Transformation of maltose into prebiotic isomaltooligosaccharides by a novel α -glucosidase from *Xantophyllomyces dendrorhous*

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1 **ABSTRACT**

2 The transglycosylation activity of a novel α -glucosidase from the basidiomycetous yeast
3 *Xanthophyllomyces dendrorhous* (formerly *Phaffia rhodozyma*) was studied using maltose as
4 glucosyl donor. The enzyme synthesized oligosaccharides with α -(1→2), α -(1→4) and α -
5 (1→6) bonds. Using 200 g/l maltose, the yield of oligosaccharides was 53.8 g/l, with prebiotic
6 oligosaccharides containing at least one α -(1→6) linkage (panose, 6-O- α -glucosyl-maltotriose
7 and 6-O- α -isomaltosyl-maltose) being the major products (47.1 g/l). The transglycosylating
8 yield was 3.6 times higher than the observed with the α -glucosidase from *Saccharomyces*
9 *cerevisiae* (53.8 vs. 14.7 g/l). Moreover, when increasing the maltose concentration up to 525
10 g/l, the maximum production of tri- and tetrasaccharides reached 167.1 g/l, without altering
11 the percentage of oligosaccharides in the mixture. Compared with other microbial α -
12 glucosidases in which the main transglycosylation product is a disaccharide, the enzyme from
13 *X. dendrorhous* yields a final product enriched in trisaccharides and tetrasaccharides.

14
15
16 **Keywords:** Glycosidase, Isomaltooligosaccharides Prebiotics, Transglycosylation, Alpha-
17 glucosidase, Oligosaccharides.

1. INTRODUCTION

Several non-digestible carbohydrates are the only prebiotic molecules known to date [1]. The two requirements of a prebiotic are that it is not hydrolyzed in the small intestine and that it is selectively fermented in the colon by certain beneficial members of the colonic microbiota (*Bifidobacterium* and *Lactobacillus* genera). There are several prebiotics in use around the world, with Japan being the leader in development and consumption. Besides the fructooligosaccharides [2] and galactooligosaccharides [3], which are commercialized in USA and Europe, Japan produces other “emerging” prebiotics (isomaltooligosaccharides, soybean oligosaccharides, lactosucrose, gentiooligosaccharides, xylooligosaccharides, to cite some) that will be likely coming soon to the market [4].

Although the synthesis of oligosaccharides *in vivo* is performed by glycosyltransferases (EC 2.4.) [5], the widely available glycosidases (glycoside hydrolases, EC 3.2.) can be also used for *in vitro* synthesis of glycosidic bonds. In order to revert their hydrolytic function towards synthesis, thermodynamic control (using high substrate concentrations) and/or kinetic control (using activated glycosyl donors) have been successfully employed [6]. Despite the broad availability of glycosidases, their synthetic applications are often limited by low yield and poor regioselectivity [7]. In this context, a group of novel, site-specifically mutated glycosidases called glycosynthases were developed [8]. A glycosynthase is a specifically-mutated retaining glycosidase in which substitution of the catalytic carboxyl nucleophile by a non-nucleophilic residue (Ala, Gly or Ser) results in an enzyme that is hydrolytically inactive but yet able to catalyse the transglycosylation reaction. Although oligosaccharide yields can reach 95-98% in some cases [6], the need of activated glycosyl fluoride donors limits their practical application.

Amylolytic enzymes are extensively used in biotechnology, as they have important applications in both the food and the pharmaceutical industries. Among them, α -glucosidases (α -D-glucoside glucohydrolases, EC 3.2.1.20) are exo-glycosidases that catalyze the release of glucose from the non-reducing end of short oligosaccharides [9-11]. On the basis of

1 aminoacid sequence homology, α -glucosidases are distributed in two groups of glycoside
2 hydrolases, i.e. GH-family 13 and 31 [12]. The former enzyme group strongly recognizes the
3 α -glucosyl-moiety in heterogeneous substrates, such as synthetic α -glucosides and sucrose.
4 The members of GH-family-31 are specific for the maltosyl-structure of maltooligosaccharides
5 rather than for the α -glucosyl-structure of heterogeneous substrates [13].

