe the current study of CTS by focusing on the synthesizing system of CTS from starch.

Isolation of CTS-producing bacteria and the producing system in B. globisporus C11.[13]

Among some 3000 bacterial strains, strain C11 was isolated from soil from Okayama, Japan, by screening for bacteria-producing nonreducing oligosaccharides. Morphological, culture, and physiological characterizations according to Bergey's Manual of Systematic Bacteriology classified strain C11 as a strain of *Bacillus globisporus*. A nonreducing oligosaccharide was purified from a reaction mixture containing Pinedex #100 (partial hydrolyzate of starch, 1.3%-hydrolysis) and the supernatant from *B. globisporus* C11. This saccharide was found to be identical

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Abbreviations: CTS, cyclic tetrasaccharide; 6GT, 6-α-glucosyltransferase; IMT, α-isomaltosyltransferase; MOS, maltooligosaccharides; CGTase, cyclomaltodextrin glucanotransferase (EC 2.4.1.19); HPLC, high-pressure liquid chromatography.

to the CTS by acid hydrolysis, FAB-MS analysis, methylation analysis, isomalto-dextranase digestion and ^{13}C-NMR analysis. CTS has been reported for enzymatic synthesis from alternan.[12] The polysaccharide alternan is a unique dextran produced from sucrose by *Leuconostoc mesenteroides* NRRL B-1355.[15] It is composed predominantly of an alternating sequence of α-1,3-linked and α-1,6-linked D-glucose residues, with approximately 10% branching. Côté *et al.* tried to obtain an enzyme hydrolyzing alternan in an endo-fashion from soil microorganisms according to the idea that such an enzyme is also a unique glycosidase. This resulted in the isolation of *Bacillus* sp. NRRL B-21195 as the producer of a novel type of end-glucanase (alternanase).[16] Furthermore, it was found that there is CTS in the reaction mixture containing purified enzyme and alternan.[12] This is the first report relating to the enzymatic synthesis of CTS. Later they examined the substrate specificity of alternanase and reported that CTS was also produced from panose by the enzyme.[17]

CTS-producing enzymes were purified from the culture supernatant of *B. globisporus* C11. As a result, it was found that CTS is produced by the sequential action of two kinds of enzymes: glucosyltransferase and glucanotransferase.[13] The former was a 6-α-glucosyltransferase (6GT) catalyzing the α-1,6-transglucosylation of one glucosyl residue to the nonreducing end of maltooligosaccharides (MOS) to produce α-isomaltosyl-MOS from MOS. The latter was an α-isomaltosyltransferase (IMT) catalyzing α-1,3-, α-1,4- and α,β-1,1-intermolecular transglycosylation of isomatosyl residues. In addition, IMT catalyzed cyclization, and produced CTS from α-isomaltosyl-(1→3)-α-isomaltosyl-MOS by intramolecular transglycosylation.

Figure 1 shows the change with time during the reaction with maltotetraose by 6GT. α-Isomaltosyl-(1→4)-α-maltotriose was preferentially synthesized from maltotetraose and accumulated in the reaction mixture. It was clear that it is hard for this transfer product to become a donor and acceptor of 6GT. These characteristics of 6GT are distinct from those of other glycosidases catalyzing the α-1,6-transglucosylation, such as dextrin dextranase (EC 2.4.1.2) and α-glucosidase (EC 3.2.1.20) from *Aspergillus niger*.

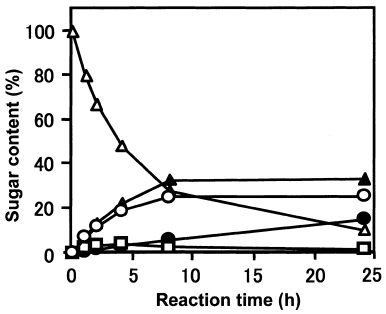

Fig. 1. Changes with time during reaction with maltotetraose by 6GT.

A reaction mixture (10 mL) containing 0.1 g of maltotetraose and 0.02 U of 6GT in 50 mM acetate buffer (pH 6.0) was incubated for the indicated time at 35°C. The sugar composition was calculated from the peak area of each sugar after HPLC with a YMC-Pack ODS AQ-303 column. ○, maltotriose; ●, α-isomaltosyl-(1→4)-α-maltose; △, maltotetraose; ▲, α-isomaltosyl-(1→4)-α-maltotriose; □, maltopentaose.

