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Oligosaccharide synthesized by glucosyltransferase activity from Pectinex ultra SP-L enzyme preparation --Manuscript Draft--

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Abstract:	Glycosyltransferase activity of Pectinex ultra SP-L (from <i>Aspergillus aculeatus</i>) commercially available enzyme preparation was studied in mono- and bisubstrate systems using different donors (maltose, lactose, sucrose) and acceptors (fructose, galactose, glucose, maltose, mannose, xylose, lactose, sucrose). Oligosaccharides consisted of three monomers were detected in the cases of maltose, sucrose and lactose as monosubstrate, thus this preparation should contain glucosyl-, fructosyl- and galactosyl-transferase activity. Generally, yields of oligosaccharides synthesized were higher (about on bisubstrate systems maltose:sucrose, maltose:lactose and sucrose:lactose) than on monosubstrates. Use of maltose:sucrose bisubstrate resulted new oligosaccharide(s) (fructosyl-maltose or glucosyl-sucrose). The optimal ratio of substrates in the case of maltose:sucrose was determined to be 1:9. Increase of the dry content of the reaction mixture induced the transfer reaction. The highest oligosaccharide content was obtained at 60 (w/v)% substrate concentration. In the case of the optimal ratio and dry content, 4.02 (w/v)% oligosaccharide (DP3) concentration was achieved. These oligosaccharides may alter prebiotic and biochemical properties in food applications.

OLIGOSACCHARIDE SYNTHETIZED BY GLUCOSYLTRANSFERASE ACTIVITY FROM
PECTNEX ULTA SP-L ENZYME PREPARATION

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Short running title: Production and evaluation of oligosaccharides

Glycosyltransferase activity of Pectinex ultra SP-L (from *Aspergillus aculeatus*) commercially available enzyme preparation was studied in mono- and bisubstrate systems using different donors (maltose, lactose, sucrose) and acceptors (fructose, galactose, glucose, maltose, mannose, xylose, lactose, sucrose). Oligosaccharides consisted of three monomers were detected in the cases of maltose, sucrose and lactose as monosubstrate, thus this preparation should contain glucosyl-, fructosyl- and galactosyl-transferase activity. Generally, yields of oligosaccharides synthesized were higher (about on bisubstrate systems maltose:sucrose, maltose:lactose and sucrose:lactose) than on monosubstrates. Use of maltose:sucrose bisubstrate resulted new oligosaccharide(s) (fructosyl-maltose or glucosyl-sucrose). The optimal ratio of substrates in the case of maltose:sucrose was determined to be 1:9. Increase of the dry content of the reaction mixture induced the transfer reaction. The highest oligosaccharide content was obtained at 60

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(w/v)% substrate concentration. In the case of the optimal ratio and dry content, 4.02 (w/v)% oligosaccharide (DP₃) concentration was achieved. These oligosaccharides may alter prebiotic and biochemical properties in food applications.

Keywords: glycosyltransferase, oligosaccharides, Pectinex ultra, maltooligosaccharide, fructooligosaccharide, galactooligosaccharide

Oligosaccharides are a major class of naturally occurring carbohydrates consisting of 3 to 10 monosaccharides. Oligosaccharides with biological background have attracted high interest both scientifically and industrially over the past years. Bioactive oligosaccharides play an important role in molecular recognition events such as immune recognition, cell-cell communication and initiation of microbial pathogenesis. Although there are some oligosaccharides, which have received particular attention because of their favourable features, being low in calories and non-cariogenic, and acting as selective energy sources for beneficial microorganisms in the intestinal flora (SHETTY et al., 2006; MUSSATTO & MANCILHA, 2007; HOMANN & SEIBEL, 2009, ANTOŠOVÁ & POLAKOVIČ, 2001). These facts consequently have stimulated an increase of demand for sufficient quantities of oligosaccharides from natural and synthetic sources. However, the isolation of oligosaccharides from natural sources is extremely difficult due to their structural complexity. Therefore, access to pure oligosaccharides for research, fully relies on the chemical and enzymatic synthesis of oligosaccharides (WEIJERS et al., 2008). Traditionally, oligosaccharides are synthesized by chemical methods. Though well developed, chemical methods of synthesis are complicated and involve a number of steps. The method needs expensive chemicals, yields are low, and scale up is difficult, which make their adaptation to

