Journal of Molecular Catalysis B: Enzymatic 58 (2009) 1–5

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Evaluation of the transglycosylation activities of a GH 39 β-D-xylosidase for the synthesis of xylose-based glycosides

M. Muzard [a], N. Aubry [b], R. Plantier-Royon [a], M. O'Donohue [b,1], C. Rémond [b,*]

[a] CNRS-URCA UMR 6229, Institut de Chimie Moléculaire de Reims, Université de Reims Champagne-Ardenne, Faculté des Sciences, BP 1039, F51687 Reims Cedex 2, France
[b] INRA-URCA UMR FARE 614. 8, rue Gabriel Voisin, BP 316, F51688 Reims Cedex 2, France

ARTICLE INFO

Article history:
Received 9 July 2008
Received in revised form
29 September 2008
Accepted 8 October 2008
Available online 25 October 2008

Keywords:
β-Xylosidase
Xylanase
Alkyl xylosides
Transglycosylation
Wheat bran

ABSTRACT

The ability of GH 39 β-D-xylosidase (XylBH39) from *Bacillus halodurans* to catalyze transxylosylation reactions, particularly for the production of alkyl xylosides, has been investigated using *p*-nitrophenyl (*p*NP) β-D-xylopyranoside, xylobiose and xylotriose. Furthermore wheat bran, being rich in xylo-oligosaccharides after enzymatic treatment with an endoxylanase, was used as a source of xylosyl donors. The autocondensation of *p*NP β-D-xylopyranoside was characterized by poor regioselectivity and the production of a variety of short (di- and trimers) oligosaccharides. Compared to xylobiose, xylotriose was a better donor molecule for XylBH39-mediated transxylosylation of primary alcohols presenting chain length from 1- to 5-carbon atoms. Using a wheat bran hydrolysate, generated using an endoxylanase, it was possible to perform XylBH39-mediated transxylosylation of propan-1-ol to produce propyl β-D-xylopyranoside. This enzymatic process led to synthesis of 0.9 g of propyl β-D-xylopyranoside from 20 g of destarched wheat bran.

1. Introduction

The rapid emergence of biorefinery concepts that aim to transform plant biomass into biofuels is raising a series of important questions concerning the management of renewable resources. In particular, the necessity to develop a whole crop approach and second generation fuels that will be derived from lignocellulosic material is now obvious [1–3]. However, this shift towards a more efficient use of biomass resources implies the use of sugars, other than glucose, as feedstock for the manufacture of added-value molecules. In this respect, the development of new product itineraries for plant-derived pentose sugars is a key challenge that must be met in order to achieve sustainable, economically viable biorefining.

Heteroxylans are widespread in plant biomass and can represent up to 35% dry weight of voluminous agro-industrial products such as wheat bran. In cereal fractions such as brans, cobs, straws and stover, heteroxylans are composed mainly of D-xylose and L-arabinose. The main-chain of these polymers is made up of β-1,4-linked D-xylopyranosyl units to which α-L-arabinofuranosyl substituents are attached through *O*-2 and/or *O*-3 linkages [4–7]. In biorefining processes, heteroxylans are found to be more labile than cellulose and their removal is normally achieved through a thermochemical pre-treatment of the biomass that solubilizes heteroxylans in the form of oligomeric or monomeric pentose residues.

Alkyl glycosides are non-ionic surfactants with useful applications in the food, pharmaceutical and cosmetic industries [8]. These compounds consist of a hydrophobic part that is usually an alkyl chain of variable length, and a hydrophilic part that can be a sugar moiety. Interestingly, because the alkyl moiety can be obtained from plant oils, and sugars are also of plant origin, alkyl glycosides are viewed as renewable, eco-friendly surfactants. The classical chemical synthesis of alkyl or aryl glycosides from free sugars is Fischer glycosylation [9]. However, this reaction suffers from several drawbacks, such as the use of large quantities of alcohol and aggressive reaction conditions (high temperatures, prolonged reactions times in the presence of strong mineral acids) that can lead to the decomposition of sugar groups and the generation of heterogeneous product mixtures (α and β anomers displaying variable degrees of polymerization). Alternatively, it is conceivable to use enzyme catalysis to circumvent these difficulties.

Glycoside hydrolases catalyze the formation of glycosidic linkages *via* reverse hydrolysis (thermodynamic control) or through transglycosylation (kinetic control) [10,11]. The production of hexose-based oligosaccharides and alkyl glycosides in reactions

* Corresponding author. Tel.: +33 3 26 35 53 63; fax: +33 3 26 35 53 69.
E-mail address: caroline.remond@univ-reims.fr (C. Rémond).
[1] Present address: LISBP, INSA, UMR INRA 792/CNRS 5504, 135 avenue de Rangueil, F31077 Toulouse Cedex 4, France.