6 Interestingly enough, some α -glucosidases exhibit transglycosylation activity, e.g.
7 those from *Aspergillus niger* [14], *Bacillus stearothermophilus* [15], *Saccharomyces*
8 *cerevisiae* [16] or brewer's yeast [15]. This transglycosylation activity is being applied to
9 produce prebiotic isomaltooligosaccharides [17;18] or to glucosylate compounds such as L-
10 menthol [15], pyridoxine [19] and hydroquinone [20]. The basidiomycetous yeast
11 *Xanthophyllomyces dendrorhous* (formerly *Phaffia rhodozyma*) was isolated from deciduous
12 trees [21], and it produces astaxanthin (3-3'-dihydroxi- β,β -carotene-4,4-dione) as the main
13 carotenoide [22]. Its amylolytic activity has been also reported, and we recently purified and
14 characterized a novel α -glucosidase from *X. dendrorhous*, which releases glucose from
15 maltooligosaccharides and soluble starch [23].

16 In this work, we have studied the transglycosylation activity of *X. dendrorhous* α -
17 glucosidase [24]. Its synthetic activity was compared with that of the α -glucosidase from
18 another yeast, namely *Saccharomyces cerevisiae*.

2. EXPERIMENTAL PROCEDURES

Materials

The α -glucosidase from *Saccharomyces cerevisiae* was from Roche. Glucose ($\geq 99.5\%$), maltose monohydrate (99%), *p*-nitrophenyl- α -D-maltoside (PNP α Malt) ($\geq 98\%$), kojibiose ($\geq 95\%$), panose ($\geq 98\%$), maltotriose ($\geq 95\%$), maltotetraose ($\geq 96\%$) and 6-O- α -glucosyl-maltotriose ($\geq 96\%$) were from Sigma. Sucrose was from Merck. All other reagents and solvents were of the highest available purity and used as purchased.

Enzyme purification and quantification

The *X. dendrorhous* α -glucosidase was obtained from 1 litre of culture filtrates prepared as previously described [23]. The filtrate was concentrated using a VivaFlow 50 tangential flow filtration module (Vivascience, Sartorius Group) with 30,000 MWCO polyethersulfone (PES) membrane, and dialyzed against 20 mM sodium phosphate buffer pH 7.0 (buffer A). It was then applied to a DEAE-Sephacel column (10 ml) equilibrated with buffer A. The protein was eluted with a 0 to 0.5 M NaCl gradient at a flow rate of 1 ml/min. Active fractions (2.5 ml) eluting at approx. 0.05 M NaCl were pooled, dialyzed against 20 mM sodium acetate pH 4.5 (buffer B), and applied to the DEAE-Sephacel column equilibrated with buffer B. The proteins were eluted with a 0 to 0.2 M NaCl gradient, active fractions (2.5 ml) eluting at 0.2 M NaCl. These fractions were pooled, dialyzed against water and stored at $-70\text{ }^{\circ}\text{C}$ until use. Silver- and Coomassie Blue-stained SDS/PAGE of the samples confirmed the enzyme purification. Protein concentration was determined with the Bio-Rad microprotein determination assay, following the manufacturer's specifications, with bovine serum albumin as standard.

Standard activity microassay

The enzymatic activity towards *p*-nitrophenyl- α -D-maltoside (PNP α Malt) was measured following *p*-nitrophenol release at 410 nm from 72 mM substrate in 0.2 M sodium acetate

1 buffer (pH 5.6) in a final reaction volume of 100 μ l and using a microplate reader (Versamax,
2 Molecular Devices). The molar absorption coefficient (ϵ) of *p*-nitrophenol at 410 nm (pH 5.6)
3 was determined to be 8620 M⁻¹ cm⁻¹. One unit (U) of activity was defined as that
4 corresponding to the hydrolysis of 1 μ mol of PNP α Malt per min.

6 **Production of isomaltooligosaccharides**

7 The reaction mixture (1 ml) consisted of 900 μ l of maltose solution in 0.2 M sodium acetate
8 buffer pH 5.6 (to reach a final maltose concentration of 200 g/l or 525 g/l) and 100 μ l enzyme
9 (to reach a final activity of 0.02 U/ml and 2.5 U/ml for *X. dendrorhous* and *S. cerevisiae* α -
10 glucosidases, respectively, measured in the hydrolysis of PNP α Malt). The mixtures were
11 incubated at 45 °C in an orbital shaker (Vortemp) at 200 rpm. At different times, 40 μ l aliquots
12 were removed from the reaction mixture, diluted with 160 μ l water and incubated 5 min at 95
13 °C to inactivate the enzyme. Samples were centrifuged 5 min at 6000 rpm using an eppendorf
14 with a 0.45 μ m Durapore® membrane (Millipore), and analyzed by HPLC.