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4 industrial scale impossible. Their application is currently limited to synthesis of some bioactive
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6 oligosaccharides for research purposes (SHETTY et al., 2007).
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9 Hence, the enzyme-catalyzed synthesis of oligosaccharides represents an interesting
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11 alternative allowing the control of both the regioselectivity and the stereochemistry of bond
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13 formation (PERUGINO et al., 2004), wide range of reaction conditions and the possibility of
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15 immobilization. Two main types of enzymes are used to catalyze oligosaccharide synthesis:
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17 hydrolases (glycosidases: EC 3.2) and transferases (glycosyl-transferases (glycosyl-transferases:
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19 EC 2.4). Most of these enzymes are of microbial origin (MONSAN & PAUL, 1995, MORACCI et
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21 al., 2001; NGUYEN et al., 1999).
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26 Glycosyl-transferases catalyze the transfer of a monosaccharide from a donor substrate
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28 (usually a carbohydrate donor) to an acceptor substrate, thereby forming glycosidic bonds. These
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30 enzymes exhibit strict regiospecificity and stereospecificity, and can transfer with either retention or
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32 inversion of configuration at the anomeric carbon of the monosaccharide (ANTOŠOVÁ&
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34 POLAKOVIČ, 2001, SHETTY et al., 2006). The enzymes are classified on the basis of the type of
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36 donor that is utilized by the enzyme and the position and stereochemistry of transfer to the
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38 acceptor (MONSAN & PAUL, 1995). Glycosidases are able to catalyze either direct coupling of
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40 glycosyl moieties by simple reversion of the hydrolysis reaction (reverse hydrolysis), or the
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42 transfer of glycosyl residue from an activated donor onto an acceptor (transglycosylation). This
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44 enzymes are synthetically attractive because these enzymes are widely available, robust and
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46 generally tolerant of organic solvents (DRUECKHAMMER et al., 1991; PALCIC, 1999). Several
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48 gluco- and fructo-oligosaccharides with high industrial interests are already marketed. However,
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50 in the last some years, the interest of new types or tailor-made oligosaccharides with specific
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52 sizes and containing specific glycopyranosyl-residues has become to front (HOMANN & SEIBEL,
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2009). In this study, glycosyltransferase activity of Pectinex ultra enzyme preparation was investigated in mind to produce new types of oligosaccharides.

1. Materials and methods

1.1. Chemicals

Pectinex ultra SP-L commercial enzyme preparation from *Aspergillus aculeatus* containing β -galactosidase and fructosyl-transferase was purchased from Sigma-Aldrich. Carbohydrates; maltose, lactose, sucrose, glucose, fructose were from Van Waters & Rogers. pH was adjusted with Soerensen buffer. All other chemicals are analytical grade and purchased either from Sigma-Aldrich or Reanal (Hungary).

1.2. Assay of enzyme activity

Hydrolytic activities of Pectinex ultra SP-L were assayed by the determination of glucose liberated by thereaction. The enzyme preparation was investigated by incubating 40 μ l, with 2 ml, 20 (w/v)% substrate solution (in Soerensen buffer pH 5.5), at 60 °C for up to 30 min. Samples (100 μ l) were taken at every 5 min and boiled for 10 minutes for stop the reaction. For the assay of β -galactosidase activity lactose and for the assay of β -fructofuranosidase activity sucrose was applied as substrate. The glucose released was measured by HPLC method. One enzyme unit was defined as the liberating 1 μ mol of glucose per minute.

1.3. Bioconversion

Oligosaccharide synthesis was investigated at 60 °C and pH 5.5 in a tube containing 5 ml of substrate solution. The tubes were kept in thermal bath. The reaction was starting by adding of 0.5 U/ml β -galactosidase or 0.01U/ml β -fructofuranosidase activity enzyme to the substrate

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4 solutions. Samples were taken at time intervals and boiled for 10 minutes for stop the reaction
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6 before analysis. The effect of ratio of substrates was monitored in range of 1:9- 9:1. The initial
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8 substrate concentration ranged from 10-70 (w/v) %.

