Introduction

Xylans, which are the main components of hemicellulose, are the world’s second most abundant renewable resource after cellulose [10]. Xylan consists of α-1,4-linked β-D-xylopyranose units in its backbone and O-acetyl, α-L-arabinofuranosyl, α-1,2-linked glucuronic or 4-O-methylglucuronic acid side-chain groups [5]. Owing to the diversity and complexity of the chemical structure of xylans, their hydrolysis requires enzymes with different catalytic activities [3, 6]. Among these enzymes, endo-1,4-β-xylanase (EC 3.2.1.8) plays the most important role in depolymerization since it directly cleaves the backbone of xylans to produce xylose and xylo-oligosaccharides by hydrolyzing β-1,4-linked glycosidic bonds. β-Xylosidase (EC 3.2.1.37) hydrolyzes xylobiose to produce xylose, whereas the other enzymes act on the side-chain cleavage [23].

A number of xylanases have been identified and characterized from different fungi, bacteria, and archaea, but most of them are active at neutral pH and moderate temperatures. The xylanases active under temperatures above 60°C could be beneficial in many industrial processes, including pulp bleaching in the paper industry.
and saccharification of lignocellulosic biomass in biofuels production [4, 10]. Performing these processes at elevated temperatures provides low viscosity that leads to increased solubility of the reaction compounds and thus increases the overall rate of the process, as well as lowers the risk of contamination from mesophilic microorganisms [9]. In particular, the pulping process at high temperatures and alkaline conditions (pH above 8) requires the search for alkali-tolerant thermophilic xylanases without side cellulase activity, to be used in the pretreatment of cooked pulp [19, 21].

Thermophilic xylanolytic microorganisms are the apparent source of thermostable xylanases. Recently, the moderately thermophilic chemo-organotrophic bacterium *Melioribacter roseus* P3M-2 (*JCM 17771*) was isolated from a microbial mat developing on the wooden surface of a chute under the influence of hot water (46°C) coming from a 2,775-m-deep oil exploration well [13]. *M. roseus* belongs to the phylum Ignavibacteria, affiliated with a “superphylum” also comprising Chlorobi and Bacteroidetes [13]. This bacterium grows at temperatures from 35°C to 60°C and at pH values from 6.0 to 8.7, and is able to utilize various polysaccharides, including cellulose, starch, and xylan [13]. Several dozens of hydrolases, representing different families of glycoside hydrolases (GHs), were identified in the genome of *M. roseus* [7]. Among them are three putative endo-1,4-β-xylanases predicted to carry N-terminal signal peptides, indicating that these enzymes could perform extracellular hydrolysis of xylan. Genes encoding GH10 family endoxylanase Mros_2091 and GH30 family endoxylanase Mros_2090 are located within a gene cluster encoding several enzymes involved in xylan metabolism: β-xylosidases (Mros_2087-2088), endoxylanases (Mros_2090-2091), α-glucuronidase (Mros_2092), and α-N-arabinofuranosidase (Mros_2093). Another probable extracellular endo-1,4-β-xylanase of the GH10 family is encoded by the distantly located gene Mros_2495. In this study, we describe the cloning, expression, and functional characterization of three xylanases from *M. roseus*.

**Materials and Methods**

**Sequence Analysis and Similarity Searches**

Bioinformatic tools were used to explore the primary structure of endo-1,4-β-xylanases from *M. roseus*. Similarity searches by BLASTP were performed on the NCBI server (http://www.ncbi.nlm.nih.gov). The location of putative signal peptides was predicted by SignalP ver. 3.0 (http://www.cbs.dtu.dk/services/SignalP). Conservative domains were identified by BLASTP searches against the NCBI’s conserved domain database [11].

**Cloning of M. roseus Endoxylanases**

Pairs of PCR primers MROS2090F (5'-CCGGATCCCATATTCCGTAAAGCCG-3')/MROS2090R (5'-TCAAGCTTCCCATGTGTAGGCTCCCTTT-3'), MROS2091F (5'-CCGGATCCATGGTGTTCACACGGCGCT-3')/MROS2091R (5'-TCAAGCTTCCCATAGCCAAACCGGACGATA-3'), and MROS2495F (5'-CCGGATCCATTTCCCGATCCGCTTGAGGACGACATGAGCTGGTCACACGGCGCT-3')/MROS2495R (5'-CGCTTCAGGACCGGAAATTAGAAAAACAGT-3') were designed to amplify the sequences of endoxylanase genes *xyl2090* (Mros_2090), *xyl2091* (Mros_2091), and *xyl2495* (Mros_2495), respectively. The forward primers were designed to target the mature parts of the endoxylanases without sequences coding for N-terminal signal peptides. The xylanase genes were amplified by PCR; the PCR products were then digested with BamHI and HindIII (*xyl2090* and *xyl2091*) or BamHI and PstI (*xyl2495*) and inserted into pQE30 (Qiagen) at the corresponding sites, yielding the plasmids pQE30_xyl2090, pQE30_xyl2091, and pQE30_xyl2495.