16 **HPLC analysis**

17 The concentration of the different products was analyzed by HPLC with a quaternary pump
18 (Delta 600, Waters) coupled to a Lichrosorb-NH2 column (4.6 x 250 mm) (Merck). The
19 column temperature was kept constant at 25 °C. Detection was performed using an
20 evaporative light-scattering detector DDL-31 (Eurosep) equilibrated at 85 °C.
21 Acetonitrile:water 85:15 (v/v), conditioned with helium, was used as mobile phase (flow rate
22 0.9 ml/min) for 8 min. Then, a gradient from this mobile phase to acetonitrile:water 75:25 (v/v)
23 was performed in 2 min, and this eluent was maintained during 7 min. Finally, a gradient from
24 this composition to acetonitrile:water 70:30 (v/v) was performed in 5 min, and maintained for
25 20 min. The data obtained were analyzed using the Millennium Software.

3. RESULTS AND DISCUSSION

Transglycosylation activity of *X. dendrorhous* α -glucosidase

In a previous study, the α -glucosidase from the yeast *X. dendrorhous* exhibited a notable hydrolytic activity towards maltooligosaccharides and soluble starch, with maltose as the best substrate [23]. No hydrolytic activity was detected towards α -(1 $\rightarrow$ 6)-glycosidic bonds (e.g. isomaltose and isomaltotriose) or α -glucosyl groups present in heterogeneous substrates (e.g. sucrose). Some α -glucosidases only exhibit hydrolytic activity [25], whereas others also show transglycosylating activity towards sugar acceptors [26] or non-glycosylated molecules [27]. For that reason, the transglycosylating activity of *X. dendrorhous* α -glucosidase was tested using maltose, the common glucosyl donor.

The transglycosylation activity was investigated with 200 g/l maltose at the optimal pH (5.5) and temperature (45 °C). Fig. 1 shows the progress of the reaction based on HPLC chromatograms. To identify the components of the mixture, the retention time of each peak was compared with those of glucosaccharide standards. Two trisaccharides (maltotriose, peak 5; panose, peak 6) were initially formed; however, the latter was resistant to hydrolysis as it contained an α -(1 $\rightarrow$ 6) bond. Two tetrasaccharides (6-O- α -glucosyl-maltotriose, peak 7; 6-O- α -isomaltosyl-maltose, peak 8), which contained mixed α -(1 $\rightarrow$ 4) and α -(1 $\rightarrow$ 6) bonds, were obtained by α -(1 $\rightarrow$ 6) glucosylation of maltotriose and panose, respectively. The amount of maltotetraose formed was negligible throughout the process. In addition, two disaccharides, kojibiose and isomaltose (peaks 3 and 4) –formed when the glucose acts as acceptor– were detected at the final stages of the reaction. Kojibiose contains an α -(1 $\rightarrow$ 2) glucosidic linkage and it is characterized by its low cariogenicity and digestibility [28].

-----Insert Fig. 1-----

Table 1 summarizes the carbohydrates present in the reaction mixture throughout the process. It is worth mentioning that the synthetic specificity of many glycosidases may differ

1 substantially from the specificity of hydrolysis [29]. Thus, various α -glucosidases –whose
2 function in nature is the hydrolysis of α -(1→4) bonds– transfer glucosyl groups to the less-
3 hindered, primary 6-OH of the acceptor, yielding products such as isomaltose and panose
4 [30]. In addition, transfer to secondary hydroxyl groups (2-OH, 3-OH, 4-OH) has been
5 reported [31]. As a result, a mixture of oligosaccharides consisting of α -(1→2), α -(1→3),
6 α -(1→4) and/or α -(1→6) bonds is usually obtained [9;15]. Our results indicate that *X.*
7 *dendrorhous* α -glucosidase is able to transfer glucosyl moieties to the 2-OH, 4-OH and 6-OH
8 of a glucose residue.

9 ----- Insert Table 1 -----
10

11 Fig. 2 illustrates the kinetics of oligosaccharide formation. Maltotriose concentration
12 reached a maximum of 16.2 g/l at 100 h, after which it was progressively hydrolyzed. The
13 maximum yield of tri- and tetrasaccharides (the kinetic maximum) with the *X. dendrorhous* α -
14 glucosidase was 53.8 g/l, which corresponded to 26.9% (w/w) of the total amount of
15 carbohydrates in the mixture. Most of them (47.1 g/l) contained α -(1→6) bonds, which are
16 responsible of the prebiotic properties of the products. The enrichment of the mixture in
17 oligosaccharides with α -(1→6) bonds is a consequence of their higher resistance to
18 hydrolysis, as the enzyme is highly α -(1→4) specific for cleavage of glycosidic bonds. In
19 addition, the formation of new disaccharides accounted for 30.6 g/l (15.3% referred to the
20 total weight of carbohydrates in the sample).