1381-1177/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2008.10.004

catalyzed by glucosidases and galactosidases has been widely documented [12–17] and the enzymatic-catalyzed synthesis of pentose-based oligosaccharides and surfactants using pentose-acting enzymes such as α-L-arabinofuranosidases, β-xylosidases and xylanases is receiving more and more attention [18–26].

β-Xylosidases belong to families GH 3, 39, 43, 52 and 54 of the glycoside hydrolase classification system [27,28] (http://afmb.cnrs-mrs.fr~pedro/CAZY/db.html). Apart from GH43, these families display a reaction mechanism that involves the formation of a glycosyl–enzyme intermediate that can be decomposed either by the action of a nucleophilic water molecule (hydrolysis) or by that of another suitable nucleophile, such as an alcohol [29,30]. Therefore, enzymes from these families are appropriate candidates for the catalysis of reactions that can lead to the production of alkyl pentosides.

Previously, we reported the production and characterization of a β-xylosidase GH 39 from *Bacillus halodurans* (XylBH39) and revealed that this enzyme can catalyze transglycosylation reactions using *p*-nitrophenyl (*p*NP) β-D-xylopyranoside (*p*NPXyl*p*) or xylotriose as substrates [31]. In the present work, we have pursued this investigation, notably to establish the usefulness of this enzyme for the synthesis of various alkyl xylosides, using primary alcohols and various donors. Moreover, striving towards an application in biorefining, we have evaluated the production of propyl β-D-xylopyranoside directly from wheat bran hydrolysates that were themselves obtained using enzyme technology.

2. Materials and methods

2.1. Xylosidase XylBH39 and xylanase Tx-Xyl11

After cloning of the corresponding gene from *B. halodurans*, recombinant xylosidase XylBH39 was produced as a C-terminally (His x 6)-tagged protein in *Escherichia coli* JM109 (DE3) and purification was performed in a one step procedure from cell lysates using IMAC technology as previously described [31]. Enzymatic activity was determined by measuring the liberation at 401 nm of *p*-nitrophenol from *p*-nitrophenyl β-D-xylopyranoside for 10 min at 45 °C in 50 mM Tris–HCl buffer, pH 7.5. One unit of β-xylosidase activity was defined as the amount of enzyme releasing 1 μmol of *p*NP per minute using the defined conditions. Kinetic parameters were obtained from reactions carried out at 45 °C with XylBH39 at 0.5 and 0.85 IU/mL in presence of xylobiose (5–100 mM) and xylotriose (1–50 mM), respectively. Data were derived from Michaelis–Menten representations using the SigmaPlot 2000 software (Version 6.1) equipped with the Enzyme Kinetics Module 1.0 (SPSS Science, Paris, France). Sequence alignment of XylBH39 (GenBank accession no. BAB04787) with xylosidase GH39 from *Geobacillus stearothermophilus* (GenBank accession no. AF098273) was performed using BLAST on the NCBI server (http://www.ncbi.nlm.nih.gov).

Endo-xylanase Tx-Xyl11 was obtained and purified from *Thermobacillus xylanilyticus* using a previously established protocol [32].

2.2. Chemicals and substrates

Alcohols and *p*-nitrophenyl β-D-xylopyranoside were purchased from Sigma–Aldrich Corp. (St. Louis, MO). Destarched wheat bran was provided by ARD, Pomacle, France. Xylobiose and xylotriose were purified from wheat bran hydrolysates that were obtained by submitting destarched wheat bran (20 g/L in water) to the action of Tx-Xyl11 (10 IU/mL) during 24 h at 60 °C. Xylo-oligosaccharides were purified from enzymatic reaction as previously reported [33].

