乳酸菌由来マルトースホスホリラーゼを用いたα－（1→4）－グルコニ糖の高収率生産

誌名
新潟大学農学部研究報告 = Bulletin of the Faculty of Agriculture, Niigata University

ISSN
03858634

著者
中井, 博之
仁平, 高則
Svensson, B.
大坪, 研一

巻/号
64巻1号

掲載ページ
p. 71-79

発行年月
2011年9月
Efficient Enzymatic Synthesis of α-(1→4)-glucosidic Disaccharides by Maltose Phosphorylase from Lactobacillus acidophilus NCFM

Hiroyuki NAKAI1*, Takanori NIHIRA2, Birte SVENSSON3 and Ken’ichi OTSUBO1,2

(Received July 6, 2011)

Summary

A gene cluster involved in maltose/maltodextrin metabolism was identified in the genome of Lactobacillus acidophilus NCFM. Enzymatic properties were described for MalP belonging to glycoside hydrolase family 65 (GH65), which is encoded by malP (GenBank: AAV43670.1) located in the gene cluster. MalP catalyses phosphorylation of maltose with inversion of the anomeric configuration releasing β-glucose 1-phosphate and glucose. Broad acceptor specificity was demonstrated by reverse phosphorylation using various carbohydrate acceptors and β-glucose 1-phosphate as donor. MalP showed strong preference for monosaccharide acceptors with equatorial 3-OH and 4-OH, and reacted also with the 2-deoxy amino sugars. By contrast none of the tested di- and trisaccharides served as acceptor. Disaccharide yields obtained from 50 mM β-glucose 1-phosphate and 50 mM glucose, glucosamine, N-acetyl glucosamine, mannose, xylose, or L-fucose were 99.80, 93.81, and 13%, respectively. Product structures were determined by NMR and ESI-MS to be α-d-glucopyranosyl(1→4)D-glucopyranose, α-D-glucopyranosyl(1→4)-D-glucosaminopyranose, α-D-glucopyranosyl(1→4)-D-mannopyranose, α-D-glucopyranosyl(1→4)-D-xylopyranose, and α-D-glucopyranosyl(1→4)-L-fucopyranose. Additionally MalP catalysed synthesis of the α-(1→4)-glucosidic disaccharides from maltose in a coupled phosphorolysis/reverse phosphorolysis one-pot reaction. Thus phosphorylation of maltose to β-glucose 1-phosphate circumvented addition of costly β-glucose 1-phosphate for reverse phosphorylation with different monosaccharide acceptors. This strategy can be applied to large-scale production of valuable oligosaccharides from low-cost carbohydrates as catalysed by phosphorylases with different substrate specificity.

Key words: β-glucose 1-phosphate, α-(1→4)-glucosidic disaccharides, glycoside hydrolase family 65, maltose phosphorylase, reverse phosphorylation

Maltose, α-D-glucopyranosyl(1→4)-D-glucopyranose, and maltodextrin, α-D-glucopyranosyl(1→4)-α-D-glucopyranosyl(1→4)-α-D-glucopyranosyl, are transported by a phosphoenolpyruvate-dependent phosphotransferase system (PTS) including NADH-dependent 6-phospho-α-glucosidase (EC 3.2.1.122) (Thompson et al., 1998; Kamionka and Dahl, 2001; Schöner et al., 2006). Furthermore, bioinformatics analysis on L. casei, L. lactis and L. casei predicted that maltose is taken up via a putative ABC transporter and degraded intracellularly by maltose phosphorylase (EC 2.4.1.8) (Nilsson and Rådström, 2001; Andersson and Rådström, 2002; Monedero et al., 2008).

Maltose phosphorylases are characterized from several bacteria (Kamogawa et al., 1973; Hüwel et al., 1997; Ehrmann and Vogel, 1998; Hidaka et al., 2005) and are found to catalyze phosphorylation of maltose with inversion of the anomeric configuration at the C-1 atom to give β-glucose 1-phosphate and glucose. Sequence analysis (Ehrmann and Vogel, 1998; Inoue et al., 2002; Hidaka et al., 2005) has assigned maltose phosphorylase to glycoside hydrolase family 65 (GH65) together with trehalose [α-D-glucopyranosyl(α1→α1)-β-D-glucopyranosyl] phosphorylase (EC 2.4.1.64) (Inoue et al., 2002; Murata et al., 2002), kojibiose [α-D-glucopyranosyl(1→2)-β-D-glucopyranosyl] phosphorylase (EC 2.4.1.220) (Yamamoto et al., 2004), and α-α-trehalase (EC 3.2.1.28) (Galm et al., 2008).