21 -----Insert Fig. 2 -----
22

23 Regarding the degree of polymerization (d.p.), it has been reported that an enzyme
24 having a poor ability to hydrolyze a tetrasaccharide is unlikely to synthesize such molecules,
25 as the binding conditions for the enzyme-substrate complex will be the same in both reactions
26 [15]. For example, the α -glucosidase from the acidophilic archaeon *Ferroplasma acidiphilum*
27 is not able to synthesize oligosaccharides with a d.p. higher than three as its hydrolytic

1 substrate specificity is restricted to disaccharides [32]. Accordingly, the *X. dendrorhous* α -
2 glucosidase is able to hydrolyze maltodextrins and also to synthesize tetrasaccharides;
3 however, pentasaccharides were not detected under our reaction conditions.

5 ***X. dendrorhous* and *S. cerevisiae* comparative analysis**

6 The transglycosylation activity of *X. dendrorhous* α -glucosidase was compared with
7 that of commercial α -glucosidase from the yeast *S. cerevisiae*, as they are good
8 representatives of yeasts α -glucosidases with different hydrolytic activity. *X. dendrorhous* α -
9 glucosidase has a narrow hydrolysis range towards maltooligosaccharides whereas the
10 aglycon specificity of *S. cerevisiae* α -glucosidase is rather broad [14]. HPLC chromatograms
11 (data not shown) clearly indicated that the main reaction catalyzed by *S. cerevisiae* α -
12 glucosidase was the hydrolysis of maltose. In the initial stages of the reaction (0.5-2 h), a
13 peak of maltotriose appeared, but this trisaccharide was quickly hydrolyzed as it only
14 contained α -(1 $\rightarrow$ 4) bonds. At the end-time of the reaction, only two transglycosylation
15 products were present, isomaltose and panose, formed, respectively, by α -(1 $\rightarrow$ 6)
16 glucosylation of glucose and maltose.

17 Fig. 3 illustrates the kinetics of the process with *S. cerevisiae* α -glucosidase. The
18 reaction rate towards maltose was much faster than with *X. dendrorhous* enzyme. This was a
19 consequence of the higher volumetric activity of the *S. cerevisiae* working solution (24.9 vs.
20 0.20 U/ml towards synthetic *p*-nitrophenyl- α -D-maltoside). However, the specific activity of *X.*
21 *dendrorhous* α -glucosidase was higher (33.0 vs. 1.1 U/mg protein), but the low protein
22 concentration in the extract (0.007 mg/ml) resulted in only a total of 0.02 enzyme units for the
23 transglycosylation experiments compared with 2.5 enzyme units with the *S. cerevisiae*
24 enzyme. It is worth mentioning that the maximum yield of oligosaccharides shows no
25 dependency with the enzyme concentration in the mixture, but it is obtained for a longer or
26 shorter time of reaction [33]. The amount of enzyme added must be a compromise to reach
27 the maximum transglycosylation rate in a relatively short time, without fast hydrolysis of the

transglycosylation products to facilitate stopping the reaction at the kinetic maximum.

-----Insert Fig. 3 -----

As shown in Fig. 3, the maltotriose concentration reached a maximum of 13.7 g/l, after which the trisaccharide was completely hydrolyzed. The *S. cerevisiae* α -glucosidase yielded a maximum of 14.7 g/l of tri- and tetrasaccharides, corresponding to a 7.4% (w/w) of the total carbohydrates in the mixture. In contrast, isomaltose and panose were not significantly hydrolyzed throughout the process, as they contained α -(1 $\rightarrow$ 6) bonds. At the end of the process, the isomaltose concentration attained 21.4 g/l.

Effect of maltose concentration on oligosaccharide production

The synthesis of isomalto-oligosaccharides from maltose is a kinetically controlled reaction that involves a glucosyl-enzyme intermediate [34]. The nucleophiles H₂O and maltose compete for the glucosyl-enzyme intermediate [35]. When H₂O is the nucleophile, the enzyme acts as a hydrolase (releasing glucose). When maltose is the nucleophile, the enzyme acts as a glucosyltransferase. The condensation products can be also hydrolyzed by the enzyme. In fact, the reaction must be monitored and stopped at the point of maximum yield of condensation products [36]. The maximum yield of oligosaccharides depends on two parameters: the concentration of maltose and the intrinsic transferase/hydrolase ratio of the enzyme [37].