2.3. Transxylosylation reactions catalyzed by XylBH39

To characterize the synthetic abilities of XylBH39 in simple conditions, transglycosylation reactions using a variety of acceptors and donors were performed in water at 45 °C. When *p*NPXyl*p* (5 mM) was used as substrate for XylBH39 (0.1 IU/mL), the synthesis of xylo-oligosaccharides was achieved after 10 min. To evaluate transxylosylation of methanol (5%, v/v), reactions were carried out using *p*NPXyl*p*, xylobiose or xylotriose (5 mM) as donors with two different concentrations of XylBH39 (0.1 and 1 IU/mL). To investigate the influence of chain length, various alcohols (5%, v/v) were incubated for 16 h at 45 °C with 1 IU/mL of XylBH39. The synthesis of propyl xyloside using crude wheat bran hydrolysates was performed in a two-stage process. First destarched wheat bran (100 g; 20 g/L in water) was incubated with Tx-Xyl11 (10 IU/mL) during 24 h at 60 °C and then a sample of the supernatant was removed. Supernatant was placed in a boiling water bath for 15 min, centrifuged at 2367 × *g* during 20 min. In order to determine total soluble xylose content, an aliquot of supernatant was acid-hydrolysed before HPAEC-PAD [33]. Propan-1-ol (5%, v/v) and XylBH39 (1 IU/mL) were added to the clarified supernatant and the transglycosylation reaction was allowed to proceed at 45 °C during 16 h. The reaction was stopped by boiling during 10 min to denature XylBH39.

2.4. Quantification, purification and characterization of the products of transglycosylation

To qualitatively analyze transglycosylation reactions, TLC was performed using Kieselgel 60 F_{254} aluminium-backed sheets (E. Merck) and EtOAc–HOAc–water (7:2:2) or BuOH–HOAc–water (2:1:1) as the mobile phase, respectively. Detection of products was achieved at 100 °C using 0.2% (v/v) orcinol in H_2SO_4 (20%, v/v). The quantification of alkyl xylosides was performed by HPLC using a NH_2 column (Nucleosil 100-5 NH_2, 250 mm × 4.6 mm) operated in reverse phase mode. Products were eluted at 1 mL/min using a mobile phase composed of an acetonitrile/water mixture (70/30 for C1 to C5 alkyl xylosides and 95/5 for C6 to C8 alkyl xylosides). The detection of eluates was performed using a dynamic light scattering detector (PL-ELS 1000, Polymer Laboratories). Following large-scale preparative reactions, compounds PXA, PXB, PXD and alkyl xylosides were purified using gel permeation chromatography (Bio-Gel P-2, Biorad Laboratories Inc.) and water as the eluent. Alkyl xylosides were further purified using silica gel chromatography (9385 Merck Kieselgel 60, 230–400 mesh) with a EtOAc/MeOH (85/15) mobile phase. These compounds were identified by NMR (D_2O, 500 MHz for ^1H and 125 MHz for ^{13}C) and MS-ESI, and used for as standards to calibrate subsequent HPLC analyses. The previously described compounds PXA and PXB [26] were identified by ^1H NMR (D_2O, 500 MHz), whereas PXD was submitted to characterization using NMR (D_2O, 500 MHz for ^1H and 125 MHz for ^{13}C) and HRMS-ESI. ^1H and ^{13}C NMR spectra, COSY and ^1H–^{13}C correlation experiments permitted structure and signal assignments.

p-Nitrophenyl [4-*O*-β-D-xylopyranosyl-2-*O*-β-D-xylopyranosyl]-β-D-xylopyranoside (PXD). ^1H NMR (D_2O, 500 MHz): δ 8.20 (d, 2H, J = 9.0 Hz, Ar–H), 7.15 (d, 2H, J = 9.0 Hz, Ar–H), 5.34 (d, 1H, J = 6.0 Hz, H-1), 4.66 (d, 1H, J = 7.8 Hz, H-1′), 4.41 (d, 1H, J = 7.8 Hz, H-1″), 4.10 (dd, 1H, J = 4.4 Hz, J = 12.3 Hz, H-5eq), 3.90 (dd, 1H, J = 5.3 Hz, J = 11.5 Hz, H-5′eq), 3.87–3.77 (m, 3H, H-2, H-3 and H-4), 3.69 (dd, 1H, J = 5.3 Hz, J = 11.5 Hz, H-5′ax), 3.60–3.52 (m, 2H, H-4′, H-5ax), 3.47 (m, 1H, H-4″), 3.38 (t, 1H, J = 9.25 Hz, H-3″), 3.36 (t, 1H, J = 9.25 Hz, H-3′), 3.26–3.17 (m, 4H, H-2′, H-2″, H-5′ax, H-5″ax); ^{13}C NMR (D_2O, 125 MHz) δ 103.9 (C-1′), 101.8 (C-1″), 98.5 (C-1), 80.7 (C-2), 75.8 (C-4), 75.5 (C-3′), 75.2 (C-3″), 73.3 (C-2′), 72.6 (C-2″ and C-3), 69.0 (C-4″), 68.9 (C-4′), 65.1 (C-5′ and C-5″), 62.6