1 Graduate School of Science and Technology, Niigata University, Niigata 950-2181, Japan
2 Faculty of Agriculture, Niigata University, Niigata 950-2181, Japan
3 Enzyme and Protein Chemistry. Department of System Biology. The Technical University of Denmark, Skolef. Plads. Building 224. DK-2800 Kgs. Lyngby, Denmark
The three-dimensional structure of *L. brevis* GH65 maltose phosphorylase was determined (Egloff et al., 2001) and shows similarity with the $(\alpha/\alpha)_4$ barrel fold of GH15 glucomylase (EC 3.2.1.3) (Aleshin et al., 1992) (20). GH94 cellobiose phosphorylase (EC 2.4.1.20) (Hidaka et al., 2006), and GH94 chitobiose phosphorylase (EC 2.4.1.1) (Hidaka et al., 2004). GH15 and GH65 together constitute glycoside hydrolase clan L (Henrissat and Davies, 1997). The reaction mechanism of GH65 enzymes has been proposed to be similar to an inverting mechanism using a general acid/base-catalyzed direct displacement (Fig. 1A), where direct nucleophilic attack by phosphate on the glycosidic bond of the substrate is assisted by proton donation from the general acid catalyst to the glycosidic oxygen. The inverting phosphorylase catalyzed by maltose phosphorylase is reversible (Tsumuraya et al., 1990), which confers the enzyme with the capacity to effectively synthesize maltose in the reverse reaction with $\beta$-glucose 1-phosphate as donor and glucose as acceptor substrates accommodated at subsites -1 and +1, respectively. However, the acceptor specificity remains to be investigated.

Here we identified *malP* encoding a putative GH65 maltose phosphorylase using bioinformatics in a gene cluster of the genome of *Lactobacillus acidophilus* NCFM, which is a well-documented and commercially available probiotic bacterium for which the full genome has previously been sequenced and annotated (Altermann et al., 2005). Furthermore, the detailed acceptor specificity of MalP was explored in reverse Phosphorolysis using $\beta$-glucose 1-phosphate as donor and various potential carbohydrate acceptors. By applying this strategy MalP was demonstrated to catalyse the efficient synthesis of six $\alpha$-glucosidic disaccharides. Additionally we combined the reverse phosphorolysis with phosphorolysis of maltose, resulting in cost efficient synthesis of the $\alpha$-(1 $\rightarrow$ 4)-glucosidic disaccharides without addition of external $\beta$-glucose 1-phosphate (Fig. 1B).

### MATERIALS AND METHODS

#### Materials

All carbohydrates were purchased from Sigma (St. Louis, MO, USA) except for xylose (Carl Roth GmbH, Karlsruhe, Germany), xylulose, xylotriose, mannotriose, laminaribiose, and laminaritriose (Megazyme, Bray, Ireland). Other reagents were of analytical grade.

#### Sequence analysis and homology modelling of MalP

Similarity searches were performed at Swiss Institute of Bioinformatics using the BLAST network service (http://expasy.org/tools/blast/). Predictions of protein localization site and signal peptide were done by PSORT-B ver. 2.0 (http://www.psort.org/psortb/) and SignalP 3.0 server (http://www.cbs.dtu.dk/services/SignalP/), respectively. Multiple sequence alignment was performed using ClustaW (http://clustalw.ddbj.nig.ac.jp/top-jhtml). The three-dimensional structure of MalP was modelled by SWISS MODEL (http://swissmodel.expasy.org/) using the structure of *L. brevis* maltose phosphorylase (PDB: 1H54) (Egloff et al., 2001) as a template. For prediction of substrate and acceptor binding to MalP, acarbose of the complex with *Thermoanaerobacterium thermosaccharolyticum* glucomylase belonging to GH15 (PDB: 1LF9) (Aleshin et al., 2003) was superimposed onto the modelled MalP structure using DeepView/Swiss-PDB viewer V. 3.7.