In order to improve the yield of transglycosylation products, a high maltose concentration (525 g/l) was assayed. Fig. 4 shows the progress of tri- and tetrasaccharide formation. As expected, the increase in maltose concentration caused an improvement in the yield of tri- and tetrasaccharides. The maltotriose concentration reached again a maximum, after which its rate of hydrolysis was higher than its rate of synthesis. On the contrary, the oligosaccharides that contained at least one α -(1 $\rightarrow$ 6) linkage were very resistant to hydrolysis. The maximum production of tri- and tetrasaccharides was 167.1 g/l, which

1 corresponded to 31.4% of the total weight of carbohydrates.

2 -----Insert Fig. 4 -----

3
4 These values indicated that the transglycosylation activity of *X. dendrorhous* α -
5 glucosidase was not significantly maltose-dependent (26.9% and 31.4% w/w tri- and
6 tetrasaccharides production at 200 and 525 g/l, respectively). Yields in kinetically controlled
7 synthesis usually range from 20 to 40% [38]. In this context, it has been reported that the ratio
8 of transglucosylating to hydrolysing activity for the α -glucosidase from *Aspergillus niger*
9 notably decreases when lowering maltose concentration [14].

10
11 -----Insert Table 2 -----

12
13 Table 2 summarizes the carbohydrate composition (in weight) of the mixture at
14 different times. The maximum percentage of trisaccharides was 20% at 200 g/l and 28.1% at
15 525 g/l maltose. In contrast, the tetrasaccharide production reached maxima of 7.4% and
16 3.6% at 200 and 525 g/l, respectively. However, the values presented in Table 2 suggest that
17 the tetrasaccharide production could be further increased, in detriment of trisaccharide yield,
18 by just allowing to progress the reaction for an extended period. The higher tetrasaccharide
19 production found at 200 g/l maltose may be explained considering that, at lower maltose
20 concentration, there is more competition between trisaccharides and maltose to accept a
21 glucosyl moiety, thus favouring the formation of high polymerization degree products [39;40].

22 The *X. dendrorhous* α -glucosidase shows a different transglycosylation pattern in
23 terms of yield and regioselectivity. Thus, the *Aspergillus niger* and *A. nidulans* α -glucosidases
24 give isomaltose as the main transglycosylating product using maltose as substrate [41]. Thus,
25 a maltose concentration of 30% (w/v) is used for *A. niger* α -glucosidase to achieve an
26 isomaltose content of 30% in the final reaction product [14]. Campa et al. [42] showed that
27 with this enzyme two trisaccharides were formed, one of them (panose) was especially fast

1 hydrolyzed and the second (isomaltotriose) presented a notably lower concentration than that
2 of isomaltose throughout the process. With the *A. nidulans* α -glucosidase B, approximately
3 50% of maltose was converted to transglycosylation products, 60% was found to be
4 isomaltose [9]. The α -glucosidase from *Acremonium* sp. synthesizes oligosaccharides
5 containing α -1,2-glucosydic bonds [43], whereas the *Paecilomyces lilacinus* enzyme forms
6 both α -1,2 and α -1,3 bonds [31]. Interestingly, a novel α -glucosidase from *Chaetomium*
7 *thermophilum* var. *coprophilum* converts 5% (w/v) maltose into trehalose and
8 maltooligosaccharides [26]. The pattern of transglycosylation products derived from maltose
9 with the α -glucosidase from *Geobacillus* sp. appeared to be different from the
10 oligosaccharides obtained with *A. niger* and *A. nidulans* enzymes, especially regarding the
11 formation of tetrasaccharides [27].

12 In conclusion, the *X. dendrorhous* α -glucosidase gives a notable yield of total
13 transglycosylating products (approx. 40% considering the di-, tri- and tetrasaccharides
14 formed). Interestingly, the transferase to hydrolase ratio of this enzyme shows low
15 dependency with maltose concentration. In addition, the mixtures obtained with *X.*
16 *dendrorhous* α -glucosidase are enriched in tri- and tetrasachharides, which contrasts with
17 other α -glucosidases that synthesize basically disaccharides.