11 12 *1.4. HPLC analysis*

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15 The respective saccharide concentrations in the sample solution were determined using Thermo
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17 Scientific Corporation Surveyor HPLC system. The system is consisted of Surveyor pump with 4
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19 channel, Surveyor automatic injector, Surveyor RI detector and Aminex HPX-87H column.
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21 Parameters of measurement: time of running was 25 minutes, eluent was 0,005 N sulphuric acid,
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23 injected volume (3-25 µl) depended of carbohydrate-concentration of samples, the temperature
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25 of the column and detector was 45 °C, the flow was 0.5 ml/min. Both internal and external
26
27 standards were prepared using various sugars such as glucose, fructose, saccharose, lactose,
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29 maltose, kestose, nystose, raffinose, stachyose, maltotriose, maltoteraose etc.

30 31 32 *1.5. TLC analysis*

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35 For qualitative separation of sugars, TLC silica gel 60 pre-coated aluminum plates (20*20 cm)
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37 was used as stationary phase. Mobile phase was chloroform/acetic acid/water (30/35/5). The
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39 staining solution consist of 3 (w/v)%N-(1-Naphtyl)-ethylen-di-amin-di-hydrochloride, 5 (V/V)%
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41 H₂SO₄, 94.7 (V/V)% methanol. 2 µl of diluted sample (0.1 - 0.5 (w/v)%) was put on plates. The
42
43 running time was 2 hours. Retention factors (R_F) of sugars: fructose-0.39; glucose-0.33; sucrose-
44
45 0.29; maltose-0.25; lactose-0.17.

46 47 48 *1.6. Statistical analysis*

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50 All data are presented as the mean of triplicate experimental runs. One-way analysis of variance
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52 (ANOVA), unpaired and paired Student's t-tests were done using Statistica V9.0 software
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package (StatSoft, USA). Generally, only $p < 0.05$ was accepted as the statistical significance level.

2. Results and discussion

2.1. Glycosyltransferase activity of *Pectinex ultra* on different bisubstrate systems

Based on our preliminary results, the commercially available Pectinex ultra SP-L enzyme preparation did not shown any activities on monosaccharide as substrate, thus glycosyltransferase activity of it was investigated using different combination of mono- and disaccharides (fructose, galactose, glucose, maltose, mannose, xylose, lactose, sucrose) as acceptor and sucrose or lactose as donor, respectively. Control experiments were carried out using only sucrose or lactose (Figure 1). In these systems, sucrose and lactose are acted as donor and acceptor in same time. Bioconversion using the combination of sucrose with maltose and lactose resulted higher trisaccharides (DP₃) than control sample (only sucrose). Several authors (TANRISEVEN & ASLAN, 2005; CSANÁDI & SISAK, 2006; GHAZI et al., 2007) have reported that Pectinex ultra has high fructosyltransferase activity and can be applied to produce different FOS using sucrose as substrate. In the case of presence of maltose 0,34 (w/v)% (about 22% higher) concentration of DP₃ was detected. Due to similar dry content of saccharides was applied (no decrease in water activity), it can be explained by fructosyltransferase activity of β -fructofuranosidase in Pectinex ultra SP-L that was able to transfer fructose molecule from sucrose to maltose synthetizing fructosylmaltose in reaction medium. GHAZI and co-workers (2007) investigated substrate specificity of fructosyltransferase (purified from Pectinex ultra SP-L) from *A. aculeatus* and found that this enzyme is able to carry fructose residue from sucrose to