When IMT reacted with panose, α-3^3-isomaltosyl panose, α-4^3-isomaltosyl panose and α-isomaltosyl β-panoside were synthesized as intermediates of final product CTS. Among the oligosaccharides, α-3^3-isomaltosyl panose had the highest CTS production rate. Therefore, this oligosaccharide can be considered to be a direct substrate for producing CTS.

Figure 2 shows the scheme for the formation of CTS from α-1,4-glucan. 6GT provides a favorable substrate to IMT for the production of CTS and IMT synthesizes CTS from this substrate in a 2-step reaction. The reaction leading to CTS-production was thus organized by a remarkable harmony with 6GT and IMT.

We examined a distribution of microorganisms having CTS-producing system similar to *B. globisporus* C11. Among some 3000 bacterial strains isolated from soil, *Arthrobacter* strains producing CTS from starch were obtained. It is interesting that there is also a similar enzymatic system in *Arthrobacter* strains, except for *Bacillus* strains. In the future, the difference of enzymes from both strains will be clarified through the study of CTS-producing enzymes from *Arthrobacter* strains. Such a CTS-producing systems may be widely distributed in other microorganisms.

The structure of the gene cluster in B. globisporus C11.

The genes for IMT (CtsY) and 6GT (CtsZ), involved in synthesis of CTS have been cloned from the genome of *B. globisporus* C11.[14] The amino-acid sequence encoded by the *ctsY* gene was composed of 1093 residues having a signal sequence of 29 residues in its N-terminus. The *ctsZ* gene encoded a protein consisting of 1284 residues with a signal sequence of 35 residues. Both of the gene products showed similarities to α-glucosidases belonging to glycoside hydrolase family 31 and conserved two aspartic acids corresponding to the putative catalytic residues of these enzymes (Fig. 3). The *ctsZ* gene lay in the rear of the *ctsY* gene through a short flanking region of

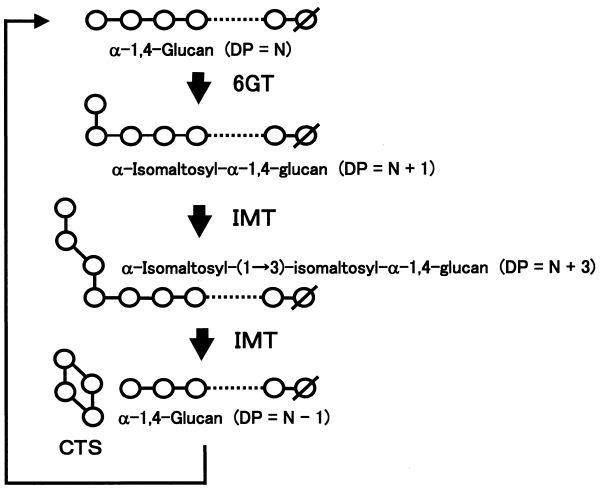

Fig. 2. Scheme for the formation of CTS from α-1,4-glucan by 6GT and IMT.

○, glucosyl residue; ∅, reducing terminal glucosyl residue; —, α-1,4-linkage; |, α-1,6-linkage; ＼, α-1,3-linkage. DP, degree of polymerization.

Enzymes	Region A	Region B
CtsW (*Bacillus globisporus* C11)	841-GFKTDGGEF	938-GWDLGGF
CtsY (*Bacillus globisporus* C11)	562-GFKTDGGEM	658-SWDMAGF
CtsZ (*Bacillus globisporus* C11)	429-GWWNDETDK	531-GMDTGGF
α-Glucosidase (*Aspergillus niger*)	486-GVWYDMSEV	687-GADTCGF
α-Glucosidase (*Candida tsukubaensis*)	522-GIWLDMNEP	769-GADICGF
α-Glucosidase (sugar beet)	465-GIWIDMNEA	595-GADICGF
Acid α-glucosidase (human lysosomal)	514-GMWIDMNEP	643-GADVCGF
Isomaltase (rabbit intestinal)	501-GLWIDMNEV	631-GADICGF

Fig. 3. Regional sequence homology among the CTS-forming enzymes and the family 31 α-glucosidases.

The putative catalytic residues are shadowed. α-Glucosidase from *Aspergillus niger* is the accession number, P56526, in Swiss-prot; from *Candida tsukubaensis*, P29064; from sugar beet, O04931. Acid-α-glucosidase from human lysosome, P10253. Isomaltase from rabbit intestine, P07768.