18 19 **ACKNOWLEDGEMENTS**

20 Projects BIO2004-03773-C04-01 and BIO2004-03773-C04-03 from Spanish Ministry of
21 Education and Science, and a grant from Genoma España –the National Foundation for
22 Promoting Genomics and Proteomics– supported this research. We thank CSIC and
23 Universidad Autonoma de Madrid (UAM) for research fellowships. D.L. was supported by a
24 Spanish FPU fellowship from the Spanish Ministry of Education and Science.

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Table 1. Carbohydrates present in the transglycosylation reactions catalyzed by the different α -glucosidases.

Peak number	No. of Glc moieties	Name	Formula
1	1	Glucose	Glc
2	2	Maltose	α -D-Glc-(1 $\rightarrow$ 4)- α -D-Glc
3	2	Kojibiose	α -D-Glc-(1 $\rightarrow$ 2)- α -D-Glc
4	2	Isomaltose	α -D-Glc-(1 $\rightarrow$ 6)- α -D-Glc
5	3	Maltotriose	α -D-Glc-(1 $\rightarrow$ 4)- α -D-Glc-(1 $\rightarrow$ 4)- α -D-Glc
6	3	Panose	α -D-Glc-(1 $\rightarrow$ 6)- α -D-Glc-(1 $\rightarrow$ 4)- α -D-Glc
7	4	6-O- α -glucosyl-maltotriose	α -D-Glc-(1 $\rightarrow$ 6)- α -D-Glc-(1 $\rightarrow$ 4)- α -D-Glc-(1 $\rightarrow$ 4)- α -D-Glc
8	4	6-O- α -isomaltosyl-maltose	α -D-Glc-(1 $\rightarrow$ 6)- α -D-Glc-(1 $\rightarrow$ 6)- α -D-Glc-(1 $\rightarrow$ 4)- α -D-Glc

Table 2. Carbohydrate composition (% w/w) of the reaction mixture using *X. dendrorhous* α -glucosidase and two initial maltose concentrations ^a.

Maltose 525 g/l					
Reaction time (h)	Maltose (%)	Glucose (%)	Other disaccharides ^b (%)	Trisaccharides (%)	Tetrasaccharides (%)
0	100	0	0	0	0
24	88.8	4.3	0	6.8	0
48	73.5	10.7	0	15.8	0
120	50.9	24.2	0	24.2	0.7
145	48.5	23.2	0	25.6	2.7
174	42.8	25.8	0	28.1	3.3
320	26.6	34.8	13.3	21.7	3.6

Maltose 200 g/l					
Reaction time (h)	Maltose (%)	Glucose (%)	Other disaccharides ^b (%)	Trisaccharides (%)	Tetrasaccharides (%)
0	100	0	0	0	0
70.5	73.6	12.0	0	13.7	0.6
94	57.3	21.4	0	19.7	1.6
168	36.7	33.3	7.5	18.2	4.3
262	19.6	43.4	11.2	20.0	5.7
286	19.0	43.6	11.9	18.2	7.3
306	16.8	41.0	15.3	19.5	7.4

^a Experimental conditions: 0.02 U/ml, 0.2 M sodium acetate buffer, pH 5.6, 45 °C.

^b Kojibiose and isomaltose.

Figure Legends

Fig. 1. HPLC progress of the transglucosylation of maltose catalyzed by *X. dendrorhous* α -glucosidase (reaction times are indicated). Conditions: 200 g/l maltose in 0.2 M sodium acetate buffer (pH 5.6), 0.02 enzyme units (33 U/mg protein), 40 °C. HPLC conditions are described in experimental section (peak assignation according to Table 1).

Fig. 2. Oligosaccharides production from maltose catalyzed by *X. dendrorhous* α -glucosidase. Experimental conditions: 200 g/l maltose, 0.02 enzyme units (33 U/mg protein), 0.2 M sodium acetate buffer, pH 5.6, 45 °C.

Fig. 3. Oligosaccharides production from maltose catalyzed by *S. cerevisiae* α -glucosidase. Experimental conditions: 200 g/l maltose, 2.5 enzyme units (1.1 U/mg protein), 0.2 M sodium acetate buffer, pH 5.6, 45 °C.

Fig. 4. Kinetics of tri- and tetrasaccharide production from maltose catalyzed by *X. dendrorhous* α -glucosidase using 525 g/l maltose. Experimental conditions: 0.2 M sodium acetate buffer, 0.02 enzyme units (33 U/mg protein), pH 5.6, 45 °C.

Fig. 1

Fig. 2

Fig. 3

Fig. 4