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4 raffinose (DP₃, galactooligosaccharide), 1-kestose (DP₃) and nystose (DP₄). All these sugars
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6 contain a β (2-1)-linked fructose in their chemical structure, thus it may play a role as a signal for
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8 recognition of substrate. Turanose, cellobiose, melibiose, leucrose, methyl- α -D-glucopyranoside
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10 and stachyose were not substrates of this enzyme (GHAZI et al., 2007). From this point of view,
11
12 maltose should not be substrate of fructosyltransferase from Pectinex ultra SP-L. According to
13
14 specification this enzyme preparation contains mainly pectintranseliminase, polygalacturonase
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16 and pectinesterase, as well as small amounts of hemicellulases and cellulases, thus it may contain
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18 β -glucosidase. Moreover, some authors also have reported β -galactosidase activity of this
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20 enzyme preparation (ASLAN & TANRISEVEN, 2007; RODRIGEZ-FERNANDEZ et al., 2011).
21
22 Interestingly, in the case of the combination of sucrose and lactose DP₃ concentration (0,65
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24 w/v%) was higher than sucrose (0,28 (w/v) %, 233 %), but less than lactose (1,42 (w/v)%, 45 %)
25
26 controls. Transgalactosyl activity of this enzyme preparation was studied RODRIGEZ-FERNANDEZ
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28 and co-workers (2011) using lactose and lactulose as substrate. They found that the formation of
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30 trisaccharides was more favored when using lactulose as substrate. In our case, lower
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32 concentration (only 0,85 (w/v) %, 59 %) of DP₃ was detected in the case of maltose and lactose as
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34 bisubstrate comparing with lactose.
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44 TLC analysis of different combinations of substrates was carried out and results are
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46 demonstrated in Figure 2. Both fructosyl- and galactosyltransferase activities were confirmed
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48 where concentration of oligosaccharides increased significantly after 4 days of bioconversion. In
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50 the case of sucrose, it was seen that all amount of fructose were successfully transferred to
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52 sucrose synthesizing oligosaccharides. Interestingly, small amounts of oligosaccharides were
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54 detected in the case of maltose as substrate while releasing glucose to the reaction medium. This
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56 may due to maltase or glucosidase activities that are not reported yet until now. In case of
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4 bisubstrate systems, only combination of maltose and sucrose resulted a new oligosaccharide,
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6 which has retention about $R_F=0.03$ different from retention of malto-oligosaccharide ($R_F:0.15$) and
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8 of fructo-oligosaccharide ($R_F:0.08$). According to HPLC analysis, nature of this oligosaccharide
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10 may be trisaccharide, thus it can be speculated that the maltose was an acceptor in
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12 fructosyltransfer or sucrose was acceptor in glucosyltransfer reaction. Maltosyl-fructose was
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14 produced by levansucrase from *Bacillus subtilis* using combination of maltose and sucrose
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16 bisubstrate (CANEDO et al. 1998). Glycosyltransferase of glucansucrase from *Streptococcus*
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18 *oralis* and fructosyltransferase of levansucrase from *Bacillus subtilis* and *Bacillus megaterium*
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20 were engineered by HELLMUTH and co-workers (2008) as well as HOMANN and co-workers
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22 (2007) and BEINE and co-workers (2008) aiming production of tailor-made sugar analogues. Our
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24 results showed that invertase in Pectinex ultra SP-L may be able to transfer fructose molecule
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26 from sucrose to maltose through mechanism in the case of levansucrase, or β -glucosidase can
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28 able to do glucosyltransfer like the glucansucrase. Intensive research works are carried out in our
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30 Lab to understand these activities.

31 32 33 34 35 36 37 38 39 2.2. Effects of ratio of sucrose to maltose on glycosyltransferase activity

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41 Different ratios of maltose to sucrose in constant of dry content were investigated. Results are
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43 demonstrated in Figure 3. Contents of oligosaccharide were varied 0.31 (w/v) % to 0.61 (w/v) %.
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45 Lowest oligosaccharide content (0.31 w/v%) was determined in the case of maltose
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47 monosubstrate and highest in the case of combination of 10 % maltose and 90 % sucrose. This
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49 value was higher than in control samples, which contain only sucrose or maltose. In the case of
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51 sucrose monosubstrate, 0.49 (w/v)% oligosaccharide was synthesized. Our optimal ratio (M1:S9)
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53 is in agreement with result published by Canedo and co-workers (1999), when optimization of
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55 ratios of substrates for production of maltosyl-fructose using levansucrase. Other combination
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4 with 40 % maltose and 60 % sucrose was also given a good result (0.53 w/v %). Generally, yield
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6 of oligosaccharides can be enhanced by optimized the ratio of donor to acceptor (SHETTY et al.,
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8 2007).
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10 11 12 2.3. Effects of substrate concentration on glycosyltransferase activity 13 14

