Efficient Enzymatic Synthesis of α-(1→4)-glucosidic Disaccharides by Maltose Phosphorylase from *Lactobacillus acidophilus* NCFM

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Summary

A gene cluster involved in maltose/maltodextrin metabolism was identified in the genome of *Lactobacillus acidophilus* NCFM. Enzymatic properties was 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 phosphorolysis of maltose with inversion of the anomeric configuration releasing β-glucose 1-phosphate and glucose. Broad acceptor specificity was demonstrated by reverse phosphorolysis 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, 53, 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)-*N*-acetyl-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 phosphorolysis of maltose to β-glucose 1-phosphate circumvented addition of costly β-glucose 1-phosphate for reverse phosphorolysis with different monosacchaide 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.

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

Maltose, α-D-glucopyranosyl-(1→4)-D-glucopyranose, and maltodextrin, α-D-glucopyranosyl-[(1 → 4)-α-D-glucopyranosyl]$_n$-(1 → 4)-D-glucopyranose, metabolisms have been widely investigated in both Gram-negative bacteria (Boos and Shuman, 1998; Dippel and Boos, 2005) and Gram-positive bacteria (Kamionka and Dahl, 2001; Nilsson and Rådström, 2001; Andersson and Rådström, 2002; Schönert *et al*., 2006; Shelburne *et al*., 2007; Shelburne *et al*., 2008). The maltose/maltodextrin regulon in *Escherichia coli* consists of 10 genes encoding four glycoside hydrolases [amylomaltase (EC 2.4.1.25), maltodextrin phosphorylase (EC 2.4.1.1), maltodextrin glucosidase (EC 3.2.1.20), periplasmic α-amylase (EC 3.2.1.1)] involved in intracellular metabolism together with an ATP-binding cassette (ABC) transporter (Boos and Shuman, 1998; Dippel and Boos, 2005). In *Bacillus subtilis* maltose/maltodextrin is transported by a phosphoenol-pyruvate-dependent phosphotransferase system (PTS) including NAD(H)-dependent 6-phospho-α-glucosidase (EC 3.2.1.122) (Thompson *et al*., 1998; Kamionka and Dahl, 2001; Schönert *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 phosphorolysis 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 [D-glucopyranosyl-(α1 → α1)-D-glucopyranose] phosphorylase (EC 2.4.1.64) (Inoue *et al*., 2002; Murata *et al*., 2002), kojibiose [α-D-glucopyranosyl-(1→2)-D-glucopyranose] phosphorylase (EC 2.4.1.230) (Yamamoto *et al*., 2004), and α , α-trehalase (EC 3.2.1.28) (Galm *et al*., 2008)

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(http://www.cazy.org/CAZY). The three-dimensional structure of *L. brevis* GH65 maltose phosphorylase was determined (Egloff *et al*., 2001) and shows similarity with the $(\alpha/\alpha)_6$ barrel fold of GH15 glucoamylase (EC 3.2.1.3) (Aleshin *et al*., 1992) [20], GH94 cellobiose phosphorylase (EC 2.4.1.20) (Hidata *et al*., 2006), and GH94 chitobiose phosphorylase (EC 2.4.1.-) (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 phosphorolysis 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 β-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 β-glucose 1-phosphate as donor and various potential carbohydrate acceptors. By applying this strategy MalP was demonstrated to catalyse the efficient synthesis of six α-glucosidic disaccharides. Additionally we combined the reverse phosphorolysis with phosphorolysis of maltose, resulting in cost efficient synthesis of the α-(1 → 4)-glucosidic disaccharides without addition of external β-glucose 1-phosphate (Fig. 1B).

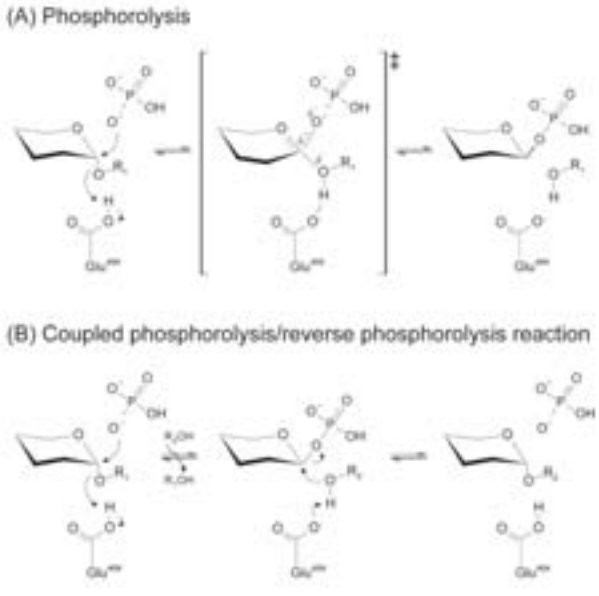

Fig.1 Reaction mechanisms of phosphorolysis (A) and coupled phosphorolysis/reverse phosphorolysis reaction (B). The general acid catalyst of MalP was predicted to be Glu484 based on sequence alignment with *L.* brevis maltose phosphorylase. R_1 and R_2 indicates glucosyl and monosaccharide acceptor residue, respectively.