**Expression and Purification of Recombinant Xylanases**

Plasmids pQE30_xyl2090, pQE30_xyl2091, and pQE30_xyl2495 were transformed into *Escherichia coli* DL1270 (DH10B derivative carrying lacI into the chromosome). Recombinant strains were grown in Luria-Bertani medium (LB) supplemented with ampicillin and induced to express recombinant xylanases by adding isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 1.0 mM at OD600 approximately 0.5, and incubated further at 30°C for 16 h. Then 15 ml of the recombinant cells was harvested by centrifugation at 3,500×g for 15 min at 4°C, washed with 20 mM Tris-HCl buffer (pH 8.0), and resuspended in 0.5 ml of 50 mM phosphate buffer (pH 7.5), 0.3 M NaCl, and 5 mM imidazole. The cell extracts after sonication were centrifuged (15,000×g, 4°C, 30 min), and the recombinant proteins were purified by metal affinity chromatography using a Ni-NTA Spin Kit (Qiagen). Upon elution from the column, the proteins (0.4 ml) were dialyzed on a Slide-A-Lyzer MINI Dialysis Unit MWCO (Thermo Scientific) against 200 ml of 25 mM phosphate buffer (pH 7.0) at 4°C for 3 h. SDS-PAGE was performed using 10.0% (w/v) acrylamide, and proteins were visualized by Coomassie Brilliant Blue R-250 staining. The concentration of the purified protein was determined by the Bradford method using BSA as a standard.

**Assay of Xylanase Activity**

The reaction mixtures contained 0.5 ml of 1.5% (w/v) birchwood xylan (Sigma) in phosphate buffer (50 mM, pH 6.5) and 0.5 ml of an appropriate dilution of enzyme (0.85 µg of Xyl2090, 0.9 µg of Xyl2091, and 0.01 µg of Xyl2495). The enzyme-substrate reaction was carried out at optimal temperature (80°C for Xyl2090, 65°C for Xyl2091, and 40°C for Xyl2495) for 30 min and the reaction was stopped by the addition of 0.5 ml of 3,5-dinitrosalicylic acid (DNSA) solution, boiled for 10 min, and then cooled on ice for color stabilization [12]. The optical absorbance was measured at 540 nm.
and the amounts of liberated reducing sugars (xylose equivalents) were estimated against a xylose standard curve. One unit of xylanase activity was defined as the amount of enzyme required to release 1 µmol of reducing sugar equivalent per minute under the assay conditions.

Biochemical Characterization of Recombinant Xylanases

A broad range of buffers (50 mM; acetate buffer (pH 4.0–5.5), phosphate buffer (pH 5.8–8.0), and Na-carbonate buffer (pH 9.0–10.8)) were used for determining the optimum pH for xylanase activity. Similarly, xylanase assay was done at various temperatures (0–95°C) to determine the optimum temperature for xylanase activity. The influence of metal ions on the activity of the xylanases was measured by including corresponding salts into the reaction mixtures followed by xylanase assay.

The thermostability of xylanase was assessed by pre-incubating the enzyme at various temperatures without the substrate. The samples were collected at the desired intervals and the residual xylanase activities determined. In addition, the stability of the enzymes in the course of xylan hydrolysis reactions performed at different temperatures was monitored by measuring the amounts of liberated reducing sugars in aliquots collected at different time intervals. Graphs displaying the amounts of liberated reducing sugars as a function of reaction time were drawn to monitor the enzyme inactivation that would result in transition of the curves from a linear to curvilinear and finally the plateau phase.

The substrate specificity of xylanase was determined by allowing the enzyme to react with 1.5% (w/v) birchwood xylan, beechwood xylan (Serva), carboxymethyl cellulose (Fluka), microcrystalline cellulose (Aldrich), locust bean gum (Sigma), guar gum (Sigma), and p-nitrophenol derivatives (0.8 mg/ml). The sugars liberated were quantified using DNSA. The amount of p-nitrophenol obtained was measured at 400 nm.