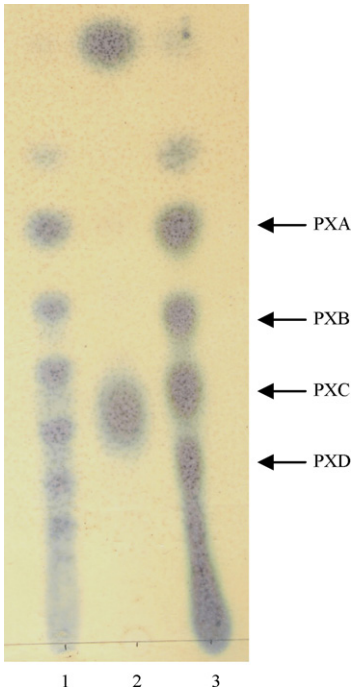


Fig. 1. TLC analysis of the xylo-oligosaccharides produced by XylBH39-mediated (0.1 IU/mL) autocondensation of *p*NPXyl*p* (5 mM) at 45 °C. Lane 1: reaction mixture obtained after 1 min of incubation; lane 2: standard mixture of xylose and *p*NPXyl*p* (1 mg/mL); lane 3: reaction mixture obtained after 10 min of incubation.

(C-5). ESI-HRMS: *m*/*z* calcd for $C_{21}H_{29}NO_{15}Na$: $[M+Na]^+$: 558.1435. Found: 558.1423.

3. Results and discussion

3.1. Autocondensation with pNP β-D-xylp

The use of *p*NP β-D-xyl*p* as substrate led to the rapid production of several products that were tentatively identified by mass spectrometry as *p*NP-derived oligosaccharides displaying

Table 1
Kinetic parameters for hydrolytic reactions catalyzed by XylBH39.

Substrate	K_M (mM)	k_{cat} (s^{-1})	k_{cat}/K_M (s^{-1} mM^{-1})
*p*NP β-D-xyl*p*[a]	8.61 ± 0.48	5.26 ± 0.78	0.60 ± 0.12
Xylobiose	80.14 ± 2.35	0.32 ± 0.01	0.0040 ± 0.0003
Xylotriose	37.27 ± 3.46	4.91 ± 0.38	0.1319 ± 0.0225

Reactions were carried out at 45 °C in 50 mM Tris–HCl buffer, pH 7.5. Data were obtained from triplicates.

[a] Data from Ref [35].

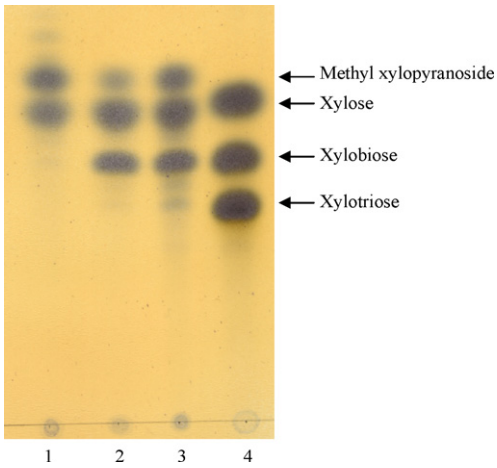
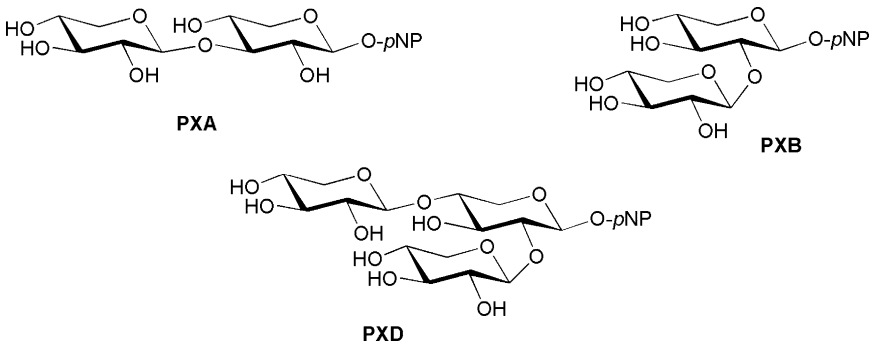


Fig. 2. TLC analysis of the products (formed after 1 h at 45 °C) of transxylosylation reactions catalyzed by XylBH39 (1 IU/mL) involving *p*NPXyl*p*, xylobiose or xylotriose (5 mM) and methanol (5%, v/v). Lane 1: with *p*NPXyl*p*; lane 2: with xylobiose; lane 3: with xylotriose; lane 4: a standard mixture of xylose, xylobiose and xylotriose (1 mg/mL).