MATERIALS AND METHODS

Materials

All carbohydrates were purchased from Sigma (St. Louis, MO, USA) except for xylose (Carl Roth Gmbh, Karlsruhe, Germany), xylobiose, xylotriose, mannobiose, 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 Bioinfomatics using the BLAST network service (http://expasy.org/tools/blast/). Predictions of protein localization site and signal peptide were done by PSRT-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 ClustalW (http://clustalw.ddbj.nig.ac.jp/top-j.html). 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* glucoamylase belonging to GH15 (PDB: 1LF9) (Aleshin *et al*., 2003) was superimposed onto the modelled MalP structure using Deep view/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′-AAACCATGGGCAAACGAATTTTTGAAATTGATCCTTGG-3′ as 5′ forward primer containing an NcoI site (underlined) and 5′- AAAGCGGCCGCAGCCTTTAAGCATTTAGTTTTACCTTCC-3′ as 3′ reverse primer containing an NotI site. The PCR product was purified by QIAquick Gel Extraction Kit (QIAGEN, Germantown, MD), digested by NcoI 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, Ebsersberg, Germany). An *Escherichia coli* BL21(DE3) (Novagen) transformant was grown at 12℃ in

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-galactopyranoside and continued at 12 ℃ for 24 h. Cells were harvested by centrifugation (9000g, 10 min, 4℃), and resuspended in 40 mL BugBuster Protein Extraction Reagents (Novagen) containing 4 μL Benzonate Nuclease (Novagen). Following 30 min incubation at R.T., the supernatant (centrifugation at 19000g, 15 min, 4℃) was filtrated (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 (Centriprep 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.

Phosphorolysis

Phosphorolysis of 40 mM α-glucosidic disaccharides and maltodextrins was measured at optimum conditions [40 mM phosphate-citrate (pH 6.2), 45 ℃] 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 comdition.

Oligosaccharide synthesis by reverse phosphorolysis

Reverse phosphorolysis was performed at optimum conditions [20 mM sodium acetate (pH 3.4), 45℃] 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 300; 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℃.

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 Thermo Accela UHPLC system equipped with a Hypercarb (100 × 2.1 mm, 3 μm) column (Thermo) eluted with a gradient of deionised water, acetonitrile and 0.2% TFA (0.4 mL/min; 70℃). MS-detection was performed in the positive mode using a spray voltage of 4.5 kV and a capillary temperature of 260℃ and auto-tuned on glucohexaose (*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, HSQC and HMBC 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*512 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 (Monedero *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* 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 glycoside 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 through 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 phosphorolyse the other α-glucosidic disaccharides [isomaltose (α-1,6), nigerose (α-1,3), kojibiose (α-1,2), and trehalose (α1, α1)] and maltodextrins of DP 3 6, indicating the *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 glycoside 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 [Trp355, Asp356, Glu484 (general acid catalyst), and Gln589], which are well-conserved in GH65 α-glucosidic disaccharide phoshorylases 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 $(\alpha/\alpha)_6$ 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 1. Acceptor specificity of MalP in reverse phosphorolysis

Acceptor	Yield (%)	Acceptor	Yield (%)
Monosaccharide		**Disaccharide**	
Glucose	99	Cellobiose	−
Mannose	93	Sophorose	−
Altrose	−	Laminaribiose	−
Galactose	−	Gentibiose	−
Talose	−	Lactose	−
Gulose	−	Lactulose	−
Idose	−	Melibiose	−
Allose	−	Xylobiose	−
Xylose	81	Mannobiose	−
Arabinose	−	Trehalose	−
L-Arabinose	−	Maltose	−
Ribose	−	Isomaltose	−
Fructose	−	Sucrose	−
Fucose	−	Turanose	−
L-Fucose	13	Arabinobiose	−
L-Rhamnose	−		
Glucosamine	80	**Trisaccharide**	−
Mannosamine	−	Raffinose	−
Galactosamine	−	Isomaltotriose	−
N-acetyl glucosamine	53	Panose	−
N-acetyl mannosamine	−	Maltotriose	−
N-acetyl galactosamine	−	Xylotriose	−
Sorbitol	−	Laminaritriose	−
Xylitol	−	Arabinotriose	−