The kinetic of the enzyme was studied by measuring enzyme action on different concentrations of birchwood xylan under standard reaction conditions using the Hanes-Woolf plot.

Analysis of Hydrolysis Products

Hydrolysis of birchwood xylan (1.5% (w/v)) or X$_r$-xylan, xylotetraose, xylopentaose (0.0625% (w/v); Megazyme) was performed in phosphate buffer (50 mM, pH 6.5) at optimal temperature (80°C for Xyl2090, 65°C for Xyl2091, and 40°C for Xyl2495). The samples were then collected at different time intervals, spotted on silica gel plate (Silica gel 60, Merck), and analyzed by thin-layer chromatography against standards (xylose, xylobiose, xylotriose, xylotetraose, and xylopentaose).

Results

Sequence Analysis of *M. roseus* Xylanase Genes

Three putative endo-1,4-β-xylanases predicted to carry N-terminal signal peptides were identified in the *M. roseus* genome (Fig. 1). The first xylanase gene, xyl2090 (Mros_2090), encodes a 633 amino acids protein, predicted to comprise the Por sorting domain (TIGR04183) associated with the sorting of proteins from the periplasm to the outer membrane in *Fibrobacteres* and *Bacteroidetes*, where they are covalently attached [17]. This domain is followed by the type 9 carbohydrate binding module (cd09619) and catalytic domain of GH30 family glycoside hydrolases (pfam02055). The CBM9 domain is known to play a role in the microbial degradation of cellulose and hemicelluloses and could facilitate binding of Xyl2090 to xylan. Xyl2090 seems to have a unique domain architecture since we found no proteins in GenBank exhibiting significant amino acid sequence similarity across the whole length of Xyl2090. Through BLASTP of the GH30 catalytic domain of Xyl2090 on GenBank, it shares the highest similarity of 40–44% with different xylanases of *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*. In particular, the catalytic domain of Xyl2090 exhibited 42% sequence identity with functionally characterized glucuronoxarabinoxylan endo-1,4-β-xylanase Xyn30A of *Bacillus licheniformis* SVD1 [15]. This enzyme was active toward both 4-O-methyl-D-glucurono-D-xylan and oat-spelt xylan [15].

The second gene, xyl2091 (Mros_2091), encodes a 781 amino acids protein, predicted to comprise the carbohydrate binding domain of CBM4 family (pfam02018), glycoside hydrolase family 10 catalytic domain GH10 (pfam00331), and C-terminal Por sorting domain (Fig. 1). Binding of CBM4 has been demonstrated with xylan, β-1,3-glucan, β-
Novel Endoxylanases of Melioribacter roseus

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1,3-1,4-glucan, β-1,6-glucan, and amorphous cellulose [22]. Xyl2091 shares the highest amino acid sequence similarity of 50% along the whole length with xylanase Xyn10A from Rhodothermus marinus of Bacteroidetes. This xylanase, like Xyl2091, comprises the Por sorting domain mediating its attachment to the cell surface [8].

The third gene, xyl2495 (Mros_2495), encodes a 374 amino acids protein comprising the signal peptide and the GH10 catalytic domain (Fig. 1). Such simple domain architecture suggests that Xyl2495 is a “free” xylanase, secreted from the M. roseus cell and acting extracellularly, not being linked to the cell surface. Xyl2495 shares the highest amino acid sequence identity of 60% with the annotated endo-1,4-β-xylanase of Solibacter usitatus (Bacteroidetes), whereas 50–58% identical enzymes were found in representatives of Proteobacteria, Bacteroidetes, Verrucomicrobia, and Acidobacteria. Xyl2495 and Xyl2091 are quite dissimilar, exhibiting only 32% amino acid sequence identity at their GH10 catalytic domains.

Overall, the sequence analysis suggests that two xylanases, Xyl2090 and Xyl2191, could be covalently linked to bacterial cells, while the third xylanase, Xyl2495, could act as a free extracellular enzyme.