DP values from 2 to 5. These products were clearly observable after 10 min of reaction, but then progressively disappeared, presumably due to secondary hydrolysis (data not shown). Although accurate quantification of the reaction products was not done, TLC analysis (Fig. 1) inferred that a *p*NP xylobioside regioisomer was the major product (PXB) and that a second one (PXA) and two *p*NP xylotrioside regioisomers (PXC and PXD) were also produced in significant quantities. NMR analysis to determine the exact structures of PXA, PXB and PXD confirmed the poor regioselectivity of reactions catalyzed by XylBH39 and revealed that the following *O*-glycosidic linkages had been formed: PXA: *p*NP 3-*O*-β-D-xylopyranosyl-β-D-xylopyranoside; PXB: *p*NP 2-*O*-β-D-xylopyranosyl-β-D-xylopyranoside; PXD: *p*NP [4-*O*-β-D-xylopyranosyl-2-*O*-β-D-xylopyranosyl]-β-D-xylopyranoside (Scheme 1). Unfortunately, due to difficulties associated with its purification, the structure of PXC could not be elucidated by NMR. Poor regioselective control of reactions catalyzed by glycoside hydrolases is quite common and is often related to relaxed hydrolytic specificity [34]. The β-xylosidase GH39 from *Thermoanaerobacterium saccharolyticum* produced xylobiosides and xylotriosides with either β-1,2 or β-1,3 or β-1,4 linkages [18], whereas the β-xylosidase GH3 from *Aspergillus* sp. is the only reported xylosidase that can catalyze regioselective synthesis of *p*NP *O*-β-1,4-D-xylooligosaccharides [19]. Interestingly, in reactions catalyzed by XylBH39, no *p*NP β-D-xylopyranosyl-(1,4)-β-D-xylopyranoside was accumulated, even though XylBH39 catalyzes the hydrolysis of β-1,4 linkages [31]. However, it seems



Scheme 1. Structures of the xylo-oligosaccharides obtained from XylBH39-mediated transxylosylation of *p*NPXyl*p*.

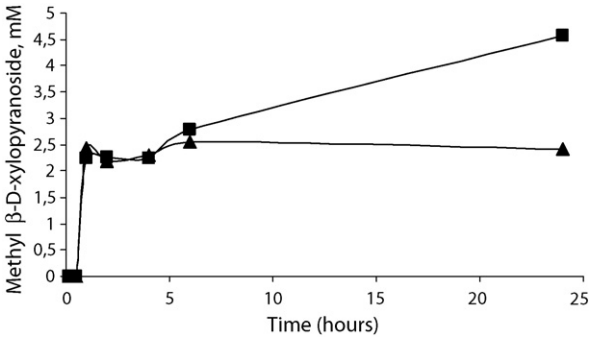


Fig. 3. Time course analysis of methyl β-D-xylopyranoside production catalyzed by XylBH39 at 45 °C with 5 mM *p*NPXyl*p* (triangles) or 5 mM xylotriose (squares) in presence of methanol (5%, v/v).

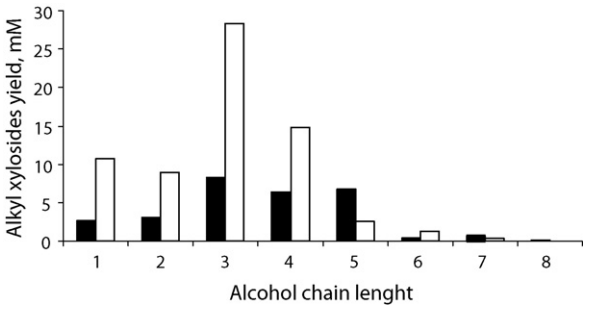


Fig. 4. Effect of alcohol chain length on the rate of production of various alkyl β-D-xylopyranosides using XylBH39 after 16 h in presence of xylotriose 5 mM (black bars) or hydrolyzate containing xylo-oligosaccharides (diluted to obtain xylotriose 5 mM) (white bars) and alcohols 5%.

likely that *p*NP 4-*O*-β-D-xylopyranosyl-β-D-xylopyranoside was rapidly synthesized and then either hydrolyzed *via* secondary hydrolysis or used as an acceptor molecule for transxylosylation, which generated PXD, a mixed linkage (β-1,4 and β-1,2 linkages) xylotrioside.