#### Cloning of malP and recombinant MalP production

The *malP* was cloned by PCR from genomic DNA of *Lactobacillus acidophilus* NCFM (ATCC SD5221; Danisco USA Inc., Madison, WI, USA). Expand High Fidelity PCR System (Roche, Basel, Switzerland) was used as DNA polymerase with the following oligonucleotides constructed based on the genomic sequence (Altermann et al., 2005): 5'-GAAACCATGGGCAAAGAATTTTTGAAATTGATCCT TGG-3' as 5' forward primer containing an Ncol site (underlined) and 5'-GACCGGCACCCGCTTTAAAGCATT TAGTTTACTTCCTC-3' as 3' reverse primer containing an NotI site. The PCR product was purified by QIAquick Gel Extraction Kit (QIAGEN, Germantown, MD), digested by Ncol and NotI (New England Biolabs, Ontario, Canada), and cloned into pET28a (Novagen, Madison, WI), which was propagated in *E. coli* DH5a (Novagen), purified by QIAprep Spin Miniprep Kit (QIAGEN), and verified by sequencing (MWG Biotech Ltd, Ebersberg, Germany). An *Escherichia coli* BL21(DE3) (Novagen) transformant was grown at 12°C in

---

**Fig. 1** Reaction mechanisms of phosphorylase (A) and coupled phosphorylase/reverse phosphorylase reaction (B). The general acid catalyst of MalP was predicted to be Glu484 based on sequence alignment with *L. brevis* maltose phosphorylase. R1 and R2 indicates glucosyl and monosaccharide acceptor residue, respectively.
4 L Luria-Bertani medium (1% tryptone, 0.5% yeast extract, 1% NaCl containing 50 μg/mL kanamycin. Expression was induced by 0.1 mM isopropyl-1-thio-β-D-galactopyranoside and continued at 17 °C for 24 h. Cells were harvested by centrifugation (9000g, 10 min, 4°C), and resuspended in 40 mL BugBuster Protein Extraction Reagents (Novagen) containing 4 μL Benzonate Nuclease (Novagen). Following 30 min incubation at RT, the supernatant (centrifugation at 19000g, 15 min, 4°C) was filtered (0.45 μm; GE Infrastructure Water & Process Technologies Life Science Microseparations, Trevose, PA, USA), and applied to 5 mL HisTrap HP column (GE Healthcare UK Ltd., Buckinghamshire, England) equilibrated with 20 mM HEPES (pH 7.5) containing 0.5 M NaCl and 10 mM imidazole. After MalP was eluted by a 22-400 mM imidazole linear gradient in the same buffer, fractions containing MalP were pooled, dialyzed against 20 mM HEPES (pH 7.0), and concentrated (Centricon YM50; Millipore Corporation, Billerica, MA). The molecular mass of purified MalP was estimated by SDS-PAGE and by gel filtration (HiLoad™ 200 Superdex™ 16/60 column: flow rate 0.5 mL/min; ÄKTA explorer: GE Healthcare, Uppsala, Sweden) equilibrated with 10 mM MES (pH 6.8) containing 0.15 M NaCl and using Gel Filtration Calibration kit HMW (GE Healthcare) as standards.

**Phosphorylase**

Phosphorylase of 40 mM α-glucosidic disaccharides and maltodextrins was measured at optimum conditions (40 mM phosphate-citrate (pH 6.2), 45 °C) by quantifying liberated glucose using the glucose oxidase/peroxidase kit (Megazyme). One unit of phosphorylase was defined as the amount of enzyme that liberates 1 μmol glucose from maltose per 1 min under the condition.

**Oligosaccharide synthesis by reverse phosphorylase**

Reverse phosphorylase was performed at optimum conditions (20 mM sodium acetate (pH 3.4), 45 °C) with 50 mM β-glucose-1-phosphate and 50 mM putative carbohydrate acceptors for 60 min. The products were quantified by high-performance ion-exchange chromatography equipped with a pulsed amperometric detector (HPAEC-PAD) using a linear 0-75 mM sodium acetate gradient in 100 mM sodium hydroxide for 35 min at a flow rate of 0.35 mL/min. For structure determination the reaction mixtures were desalted using Amberlite MB20 (Sigma) and products were purified by HPLC (UltiMate 3000; Dionex, CA, USA) equipped with refractive index detector (Shodex RI-101; Showa Denko K.K., Kanagawa, Japan) and a TSKgel Amide-80 column (4.6 × 250 mm; Tosoh Bioscience, Tokyo, Japan) under a constant flow (1.0 mL/min) of mobile phase (acetonitrile/water of 75/25, v/v) at 65°C.