15 For the determination of optimal substrate concentration of transfer activity (on maltose:sucrose
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17 bisubstrate system) the oligosaccharide synthesis was monitored from 20-70 (w/V)%. Based on
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19 previous results ratio 1:9 of maltose:sucrose was applied. The optimal substrate concentration is
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21 60 (w/v)% (Figure 4). In this case 4.02 (w/v)% oligosaccharide concentration was achieved on
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23 the 5th day of bioconversion. This result totally agrees with that published by HANG &
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25 WOODAMS (1995). They found that maximal yield of fructooligosaccharides by
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27 fructosyltransferase from *Aspergillus foetidus*, *Aspergillus niger* and *Aureobasidium pullulans*
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29 was reported to be in the range 55-60 (w/v)%. Generally, the higher substrate concentration
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31 results higher glycosyltransferase activity (CRITTENDEN & PLAYNE, 1996), because it may
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33 decrease the hydrolytic activities of carbohydrases. It is also worth to remark that application
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35 of 60 (v/w)% monosubstrate concentration, only 2 (w/v)% oligosaccharide was yielded (data are
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37 not shown). Oligosaccharides with higher polymerization degree were not detected in reaction
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39 mixtures.
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52 3. Conclusion 53 54

55 Pectinex ultra SP-L commercial enzyme preparation is capable to catalyze glycosyltransfer
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57 reactions in different mono- and bisubstrate systems for production of various types of
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59 oligosaccharides. This enzyme does not have only fructosyltransferase and galactosyltransferase
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activities are contains, but it also able to hydrolyze and transfer glucose molecule from maltose to maltose yielding maltooligosaccharide. In the case of maltose:sucrose bisubstrate a new type of oligosaccharide was detected that distinguishes from well-known fructooligosaccharides and maltooligosaccharides. It is should be fructosyl-maltose or glucosyl-sucrose due to nature of applied substrates. Use of the ratio of maltose:sucrose in 1:9 at 60 (w/v) % dry content results higher concentration of oligosaccharide (4 w/v%) after five day of bioconversion. Further studies are needed for understanding new types of oligosaccharides, but results gained are very promising for development of technology for synthesis of novel productsfor pharmaceutical and functional food applications.

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References

- Antošová M. & Polakovič M. (2001): Fructosyltransferases: The enzymes catalyzing production of fructooligosaccharides. *Chem. Pap.*, 55, 350-358
- Aslan Y. & Tanriseven A. (2007): Immobilization of Pectinex Ultra SP-L to produce galactooligosaccharides. *Journal of MolecularCatalysis B: Enzymatic*, 45, 73-77

- 1
2
3
4 Beine R., Moraru R., Nimtz M., Na'Amnieh S., Pawlowski A., Buchholz K. & Seibel J. (2008):
5
6 Synthesis of novel fructooligosaccharides by substrate and enzyme engineering. *J.*
7
8 *Biotechnol.*, 138, 33–41
9
- 10
11 Canedo M., Jimenez-Estrada M., Cassani J. & López-Munguía A. (1999): Production of
12
13 maltosylfructose (erlose) with levansucrase from *Bacillus subtilis*. *Biocatalysis and*
14
15 *Biotransformation*, 16, 475-485
16
17
- 18
19 Crittenden R.G. & Playne M. J. (1996): Production, properties and applications of food-grade
20
21 oligosaccharides. *Trends in Food Science & Technology*, 7, 353-361
22
23
- 24
25 Csanádi Zs. & Sisak Cs. (2006): Immobilization of pectinex ultra sp-l pectinase and its
26
27 application to production of fructooligosaccharides. *Acta Alimentaria*, 35, 205-212
28
29
- 30
31 Drueckhammer D.G., Hennen J.W., Pederson R.L., Barbas C.F., Gautheron C.M., Krach T. &
32
33 Wong C.H. (1991): Enzyme catalysis in synthetic carbohydrate chemistry. *Synthesis*, 499-
34
35 525
36
- 36
37 Ghazi I., Fernandez-Arrojo L., Garcia-Arellano H., Ferrer M., Ballestoros A. & Plou F.J. (2007):
38
39 Purification and kinetic characterization of fructosyltransferase from *Aspergillus aculeatus*.
40
41 *Journal of Biotechnology*, 128, 204-211
42
- 43
44 Hang Y.D. & Woodams E.E. (1995): Optimization of enzymatic production of fructo-
45
46 oligosaccharides from Sucrose. *Lebensm.-Wiss. u.-Technol.*, 29, 578-580
47
- 48
49 Hellmuth H., Wittrock S., Kralj S., Dijkhuizen L., Hofer B. & Seibel J. (2008): Engineering the
50
51 glucansucrase GTFR enzyme reaction and glycosidic bond specificity: toward tailor-made
52
53 polymer and oligosaccharide products. *Biochemistry*, 47, 6678–6684
54
55
56
57
58
59
60
61
62
63
64
65