3.2. Transxylosylation with various primary alcohols

3.2.1. The influence of the donor sugar on the synthesis of methyl β-D-xylopyranoside

In the initial phase of this study, xylobiose, xylotriose and *p*NPXyl*p* were used as substrates for reactions catalyzed by XylBH39 in the presence of methanol. TLC analysis of the products, which were formed after 1 h of reaction, revealed that all three molecules were able to act as donors for transxylosylation reactions (Fig. 2). Compared to xylobiose, xylotriose was a better donor group and was completely consumed after 1 h of reaction. Kinetic parameters calculated for reactions involving xylobiose and xylotriose revealed that dissimilar values for k_{cat} and K_M translated into a 33-fold difference in catalytic efficiency (Table 1). Nevertheless, *p*NPXyl*p* was the most efficient donor group, because the catalytic efficiency of XylBH39 on this substrate was 4.5- and 150-fold higher than on xylotriose or xylobiose, respectively.

A time course analysis of the enzyme-catalyzed production of methyl D-xyloside using either *p*NPXyl*p* or xylotriose revealed that synthesis occurred at the same level during the 6 first hours of reaction (Fig. 3). In case of *p*NPXyl*p* as donor, maximum concentration of methyl D-xyloside was produced within 6 h. Approximatively 50% of the donor initially present (5 mM) were converted into methyl D-xyloside (2.4 mM). When xylotriose was used as donor, no plateau was observed and after 24 h, methyl D-xyloside concentration was 2-fold higher (4.6 mM) than maximal concentration obtained with *p*NPXyl*p*. No secondary hydrolysis of methyl β-D-xylopyranoside occurred.

Table 2
Quantitative evaluation of an enzymatic two-step process to produce propyl β-D-xylopyranoside from wheat bran and propan-1-ol.

	Quantification (g)
Wheat bran	20
Xylans	8
Hydrolysis with Tx-Xy111	
Xylo-oligosaccharides produced	3.15
Transxylosylation with Xyl BH39 and propan-1-ol	
Purified propyl β-D-xylopyranoside	0.9

3.2.2. Enzyme-mediated synthesis of alkyl β-D-xylopyranosides using xylotriose and various primary alcohols

Primary alcohols displaying chain lengths in the range C1 to C5 were tested in XylBH39-catalyzed reactions containing xylotriose (Fig. 4). These proved to be good acceptors, although the use of higher concentrations (>5%, v/v) did not improve product yields (data not shown). Maximal concentration of alkyl β-D-xyloside (8.3 mM) was obtained with propan-1-ol as acceptor. With propan-1-ol, butan-1-ol and pentan-1-ol, concentrations of alkyl β-D-xylosides were superior to the initially concentration of xylotriose (5 mM). This can be attributed to the fact that one mole of xylotriose will produce one mole each of alkyl D-xyloside and xylobiose, which in turn can be used as a donor sugar for the production of a second mole of alkyl β-D-xylopyranoside. As mentioned above, xylobiose conversion will be much slower than the one involving xylotriose, but over long periods it will have a positive impact on product yield.

Alcohols displaying longer chain lengths (C6–8) were unsuitable as acceptors. Similar results have already been obtained with other β-xylosidases [21,35], notably in the case of the GH3 β-xylosidase from *Aspergillus awamori* that showed a clear acceptor preference for propan-1-ol. Preference for shorter alkyl chains has been attributed to the presence of hydrophobic residues in the enzyme's active site. In this context, it is noteworthy that 3D struc-

```
XylBH39  74   VEPFYNFTYIDRIFDTFLELNIRPFVEIGFMPKLLASGEQTIFDWQGNVTPPKDYDQWKQ  133
XylGS39  73   MKPFYNFTYIDRIVDSYLALNIRPFIEFGFMPKALASGDQTVFYWKGNVTPPKDYNKWRD  132

XylBH39  134  LIQAVISHFIDRYGVEEVTKWPFEIWNEPNLINFWQHADKKEYFKLYKITARAIKEVHPY  193
XylGS39  133  LIVAVVSHFIERYGIEEVRTWLFEVWNEPNLVNFWKDANKQEYFKLYEVTARAVKSVDPH  192

XylBH39  194  IQVGGPAICGGSDEWITDFLQFCHKEEVPVDFVSRHAYTSAKPHKVTPDYYYQELYENTH  253
XylGS39  193  LQVGGPAICGGSDEWITDFLHFCAERRVPVDFVSRHAYTSKAPHKKTFEYYYQELEPPED  252

XylBH39  254  MLDELKSVKELIQQSPFPNLPFHITEYNTSYSPINPVHDTVLNAAYLARILSEAGDIVDS  313
XylGS39  253  MLEQFKTVRALIRQSPFPHLPLHITEYNTSYSPINPVHDTALNAAYIARILSEGGDYVDS  312
```

Fig. 5. Partial sequence alignment corresponding to the aglycon sites of XylBH39 and of GH39 xylosidase from *Geobacillus stearothermophilus* (XylGS39). Conserved residues conferring hydrophobicity are underlined in grey.

tural data for the GH39 β-xylosidase from *G. stearothermophilus* shows that the aglycon binding pocket contains several hydrophobic residues (Phe115, Leu163, Phe166 and Tyr283) [36]. Therefore, the presence of homologous residues in XylBH39 (Phe116, Leu164, Phe167 and Tyr284) might explain its substrate preference (Fig. 5).