**Electrospray ionization mass spectrometry (ESI-MS)**

ESI-MS was performed using a LTQ XL Ion Trap MS (Thermo Scientific, San Jose CA, USA). Samples were introduced through a nano Acetta UHPLC system equipped with a Hypercarb (100 × 21 mm, 3 μm) column (Thermo) eluted with a gradient of deionised water, acetonitrile and 0.2% TFA (0.4 mL/min; 70°C). MS-detection was performed in the positive mode using a spray voltage of 4.5 kV and a capillary temperature of 260°C and auto-tuned on glycinehexose (m/z 1013).

**Nuclear Magnetic Resonance (NMR) analysis**

NMR spectra were recorded on a Bruker DRX 600 spectrometer in 5 mm NMR tubes at 300K. Relative amounts were obtained by integration of one-dimensional proton spectra. A series of 2D homo- and heteronuclear correlated spectra were obtained using Bruker standard experiments COSY, NOESY, TOCSY, I1SQC and HMQC spectra. The following parameters were used: acquisition time 0.4 s, NOESY mixing time 0.8 s, 0.12 s TOCSY spinlock, and data points 4096×128 with zero filling in F1 dimension.

**RESULTS AND DISCUSSION**

The amino acid sequence of MalP deduced from *L. acidophilus NCFM* malP (GenBank AAV43670.1) showed similarity to α-glucosidic disaccharide phosphorylase belonging to GH65, in particular to maltose phosphorylases from *Lactobacillus* species (Menendez et al., 2008; Ehrmann and Vogel, 1998). Additionally no signal peptide was found in the deduced sequence, suggesting that MalP plays a role in the maltose metabolism in the cytoplasm. The *malP* gene is located in a gene cluster in the genome of *L. acidophilus NCFM* (Altermann et al., 2005). The gene cluster contains a ATP-binding protein (MsmK, AAV43667.1), a carbohydrate binding protein (MalE, AAV43666.1), and two ABC transport system permeases (MalF, AAV43665.1; MalG, AAV43664.1). The putative ABC transporter showed 40 44% sequence identity to those of *B. subtilis* (Kamionka and Dahl, 2001; Schönert et al., 2006) for uptake of maltose and maltodextrin. This comparison suggests that maltose and maltodextrin are taken up by *L. acidophilus NCFM via the ABC transporter system. Furthermore, three genes encoding a putative neopullulanase (MalN, AAV43671.1: EC 3.2.1.135), oligo-1,6-glucosidase (MalL, AAV43672.1: EC 3.2.1.10), and acetate kinase (AckA, AAV43673.1: EC 2.7.2.1) are found to be localized downstream of the *malP*. The two glycose hydrolases (MalN and MalL) are predicted to be involved in the intracellular metabolism of maltose and maltodextrin. Additionally a *pgmB* encoding β-phosphoglucomutase (PgmB, AAV43669.1: EC 5.4.2.2) is situated upstream of the *malP*. This sequence analysis suggests the maltose and maltodextrin transported by the ABC transporter system is converted intracellularly by the glycosidases to glucose and β-glucose-1-phosphate, which can enter the glycolysis conversion to glucose 6-phosphate by the β-phosphoglucomutase. The acetate kinase (AckA) can produce pyruvic acid in the glycolysis.

Recombinant MalP was produced in *E. coli* BL21(DE3) as a His-tag fusion protein and purified by nickel-chelating chromatography system in a yield of 58 mg from 4 L culture.
MalP migrated in SDS-PAGE as a single band with an estimated size of 88 kDa. However, the molecular mass was estimated by gel filtration to 176 kDa, indicating that MalP is a dimer in solution. The purified MalP was strictly specific for maltose with a specific activity of 52 U/mg and did not phosphorolise the other α-glicosidic disaccharides [isomaltose (α-1.6), nigerose (α-1.3), kojibiose (α-1.2), and trehalose (α/l. α/I)] and maltodextrins of DP 3 to 6, indicating that malP localized in the gene cluster of L. acidophilus NCFM encodes maltose phosphorylase. In an attempt to understand why maltodextrins were not substrates for MalP, the three-dimensional structure of MalP was modelled using that of the 59% sequence identical L. brevis maltose phosphorylase (Egloff et al., 2001) as a template, and an inhibitory pseudomaltotetrasaccharide acarbose in a complex with T. thermosaccharolyticum glucoamylase of GH15 (Aleshin et al., 2003) constituting glycside hydrolase clan L together with GH65 (Henrissat and Davies, 1997) was superimposed onto the MalP modelled structure, since no structure of ligand complex has been available in GH65. In this MalP/acarbose complex, four amino acid residues [Tir335, Asp356, Glu484 (general acid catalyst), and Gln589], which are well-conserved in GH65 α-glucosidic disaccharide phosphorylases including trehalose phosphorylase (Inoue et al., 2002; Murata et al., 2002) and kojibiose phosphorylase (Yamamoto et al., 2004) (Fig. 2A), were predicted to be involved in the recognition of the glucose unit at subsite 1 (Fig. 2B). Noticeably, the loop3 (His413-Glu421) between α3 and α4 of the (α/α)₆ barrel domain of MalP appeared to block binding of maltodextrin longer than maltose (Fig. 2C), thus conforming with the strict specificity of MalP towards maltose.