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℃ 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 glucosamine, 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, galactosamine, and *N*-acetyl galactosamine as well as sugars with axial hydroxyl at C-3, *i.e.*, altrose, allose, arabinose, 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. NMR linkage analysis based on chemical shifts of the products was done using two-dimensional NMR. The α-(1 → 4) linkages were in each case identified by long range proton-carbon tree bond correlation from the non-reducing anomeric proton to C-4 at the substitution site, and confirmed by inter NOE correlation. These results show that MalP with high regioselectivity produced the α-(1→4) linked disaccharides

α-D-glucopyranosyl-(1→4)-D-glucopyranose,

α-D-glucopyranosyl-(1→4)-D-glucosaminopyranose,

α-D-glucopyranosyl-(1→4)-*N*-acetyl-D-glucosaminopyranose,

α-D-glucopyranosyl-(1 → 4)-D-mannopyranose, α-D-glucopyranosyl-(1 → 4)-D-xylopyranose, and α-D-glucopyranosyl-(1→4)-L-fucopyranose. The acceptor specificity was explained by the modelled three-dimensional structure of MalP in complex with acarbose (Fig. 2). The quinovose unit of acarbose situated at subsite +1 suggested formation of several direct hydrogen bonds to MalP, the O-3 hydroxyl group being hydrogen bonding with Lys588 and Glu484. This modelled MalP/acarbose complex is in agreement with the observations that carbohydrates with axial hydroxyl group at C-3 are not acceptors for MalP. Noticeably, His413 and Glu415 hydrogen bond with the O-1 hydroxyl of the α-anomer of the quinovose unit at subsite +1, suggesting that MalP specifically recognizes the α-anomeric configuration of maltose. Moreover MalP has no contact with the O-2 hydroxyl at subsite +1 and a relatively large space was found in accordance with mannose, 2-glucosamine and 2-*N*-acetyl glucosamine being accommodated as acceptors at subsite +1. Additionally the loop3, His413-Glu421 between α3 and α4 of the $(\alpha / \alpha)_6$ catalytic domain, is predicted to block binding of substrates longer than maltose (Fig. 2C), thus MalP can only use monosaccharides as acceptors and hence produces disaccharides.

For large scale production of the α-(1 → 4)-glucosidic disaccharides, one-pot enzymatic procedure from maltose and the corresponding monosaccharides was developed in this study (Fig. 1B). In the coupled phosphorolysis/reverse phosphorolysis reaction β-glucose 1-phosphate is initially formed by Phosphorolysis, and then consumed by reverse phosphorolysis. This advantageous coupled reaction was firstly demonstrated in 0.1 M phosphate-citrate (pH 6.2) from 0.1 M maltose and 0.3 M *N*-acetyl glucosamine, glucosamine, mannose, L-fucose, or xylose. The optimum pH was 6.2 as assessed from amounts of α-D-glucopyranosyl-(1 → 4)-D-xylopyranose 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 glucosamine, 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 phosphorylases.