3.3. Direct enzymatic synthesis of propyl β-D-xylopyranoside from wheat bran hydrolyzate

As previously reported, the incubation of destarched wheat bran with the endo-xylanase from *T. xylanilyticus* (Tx-Xyl11) allowed the production of a mixture of xylobiose, xylotriose and soluble arabino-xylo-oligosaccharides [37]. After clarification of this mixture and the addition of various alkyl alcohols, XylBH39 was able to catalyze the production of various alkyl D-xylopyranosides (Fig. 4). The use of propan-1-ol procured maximum product concentration (28.4 mM) and, interestingly, the crude mixture of xylo-oligosaccharides, diluted to obtain a final concentration of 5 mM xylotriose, gave better product yields than pure xylotriose used at the same concentration. This data confirms the observation already mentioned above, XylBH39 catalyzes transxylosylation preferentially with xylotriose as donor but other xylo-oligosaccharides present in wheat bran hydrolysate (xylobiose, xylo-oligosaccharides with higher degree of polymerization) could act as donor molecules.

To scale-up the enzyme-catalyzed synthesis of propyl β-D-xylopyranoside from wheat bran, a multi-gram experiment was performed and results are summarized in Table 2. After the treatment of 20 g of wheat bran with Tx-Xyl, 39% of wheat bran xylans were converted in a soluble form (xylo-oligosaccharides). After addition of propan-1-ol (5%, v/v) to the hydrolyzate containing 3.15 g of soluble xylo-oligosaccharides, XylBH39 catalyzed the synthesis of 0.9 g of pure propyl β-D-xylopyranoside, which corresponds to yield of 112 mg/g of xylan. This is lower compared to the reported synthesis of hexyl β-D-xylopyranoside using a crude mixture of a xylanase and xylosidase from *Thermotoga neopolitana* that produced 228 mg/g of xylan [38] and is better than the enzyme-mediated synthesis of octyl β-D-xylopyranoside from xylan and octan-1-ol using an endo-xylanase from *Aurebasidium pullulans*, which procured a yield of only 35 mg/g of xylan [24].

4. Conclusion

In the present study, we have sought to investigate the usefulness of XylBH39 for the synthesis of alkyl D-xylosides. Our data indicate that this enzyme could be useful for the production of short alkyl chain xylosides and that it is suitable for use in combination with xylanases for the direct synthesis of alkyl chain xylosides from arabinoxylan-rich agricultural co-products. One drawback that is underlined in this study is the poor ability of XylBH39 to use longer chain alkyl alcohols as acceptors. However, it is plausible that the attenuation through enzyme engineering of hydrophobicity in the aglycon site of XylBH39 could lead to better performances with longer chain alkyl alcohols. Protein engineering work aimed at achieving this goal is now underway.