Whereas MalP phosphorolysed only maltose, the acceptor specificity in reverse phosphorolysis has not been investigated so far. Therefore we investigated the detail acceptor specificity by quantifying products generated using various carbohydrate acceptors in reverse phosphorolysis (Table 1), resulting in production of six disaccharides with the

<table>
<thead>
<tr>
<th>Table 1. Acceptor specificity of MalP in reverse phosphorolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acceptor</strong></td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Monosaccharide</td>
</tr>
<tr>
<td>Glucose</td>
</tr>
<tr>
<td>Mannose</td>
</tr>
<tr>
<td>Altrose</td>
</tr>
<tr>
<td>Galactose</td>
</tr>
<tr>
<td>Talose</td>
</tr>
<tr>
<td>Gulose</td>
</tr>
<tr>
<td>Idose</td>
</tr>
<tr>
<td>Allose</td>
</tr>
<tr>
<td>Xylose</td>
</tr>
<tr>
<td>Arabinose</td>
</tr>
<tr>
<td>L-Arabinose</td>
</tr>
<tr>
<td>Ribose</td>
</tr>
<tr>
<td>Fructose</td>
</tr>
<tr>
<td>Fucose</td>
</tr>
<tr>
<td>L-Fucose</td>
</tr>
<tr>
<td>L-Rhamnose</td>
</tr>
<tr>
<td>Glucosamine</td>
</tr>
<tr>
<td>Mannosamine</td>
</tr>
<tr>
<td>Galactosamine</td>
</tr>
<tr>
<td>N-acetyl glucosamine</td>
</tr>
<tr>
<td>N-acetyl mannosamine</td>
</tr>
<tr>
<td>N-acetyl galactosamine</td>
</tr>
<tr>
<td>Sorbitol</td>
</tr>
<tr>
<td>Xyitol</td>
</tr>
</tbody>
</table>

MalP was incubated with 50 mM β-glucose 1-phosphate and 50 mM each acceptor in 20 mM sodium acetate (pH 3.4) at 45°C for 60 min. Products were quantified by HPAEC-PAD. The yields were calculated based on the β-glucose 1-phosphate (50 mM). - product was not detected.
acceptors glucose, glucosamine, N-acetyl glucosamin. L-fucose, mannose, and xylose. Thus MalP was unable to utilize sugars with axial hydroxyl at the C-4 position, i.e., galactose, talose, gulose, L-rhamnose, galacosamine, and N-acetyl galactosamin. as well as sugars with axial hydroxyl at C-3, i.e., altrose, alloze, arabinoze, and ribose. Moreover, none of the 15 disaccharides and seven trisaccharides served as acceptor in MalP catalysed reverse phosphorolysis. Single disaccharide products with each of the above six acceptors and the regioselectivity of MalP were determined by structural analysis of the products after purification by HPLC. Mass spectrometric analyses gave signals for all products, corresponding to the calculated molecular mass for sodium adducts of the acceptor conjugate of glucose. glucosamine. man nose. L-fucose. man nose. or xylose. The optimum pH was 6.2 as assessed from amounts of α-D-glucopyranosyl-(1 → 4)-βxylopyranose generated in the pH range 2.5-8.1 using 0.1 M phosphate-citrate. 0.1 M maltose and 0.3 M xylose. At optimum pH 6.2 the product yield was found to be highest (71%) in 0.2 M phosphate-citrate (the range 0.01-0.2 M was tested). Moreover, the concentration of N-acetyl glucosamin, glucosamine, mannose, L-fucose, or xylose acceptor influenced the yield of each product in 0.2 M phosphate-citrate (pH 6.2), indicating that the acceptors show different affinity at subsite +1 in reverse phosphorolysis. The optimized reaction was saturated without phosphorolysis of produced α-(1→4)-glucosidic disaccharides by extension of reaction time, resulting in 84-91% yields (Table 2). This approach using the cheap starting material maltose instead of β-glucose 1-phosphate for production of valuable α-(1 → 4)-glucosidic disaccharides can be extended to include cost efficient preparation of the other oligosaccharides by several phosphorolyses.