- 1
2
3
4 Homann A., Biedendick R., Götze S., Jahn D. & Seibel J. (2007): Insights into polymer versus
5
6 oligosaccharide synthesis: mutagenesis and mechanistic studies of a novel levansucrase
7
8 from *Bacillus megaterium*. *Biochem J.*, 407, 189–198
9
- 10
11 Homann A. & Seibel J. (2009): Towards tailor-made oligosaccharides- chemoenzymatic
12
13 approaches by enzyme and substrate engineering. *Appl. Microbiol. Biotechnol.*, 83, 209-
14
15 216
16
17
- 18
19 Monsan P. & Paul F. (1995): Enzymatic synthesis of oligosaccharides. *FEMS Microbiology*
20
21 *Reviews*, 16, 187-192
22
- 23
24 Moracci M., Trincone A. & Rossi M. (2001): Glycosynthases: new enzymes for oligosaccharide
25
26 synthesis. *Journal of Molecular Catalysis B: Enzymatic*, 11, 155-163
27
- 28
29 Mussatto S. I., Mancilha I. M. (2007): Non-digestible oligosaccharides: A review. *Carbohydrate*
30
31 *Polymers*, 68, 587–597
32
- 33
34 Nguyen D.Q., Mattes F., Hoschke Á., Rezessy-Szabó J. & Bhat M.K. (1999): Production,
35
36 purification and identification of fructooligosaccharides produced by β -fructofuranosidase
37
38 from *Aspergillus niger* IMI 303386. *Biotechnology Letters*, 21, 183-186
39
- 40
41 Palcic M.M. (1999): Biocatalytic synthesis of oligosaccharides. *Current opinion in*
42
43 *Biotechnology*, 10, 616-624
44
- 45
46 Perugino G., Trincone A., Rossi M. & Moracci M. (2004): Oligosaccharide
47
48 synthesis by glycosynthases; *Trends in Biotechnology*, 22, 31-37
49
- 50
51 Rodrigez-Fernandez M., Cardelle-Cobas A., Villamiel M. & Banga J.R. (2011): Detailed kinetic
52
53 model describing new oligosaccharides synthesis using different β -galactosidases. *Journal*
54
55 *of Biotechnology*, 153, 116-124
56
57
58
59
60
61
62
63
64
65

- 1
2
3
4 Shetty K., Paliyath G., Pometto A.L. & Levin R.E. (2007): Enzymatic synthesis of
5
6 oligosaccharides: Progress and recent trends. *Functional Foods and Biotechnology*, CRC
7
8 Press, Taylor & Francis Group, Boca Raton U.S., 473-500
9
10
11 Tanriseven A.& Aslan Y. (2005): Immobilization of Pectinex Ultra SP-L to produce
12
13 fructooligosaccharide. *Enzyme and Microbial Technology*, 36, 550–554
14
15
16 Weijers C.A.G.M., Franssen M.C.R & Visser G.M. (2008): Glycosyltransferase-catalyzed
17
18 synthesis of bioactive oligosaccharides. *Biotechnology Advances*, 26, 436-456
19
20
21
22
23
24
25
26
27
28
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List of figures

Figure 1 Oligosaccharide yield in case of sucrose and lactose donor and different acceptormolecules on the 4th day of bioconversion. Ratio of donor and acceptor molecules was 1:1

Figure 2 TLC picture of 0 and 4th day of bioconversion using mono- and bisubstrate systems

Figure 3 Oligosaccharide content on the 1st day of enzyme reaction in case of different ratio of maltose and sucrose with 20 (w/V)% total carbohydrate concentration (M: maltose, S: sucrose)

Figure 4 Oligosaccharide synthesis in case of different substrate concentration. Ratio of maltose:sucrose was 1:9