55 bases. The two genes were linked together, forming *ctsYZ*. A transcription terminator-like sequence lay in the rear of the *ctsZ* gene.

The DNA sequence of 16,515 bp cloned in this study contained four open reading frames (ORF1-4) upstream of *ctsYZ*.

ORF-1 (*ctsU*) was an incomplete ORF missing the 5′-region. The amino-acid sequence (209 residues) deduced from the ORF showed similarities to the C-terminal regions of a hypothetical permease of the sugar transport system encoded by the gene BH1926 from *Bacillus halodurans* C-125[18] and a CD-binding protein-dependent permease from *Thermococcus* sp. B1001.[19] These sequence similarities suggest that the ORF-1 products may be part of the CTS-binding protein-dependent ABC transport system.

ORF-2 (*ctsV*) lay in the rear of the incomplete ORF-1 through a 98-bp flanking region. The structural gene encoded a protein with 449 amino-acid residues (calculated molecular mass of 49,553 Da). The putative protein was similar to a CD-binding protein from *Thermococcus* sp. B 1001[19] and to a periplasmic solute-binding protein of the sugar uptake ABC transporter from *Rhizobium meliloti*.[20] These sequence similarities suggest that the ORF-2 products may be CTS-binding proteins.

ORF-3 (*ctsW*) encoded a protein with 1237 amino-acid residues not having a signal sequence (calculated molecular mass of 138,846 Da). The C-terminus amino-acid sequence of the ORF-3 protein showed a significant similarity to the N-terminus part of CtsY. The expression experiment showed that the ORF-3 protein produces CTS from panose but not from either 6-glucosylated glycogen or starch. It was suggested that the protein is a kind of IMT preferring low-molecular-mass substrate to high-molecular-mass ones. The difference of substrate-preference between ORF-3 protein and IMT may be caused by the lack of the putative sugar-binding C-terminal domain. The ORF-3 protein may be an intracellular protein and catalyze another CTS-producing reaction differently from the extracellular IMT.

ORF-4 (*ctsX*) lay between ORF-3 and *ctsY* genes, and encoded a small protein of 144 amino-acid residues (16,438 Da). The similarity was insufficient for characterization of the ORF-4 product, and it remains an unknown protein.

As described above, it seems that *ctsUVWXYZ* is a

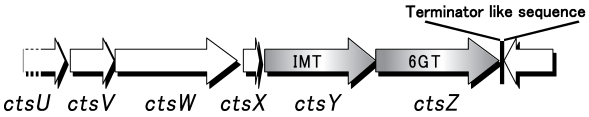

Fig. 4. Structure of the gene cluster containing genes for synthesis and transport of CTS in *B. globisporus* C11.

Arrows show the localization of each gene and the orientation of the coding sequences.

gene cluster related to CTS (Fig. 4). In the case of CDs, the *cgtBACDE* cluster was found in the genome of *Thermococcus* sp. B1001: *cgtB* for the intracellular CD-degradation enzyme, *cgtA* for the extracellular CD-synthesis enzyme (CGTase), *cgtC* for the CD-binding protein, and *cgtDE* for the membrance transporter proteins.[19] It has been reported that *Klebsiella oxytoca* M5a1 has a CD-synthesis/uptake/degradation system similar to that of *Thermococcus*.[21]

Examination of reaction conditions for synthesizing CTS from starch.

The reaction conditions for CTS synthesis were examined using 6GT and IMT from *B. globisporus* C11.[13,22] When the substrate was MOS, the yields of CTS increased with increasing degree of polymerization of MOS.[13] Furthermore, when the substrate was partial hydrolyzate of starch, the yields of CTS increased with decreasing hydrolysis percentage.[22] Interestingly, glycogen was the most suitable substrate for CTS synthesis and the CTS yields reach 80% or more. It is known that when the substrates are debranched by isoamylase, the CTS yields decrease significantly. These suggest that the α-1,6-branched structure proves to be favorable to the CTS synthesis. The optimum reaction conditions to obtain CTS from Pinedex #100 (partial hydrolyzate of starch, 1.3%-hydrolysis) were the following: substrate concentration, 3%; pH, 6–7; temperature, 30°C; enzyme dosages, 1 U/g-dry solid 6GT, 10 U/g-dry solid IMT. In these optimum conditions the CTS yields reached 62% at the reaction time of 48 h.[22]