ACKNOWLEDGEMENT

This study was supported by the Danish Strategic Research Council’s Committee on Food and Health (FoSu, to the project "Biological Production of Dietary Fibres and Prebiotics" no. 2101-06-0067), the Center for Advanced Food Studies (LMC), the Carlsberg Foundation, and the Danish Research Council for Natural Sciences.

REFERENCES


Fig. 2 Structural elements of MalP involved in the substrate and acceptor specificities. (A) Sequence alignments of the loop 3 between α3 and α4 of the (α/α)6 barrel catalytic domain in GH65. Maltose phosphorylases: LaMP, L. acidophilus NCFM; BsMP, Bacillus sp. RK-I; LbMP, L. brevis; LcMP, L. casei, LsMP, L. sanfranciscensis, PsMP, Paenbacillus sp. SH-55. Trehalose phosphorylases: TbTP, Thermoanaerobacter brockii; GsTP, Geobacillus stearothermophilus. Kojibiose phosphorylase: TbKP, T. brockii. Trehalose hydrolase: SvTH, Streptomyces verticillus. (B) Prediction of the substrate and acceptor recognition of MalP by stereo representation of the active site superimposed acarbose, in pink, from Thermoanaerobacter thermosaccharolyticum glucoamylase (PDB: 1LF9). Hydrogen bonds are shown as black dotted lines. (C) Surface model of the MalP active site. The loop 3 (His413-Glu421) between α3 and α4 of the catalytic domain is drawn as cartoon ribbon.
Table 2. α-(1→4)-Glucosidic disaccharides produced by MalP catalysed coupled reaction of phosphorylase/reverse phosphorylase from maltose and monosaccharide acceptors

<table>
<thead>
<tr>
<th>Acceptor</th>
<th>Optimum acceptor conc. (M)a</th>
<th>Product</th>
<th>Yield (%)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-acetyl glucosamine (GlcNAc)</td>
<td>0.5</td>
<td>α-Glc-(1→4)-GlcNAcp</td>
<td>89</td>
</tr>
<tr>
<td>Glucosamine (GlcN)</td>
<td>0.1</td>
<td>α-Glc-(1→4)-GlcNp</td>
<td>91</td>
</tr>
<tr>
<td>Mannose (Man)</td>
<td>0.1</td>
<td>α-Glc-(1→4)-Manp</td>
<td>88</td>
</tr>
<tr>
<td>L-Fucose (L-Fuc)</td>
<td>1.0</td>
<td>α-Glc-(1→4)-L-Fucp</td>
<td>86</td>
</tr>
<tr>
<td>Xylose (Xyl)</td>
<td>0.5</td>
<td>α-Glc-(1→4)-Xylp</td>
<td>84</td>
</tr>
</tbody>
</table>

a The most efficient concentration of monosaccharide acceptor was determined by HPAEC-PAD monitoring of each product formation from 0.1 M maltose and 0.1-1 M acceptors in 0.2 M phosphate-citrate (pH 6.2) at 45°C for 60 min.
b The yields were calculated based on the maltose concentration (0.1 M).


Tsumuraya, Y., C.F. Brewer, E.J. Hehre. 1990. Substrate-

乳酸菌由来マルトースホスホリラーゼを用いたα-(1→4)-グルコン二糖の高収率生産

中井博之12*・仁平高則2・Birte Svensson3・大坪研究12

(平成23年7月6日受付)

要約

Lactobacillus acidophilus NCFMのゲノム内に、マルトース/マルトデキストリン代謝に関与する遺伝子クラスターを見出した。このクラスターには、グルコースハイドロレーゼファミリー-65に属する機能未知な糖質亜基酵素（MalP）をコードする遺伝子が含まれている。そこで大腸菌を宿主として組み換え酵素を生産し、その酵素の分離と活性測定を行った結果、MalPはマルトースに特異的に作用する糖質亜基酵素であることが明らかになった。加えてMalPが触媒する糖質合成反応を用いて、溶のα-(1→4)-グルコン二糖を高収率生産に成功した。

キーワード：β-グルコース、α-リノ酸、α-(1→4)-グルコン二糖、グルコースハイドロラーゼファミリー-65、マルトースホスホリラーゼ

1 新潟大学大学院自然科学研究科
2 新潟大学農学部
3 Department of System Biology, The Technical University of Denmark