Figure 1

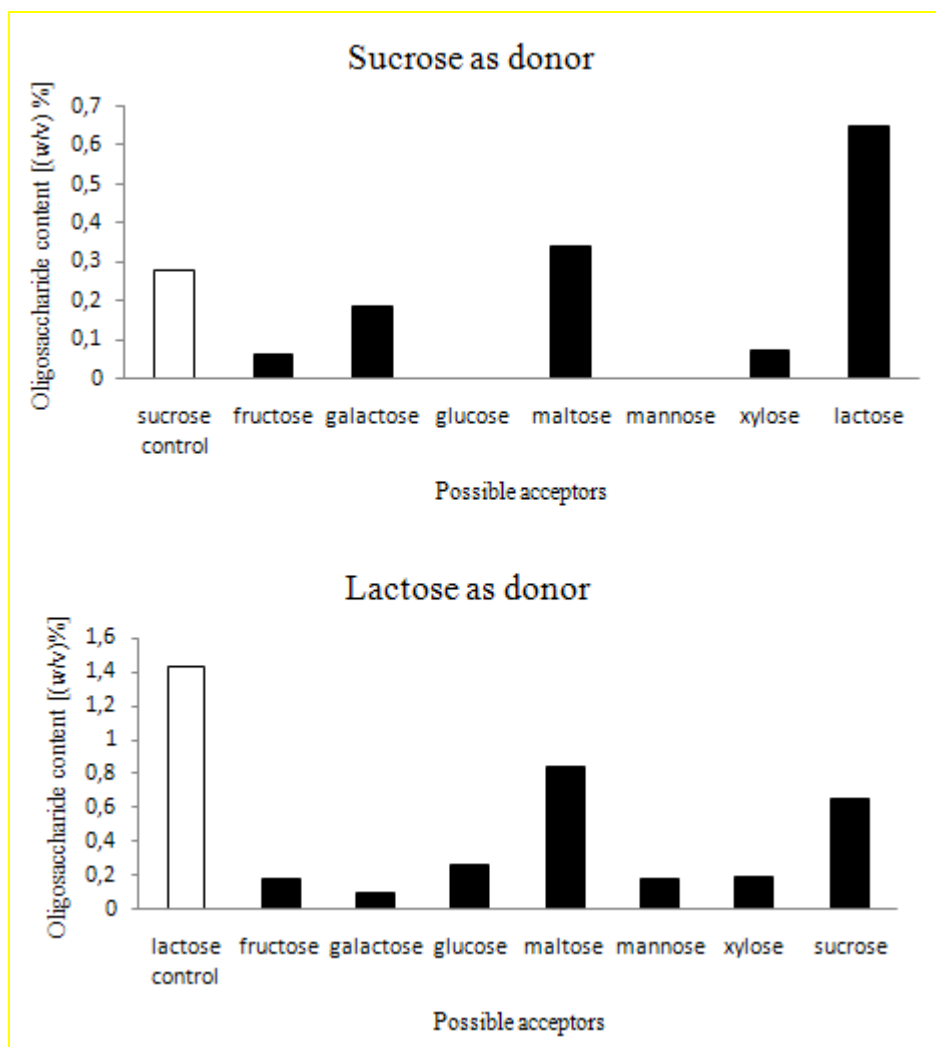


Figure 2

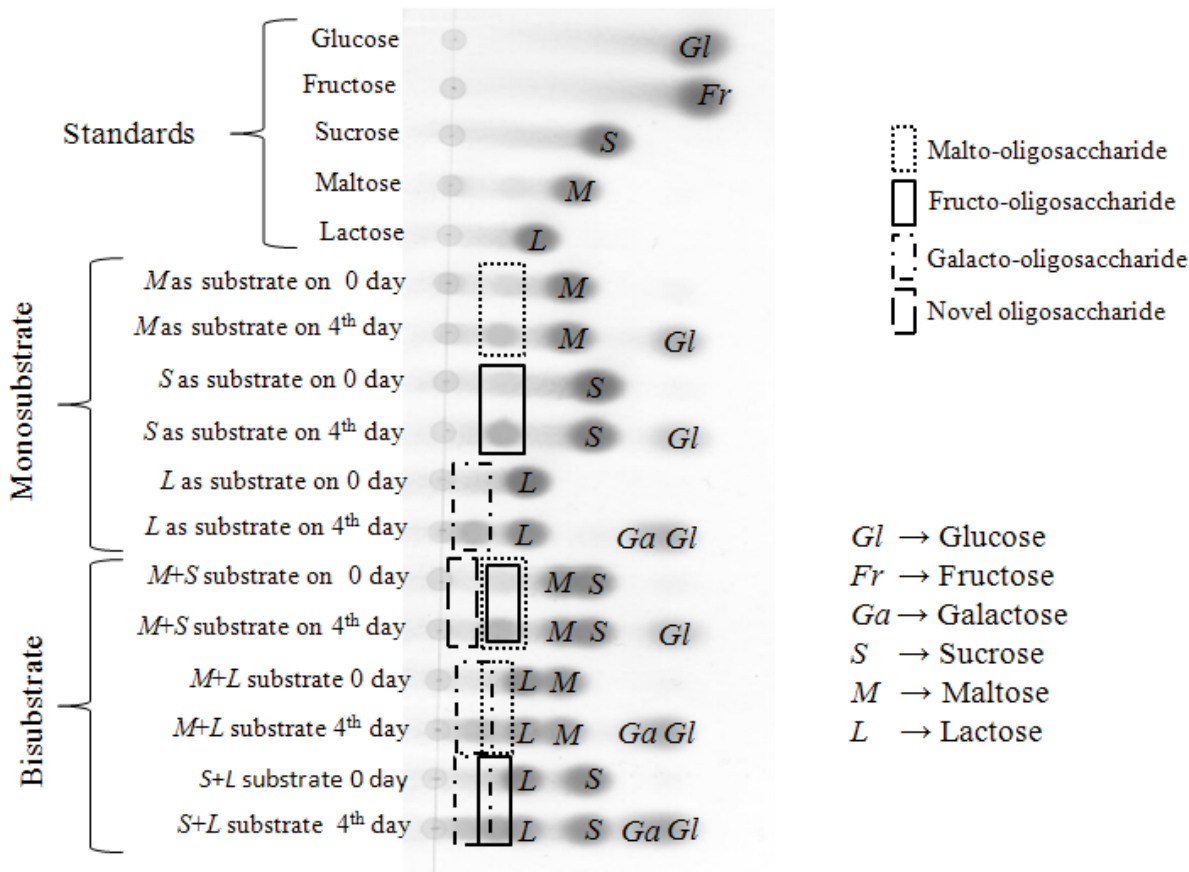