Two kinds of branched CTS resisting glucoamylase and α-amylase were isolated in the reaction mixture containing of 6GT, IMT and starch.[22] One is α-1,4-glucosyl branched CTS, *cyclo*{→6)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→6)-[α-D-Glc*p*-(1→4-)]-α-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→} (Fig. 5). The other is α-1,3-isomaltosyl branched CTS, *cyclo*{→6)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→6)-[α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→3)]-α-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→} (Fig. 5). These branched CTS are considered to be synthesized by transglycosylation of 6GT and IMT to CTS. The conversion of branched CTS into CTS and the use of thermostable enzymes are necessary for the development of a mass-production method at a reasonable cost. The simultaneous action of glucoamylase and α-glucosidase was effective for former subject. To solve the latter subject, we re-screened bacteria producing both 6GT and IMT more stable than the C11 enzymes, and consequently obtained strain N75 belonging to the same bacterial species.[23] CTS formation by the N75 enzymes was compared with that by the C11 enzymes. Figure 6 shows the CTS yields by the N75 enzymes, together with the re-

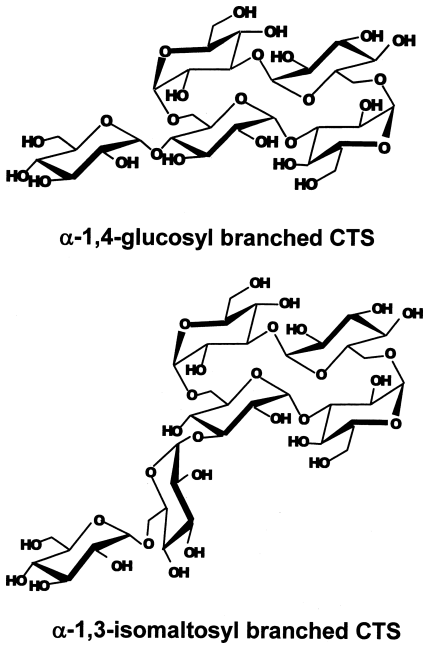

Fig. 5. Structures of branched CTS derivatives synthesized enzymically.

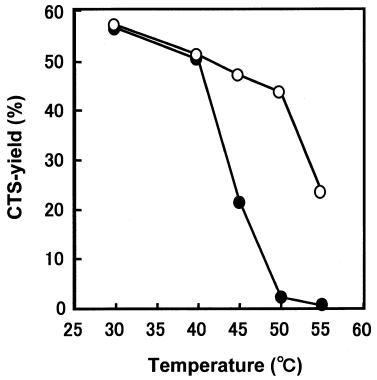

Fig. 6. Effect of temperature on CTS formation.

Reaction mixtures containing 2% (w/v) dextrin (Pinedex #100) and the enzymes (2 U/g dry solid of CTS-forming activity) in 20 mM acetate buffer (pH 6.0) and 1 mM $CaCl_2$ were incubated at several temperatures from 30°C to 55°C for 48 h. CTS content was measured by the method of Aga.[23] ○, N75 enzymes; ●, C11 enzymes.

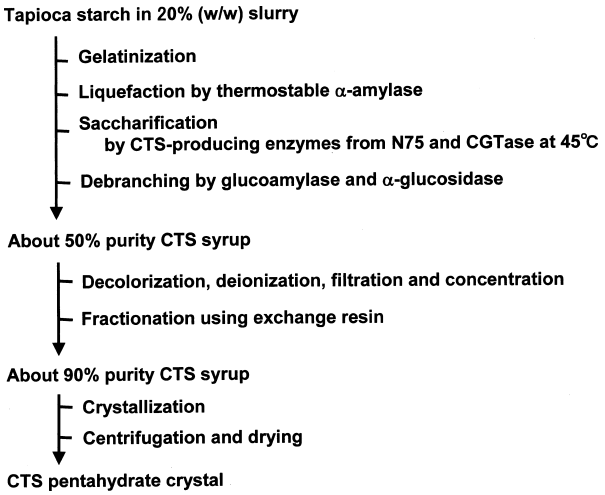

Fig. 7. Procedure for manufacturing CTS pentahydrate crystal from starch.

sults from the C11 enzymes. The yields by N75 enzymes up to 50°C were more than 40% and gradually decreased depending on the reaction temperature, although the yield fell remarkably at 55°C. On the other hand, the yield by the C11 enzymes decreased sharply at 45°C because of inactivation of the enzymes. Thus, the N75 enzymes could produce CTS at 10°C higher temperature than the C11 enzymes. The CTS yield decreased with increasing the concentration of the substrate. As a result of the additional examination of several enzymes, we found that the CTS yield from the high-concentration substrate increased with the addition of cyclomaltodextrin glucanotransferase (CGTase).[23] Figure 7 shows the procedure for manufacturing CTS pentahydrate crystal from starch. We succeeded in a preparation of about 1000 kg CTS pentahydrate crystal from starch according to this procedure.