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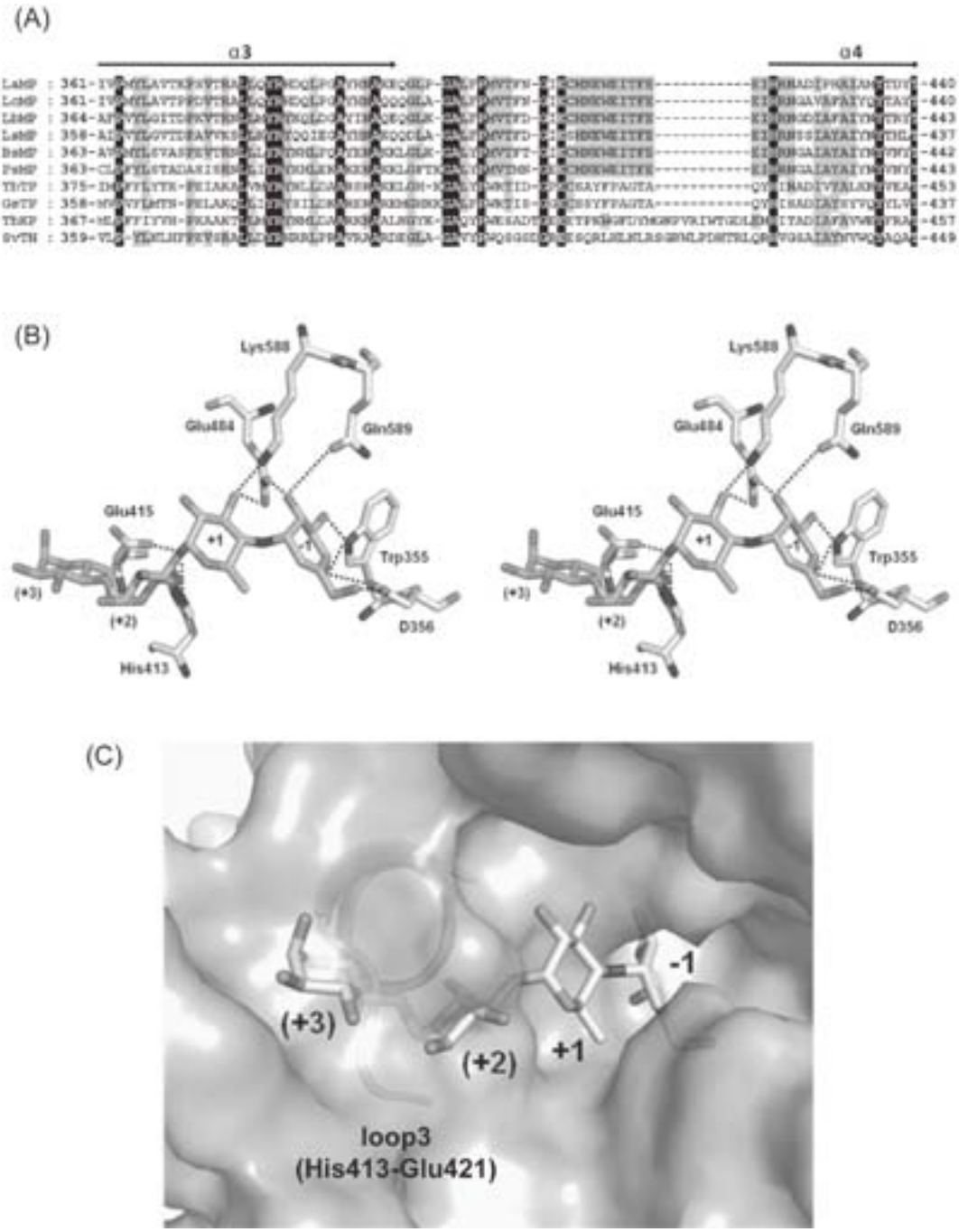

Fig. 2 Structural elements of MalP involved in the substrate and acceptor specificities. (A) Sequence alignments of the loop 3 between $\alpha 3$ and $\alpha 4$ of the $(\alpha/\alpha)_6$ barrel catalytic domain in GH65. Maltose phosphorylases: LaMP, *L. acidophilus* NCFM; BsMP, *Bacillus* sp. RK-1; LbMP, *L. brevis*; LcMP, *L. casei*, LsMP, *L. sanfranciscensis*, PsMP, *Paenibacillus* 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 *Thermoanaerobacterium 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 $\alpha 3$ and $\alpha 4$ of the catalytic domain is drawn as cartoon ribbon.

Table 2. α-(1→4)-Glucosidic disaccharides produced by MalP catalysed coupled reaction of phosphorolysis/reverse phosphorolysis from maltose and monosaccharide acceptors

Acceptor	Optimum acceptor conc. (M)[a]	Product	Yield (%)[b]
N-acetyl glucosamine (GlcNAc)	0.5	α-Glcp-(1→4)-GlcNAcp	89
Glucosamine (GlcN)	0.1	α-Glcp-(1→4)-GlcNp	91
Mannose (Man)	0.1	α-Glcp-(1→4)-Manp	88
L-Fucose (L-Fuc)	1.0	α-Glcp-(1→4)-L-Fucp	86
Xylose (Xyl)	0.5	α-Glcp-(1→4)-Xylp	84

[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℃ for 60 min.
[b] The yields were calculated based on the maltose concentration (0.1 M).

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乳酸菌由来マルトースホスホリラーゼを用いたα-(1→4)-グルコ二糖の高収率生産

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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。本クラスター中には、グルコシドハイドロレースファミリー65に属する機能未知な糖質関連酵素（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、その酵素化学的諸性質を調査した結果、MalPはマルトースに特異的に作用する糖質加リン酸分解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、6種のα-(1→4)-グルコ二糖を高収率生産に成功した。

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