Figure 3

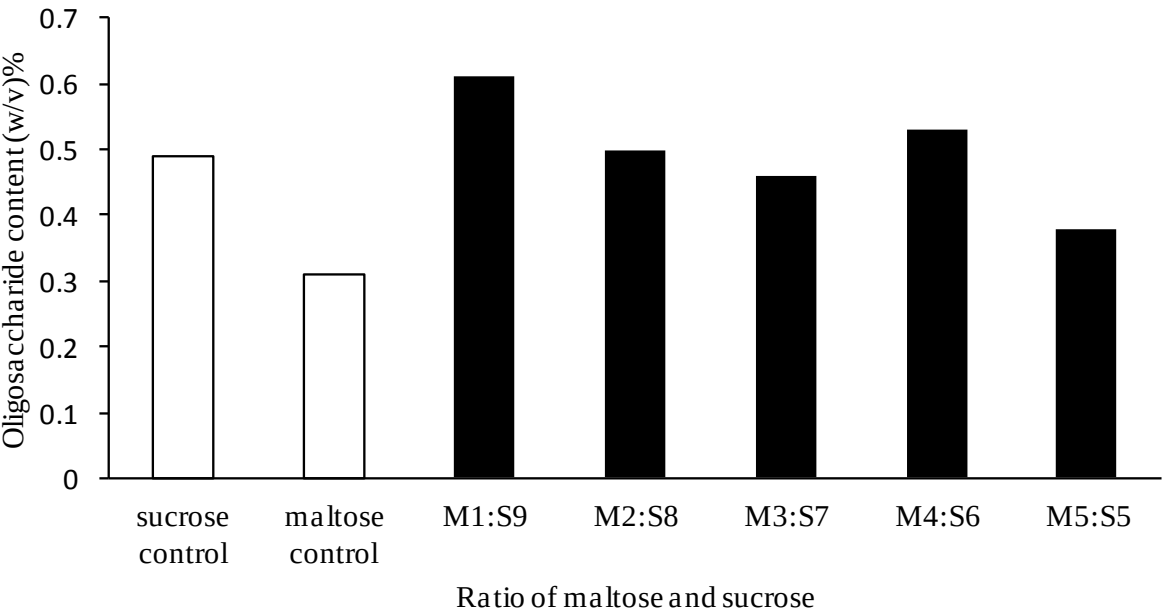


Figure 4