Some properties of CTS.

The CTS crystal is accessible from a water solution.[12,13,22] The crystal structure is determined by X-ray analysis.[24] The asymmetric unit in the crystal contains one CTS together with five water molecules. One of the water molecules was found in the dished-shape cavity of CTS. CTS adopts a plate-like overall shape with a very shallow depression on one side. Accordingly CTS is considered to not have a hydrophobic cavity region to form inclusion compounds, similar to cyclodextrins and the cyclodextrin molecule. Recently, we found CTS anhydrous and monohydrate crystals in addition to the pentahydrate crystal. CTS pentahydrate crystal is nonhygroscopic under the relative humidity of 95%. On the other hand, CTS anhydrous and monohydrate crystals changed to pentahydrate crystal under the relative humidity of 80% or above.

The solubility of CTS pentahydrate crystal in water is relatively high (46.1 g/100 g water at 20°C) and the sweetness is 27% of that of sucrose. CTS does not exhibit maillard reaction at all and is very stable under alkaline conditions because CTS is a nonreducing saccharide.[25]

CTS is expected to offer strong resistance to hydrolysis by glycosidases from its structure. In our examination, CTS was finally hydrolyzed to isomaltose by IMT and isomalto-dextranase (The CTS hydrolytic activity of IMT is a very weak). There was a difference between the reaction specificity of the two enzymes. With isomalto-dextranase, an open-chain intermediate was detected in the reaction mixture, while with IMT, this saccharide was not detected. This was probably caused by a difference in the reaction rates of both enzymes on the open-chain intermediate. This action pattern of alternanase on CTS is analogous to that of IMT.[16] Other enzymes hydrolyzing α-1,6-glucosidic linkage in an endo-fashion, such as dextranase (EC 3.2.1.11), isoamylase (EC 3.2.1.68), pullulanase (EC 3.2.1.41) did not act on CTS at all. No hydrolysis of CTS was observed in an *in vitro* digestive test using salivary, artificial gastric juice, pancreatic amylases or small intestinal enzymes. CTS was not utilized by typical human intestinal bacteria. These facts indicate that CTS is a highly indigestible saccharide.[25] CTS was not fermented to an acid and did not synthesize water-insoluble glucan by *Streptococcus mutans*. This suggests that CTS is not responsible for dental caries in humans.

The production of saccharides using CTS, 6GT or IMT.

It is possible to produce isomaltose and nigerose by limited degradation of CTS. The α-1,3-glucosidic linkage present in CTS is hydrolyzed by isomalto-dextranase. As a result, highly purified isomaltose is produced from CTS. On the other hand, after selective degradation of the α-1,6-glucosidic linkage present in CTS by acetolysis, highly purified nigerose can be obtained by deacetyl reaction and column chromatography.

Both 6GT and IMT are very attractive enzymes. The distinctive feature of 6GT is that it has a weak hydrolytic activity toward transfer products compared with α-glucosidase from *A. niger* using the manufacture of isomalto-oligosaccharids. It is possible that a new procedure for manufacturing isomalto-oligosaccharides can be achieved using 6GT instead of α-glucosidase. We have already developed a novel method of isomaltose production with 6GT and isomalto-dextranase from starch. IMT may be able to efficiently synthesize various transfer products having the α-1,3-glucosidic linkage. For example, α-isomaltosyl-(1→3) trehalose was synthesized from trehalose and panose. These detailed results will be reported in our next papers.

Conclusion and prospects.

It is obvious that a starch-degradation pathway controlled by 6GT and IMT exists in *B. globisporus* C11. Indeed, during the culture of the strain using soluble starch as the carbon source, CTS was synthesized with the decrease of starch in medium and it was all consumed in time (Nishimoto *et al*., unpublished data). This indicates that the strain synthesizes CTS to utilize starch. To clarify the whole starch-degradation pathway in *B. globisporus* C11, the structure of the gene cluster has to be investigated in further detail. The CTS gene cluster so far clarified is lacking in the genes encoding a part of ABC transport system and a CTS-hydrolyzing enzyme.