References

[1] J.H. Clark, J. Chem. Technol. Biotechnol. 82 (2007) 603–609.
[2] B. Kamm, M. Kamm, Appl. Microbiol. Biotechnol. 64 (2004) 137–145.
[3] A.J. Ragauskas, C.K. Williams, B.H. Davison, G. Britovsek, J. Cairney, C.A. Eckert, W.J. Frederick Jr., J.P. Hallett, D.J. Leak, C.L. Liotta, J.R. Mielenz, R. Murphy, R. Templer, T. Tschaplinski, Science 311 (2006) 484–489.
[4] J.M. Brillouet, J.P. Joseleau, L.P. Utille, D. Lelievre, J. Agric. Food Chem. 30 (1982) 488–495.
[5] J.M. Brillouet, Carbohydr. Res. 159 (1987) 109–126.
[6] G.A. Adams, Can. J. Chem. 33 (1955) 56–67.
[7] J. Schmorak, C.T. Bishop, G.A. Adams, Can. J. Chem. 35 (1957) 108–114.
[8] W. von Rybinski, K. Hill, Angew. Chem. Int. Ed. 37 (1998) 1328–1345.
[9] J. Garegg Per, Adv. Carbohydr. Chem. Biochem. 59 (2004) 69–134.
[10] D.H.G. Crout, G. Vic, Curr. Opin. Chem. Biol. 2 (1998) 98–111.
[11] F. van Rantwijk, M. Woudenberg-van Oosterom, R.A. Sheldon, J. Mol. Catal. B: Enzym. 6 (1999) 511–532.
[12] A. Basso, A. Ducret, L. Gardossi, R. Lortie, Tetrahedron Lett. 43 (2002) 2005–2008.
[13] S. Das-Bradoo, I. Svensson, J. Santos, F. Plieva, B. Mattiasson, R. Hatti-Kaul, J. Biotechnol. 110 (2004) 273–285.
[14] A. Giordano, A. Tramice, G. Andreotti, E. Molo, A. Trincone, Bioorg. Med. Chem. Lett. 15 (2005) 139–143.
[15] T.-H. Park, K.-W. Choi, C.-S. Park, S.-B. Lee, H.-Y. Kang, K.-J. Shon, J.-S. Park, J. Cha, Appl. Microbiol. Biotechnol. 69 (2005) 411–422.
[16] P. Simerska, M. Kuzma, D. Monti, S. Riva, M. Mackova, V. Kren, J. Mol. Catal. B: Enzym. 39 (2006) 128–134.
[17] P. Turner, D. Svensson, P. Adlercreutz, E.N. Karlsson, J. Biotechnol. 130 (2007) 67–74.
[18] S. Armand, C. Vieille, C. Gey, A. Heyraud, G. Zeikus, B. Henrissat, Eur. J. Biochem. 236 (1996) 706–713.
[19] E.V. Eneyskaya, H. Brumer, L.V. Backinowsky, D.R. Ivanen, A.A. Kulminskaya, K.A. Shabalin, K.N. Neustroev, Carbohydr. Res. 338 (2003) 313–325.
[20] Z. Jiang, Y. Zhu, L. Li, X. Yu, I. Kusakabe, M. Kitaoka, K. Hayashi, J. Biotechnol. 114 (2004) 125–134.
[21] M. Kurakake, T. Fujii, M. Yata, T. Okazaki, T. Komaki, Biochim. Biophys. Acta 1726 (2005) 272–279.
[22] S. Matsumura, K. Sakiyama, K. Toshima, Biotechnol. Lett. 19 (1997) 1249–1253.
[23] S. Matsumura, K. Sakiyama, K. Toshima, Biotechnol. Lett. 21 (1999) 17–22.
[24] T. Nakamura, K. Toshima, S. Matsumura, Biotechnol. Lett. 22 (2000) 1183–1189.
[25] C. Rémond, M. Ferchichi, N. Aubry, R. Plantier-Royon, C. Portella, M.J. O'Donohue, Tetrahedron Lett. 43 (2002) 9653–9655.
[26] C. Rémond, R. Plantier-Royon, N. Aubry, E. Maes, C. Bliard, M.J. O'Donohue, Carbohydr. Res. 339 (2004) 2019–2025.
[27] B. Henrissat, Biochem. J. 280 (1991) 309–316.
[28] B. Henrissat, A. Bairoch, Biochem. J. 293 (1993) 781–788.
[29] C.S. Rye, S.G. Withers, Curr. Opin. Chem. Biol. 4 (2000) 573–580.
[30] S.G. Withers, Carbohydr. Polym. 44 (2001) 325–337.
[31] I. Smaali, C. Rémond, M. O'Donohue, Appl. Microbiol. Biotechnol. 73 (2006) 582–590.
[32] M. Debeire-Gosselin, M. Loonis, E. Samain, P. Debeire, in: J. Visser, G. Beldman, M.A. Kusters-van Someren, A.G. Voragen (Eds.), Xylans and Xylanases, Elsevier, Amsterdam, 1992, pp. 463–466.
[33] C. Rémond, I. Boukari, G. Chambat, M. O'Donohue, Carbohydr. Polym. 72 (2008) 424–430.
[34] S. Mala, H. Dvorakova, R. Hrabal, B. Kralova, Carbohydr. Res. 322 (1999) 209–218.
[35] H. Shinoyama, Y. Kamiyama, T. Yasui, Agric. Biol. Chem. 52 (1988) 2197–2202.
[36] M. Czjzek, A. Ben David, T. Bravman, G. Shoham, B. Henrissat, Y. Shoham, J. Mol. Biol. 353 (2005) 838–846.
[37] S. Benamrouche, D. Crônier, P. Debeire, B. Chabbert, J. Cereal Sci. 36 (2002) 253–260.
[38] A. Tramice, E. Pagnotta, I. Romano, A. Gambacorta, A. Trincone, J. Mol. Catal. B: Enzym. 47 (2007) 21–27.