Extended research is needed for the identification of other functions and applications of CTS. The investigation of its function as soluble food fiber is now in progress. In oral administration of CTS, we expect it to result in good effects for human health.

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6-α-Glucosyltransferase と α-Isomaltosyltransferase による環状四糖生成

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環状四糖（CTS；*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→}）は，四つのグルコース残基がα-1,3とα-1,6で交互に結合した環状オリゴ糖である．CTSの酵素合成は既に報告されており，*Leuconostoc mesenteroides* NRRL B-1355アルタナンスクラーゼがスクロースから合成する多糖アルタナンに，*Bacillus* sp. NRRL B-21195アルタナナーゼ（エンド-グルカナーゼ）を作用させることで生成する．我々は独自に土壌由来の細菌をスクリーニングし，非還元性オリゴ糖を生成する微生物を調査した．その結果，*Bacillus globisporus* と同定されたC11株が澱粉からCTSを生成することを見出した．本株の培養上清から酵素を精製したところ，2種類の糖転移酵素，6-α-グルコシルトランスフェラーゼ(6GT)とα-イソマルトシルトランスフェラーゼ(IMT)を単離することができた.両酵素が協同的に作用し，α-1,4-グルカンからCTSを合成することを明らかにした．*B. globisporus* C11株からIMT遺伝子（*ctsY*）と6GT遺伝子（*ctsZ*）をクローン化した．両酵素遺伝子は染色体上で近接し*CtsYZ*の順で存在していた．両酵素のアミノ酸配列は糖加水分解酵素ファミリー31に属するα-グルコシダーゼと相同性があり，ファミリー31の触媒残基と提唱されている二つのアスパラギン酸も保存していた．*ctsYZ* の上流には少なくとも四つの遺伝子（*ctsUVWX*）が存在し，遺伝子クラスター *ctsUVWXYZ* を形成していた．*B. globisporus* C11由来6GTとIMTを用いてCTS生成の反応条件を調べた．Pinedex #100（澱粉部分分解物；加水分解率　1.3%）を用いた場合，CTS生成の最適条件は，基質濃度；3%，pH　6–7，温度；30℃，6GT；1 U/g-DS，IMT；10 U/g-DSであった．48時間反応後のCTS生成率は62%にも達した.さらに,澱粉から高純度CTS結晶粉末の大量製造法を確立し，CTS機能・特性を検討した．

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〔質　問〕　信州大　北畑

1）Panoseを基質に用いたときには1,3結合での転移以外に1,4，1,1結合での転移が相当量起こっているようですが，Panoseを基質に用いたためでしょうか？デンプンを基質に用いたときにも起こっていますか？

2）CTSの収量が50%程度とのことですが，生成物中に分岐CTSが多量生成しており，α-glucosidase処理により，グルコースが多量生成しているためでしょうか？

〔答〕

1）実際に確認はしておりませんが，デンプンを基質に用いたときにも1,3結合での転移以外に1,4，1,1結合での転移が起こっていると考えています．

2）糖濃度30%(w/w)で糖化反応を行い，α-glucosidase処理により分岐糖鎖を加水分解した後のCTS収率が約50%になります．CTS収率が約50%に制限されるのは，IMTによるα-1,3転移以外の転移（α-1,4，α,β-1,1転移）生成物および6GTによるα-1,6転移以外の転移生成物の蓄積が影響しているものと考えています．また，特に糖濃度が高くなると，IMTによる分子間糖転移反応が促進されることも要因であると思われます．

〔質　問〕　中部大　谷口

反応液中のCTSと枝付きCTSの割合はどれ位ですか．

〔答〕

反応液中のCTSと枝付きCTSの割合は，反応時の糖濃度により異なります．糖濃度が高くなるに従い，枝付きCTSの割合が増加すると共にα-glucosidase処理後のCTS収率は低下します．糖濃度20%（w/w）で反応した場合，反応液中のCTSは全糖に対して約35%で，α-glucosidase処理により約50%に上昇します．よって，およそ25%分のCTSに種々の分岐鎖が結合した形で存在することになります．ですから，遊離のCTSと枝付きCTSを含めた生成率は50%よりかなり高い値になると考えられます．