Cloning and Expression of Xylanase Genes

For functional analysis of the recombinant enzymes, the xylanase genes xyl2090 (Mros_2090), xyl2091 (Mros_2091), and xyl2495 (Mros_2495) were expressed in E. coli DLT1270. Since all three native genes were predicted to encode N-terminal signal peptides, gene fragments coding for mature proteins lacking these signals were cloned and expressed. The heterologous proteins were overexpressed by inducing cells with IPTG (Fig. 2). The recombinant xylanases were purified through Ni-NTA affinity chromatography. The purified proteins appeared in SDS-PAGE analysis as pure bands at molecular masses expected for the mature enzymes.

Table 1. Properties of recombinant endoxylanases from M. roseus.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Xyl2090</th>
<th>Xyl2091</th>
<th>Xyl2495</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH family</td>
<td>GH30</td>
<td>GH10</td>
<td>GH10</td>
</tr>
<tr>
<td>CBM domain</td>
<td>CBM9</td>
<td>CBM4</td>
<td>-</td>
</tr>
<tr>
<td>Mol. mass</td>
<td>68 kDa</td>
<td>86 kDa</td>
<td>42 kDa</td>
</tr>
<tr>
<td>pH optimum</td>
<td>6.5</td>
<td>6.5</td>
<td>6.5</td>
</tr>
<tr>
<td>Temp. optimum</td>
<td>80°C</td>
<td>65°C</td>
<td>40°C</td>
</tr>
<tr>
<td>Vmax (U/mg)</td>
<td>92.1</td>
<td>130.2</td>
<td>3,230.8</td>
</tr>
<tr>
<td>Km (mg/ml)</td>
<td>10.50</td>
<td>3.0</td>
<td>1.05</td>
</tr>
<tr>
<td>Specific activity against birchwood xylan (U/mg)</td>
<td>41.2</td>
<td>75.3</td>
<td>1,920.0</td>
</tr>
<tr>
<td>Specific activity against beechwood xylan (U/mg)</td>
<td>55.3</td>
<td>41.8</td>
<td>1,352.1</td>
</tr>
<tr>
<td>Products of hydrolysis of birchwood xylan</td>
<td>&gt;X5</td>
<td>X1, X2</td>
<td>X1, X2</td>
</tr>
</tbody>
</table>

X1, xylose; X2, xylobiose; >X5, xylose oligomers longer than xylopentaose.
proteins without the signal peptides but carrying N-terminal histidine tags; 68 kDa for Xyl2090, 86 kDa for Xyl2091, and 42 kDa for Xyl2495 (Fig. 2, Table 1).

**Functional Characterization of Recombinant Xylanases**

All three xylanases are active at a broad range of pH with an optimum at pH 6.5 (Fig. 3A). Xyl2090 and Xyl2495 are also active at alkaline pH (retain about 50% activity up to pH 9), whereas Xyl2091 has a more narrow pH range shifted towards acidic conditions.

The temperature profiles of three xylanases appeared drastically different (Fig. 3B). The recombinant Xyl2495 enzyme has maximum activity at 40°C and retains more than 50% of maximal activity between 10°C and 50°C. The

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**Fig. 3.** Effects of pH (A) and temperature (B) on the activity of the recombinant xylanases.

**Fig. 4.** Effects of temperature on the stability of the recombinant xylanases. (A) Purified xylanase was pre-incubated at various temperatures (0–90°C) before the xylanase assay. (B) Xylanase assays were run at different temperatures and the liberated reducing sugars were measured in aliquots collected at different time points.
Table 2. Effects of metal ions on the activity of the recombinant xylanases.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Xyl2090</th>
<th>Xyl2091</th>
<th>Xyl2495</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>2 mM</td>
<td>10 mM</td>
<td>2 mM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Hg&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>73</td>
<td>9</td>
<td>23</td>
</tr>
<tr>
<td>Cd&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>21</td>
<td>52</td>
<td>10</td>
</tr>
<tr>
<td>Zn&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>39</td>
<td>46</td>
<td>5</td>
</tr>
<tr>
<td>Ni&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>63</td>
<td>60</td>
<td>28</td>
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<tr>
<td>Cu&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>77</td>
<td>35</td>
<td>0</td>
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<tr>
<td>Pb&lt;sup&gt;2+&lt;/sup&gt;</td>
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<td>100</td>
<td>100</td>
<td>100</td>
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<td>Na&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>103</td>
<td>100</td>
</tr>
<tr>
<td>K&lt;sup&gt;+&lt;/sup&gt;</td>
<td>99</td>
<td>94</td>
<td></td>
</tr>
</tbody>
</table>

The activity of the enzyme without adding metal ions was defined as 100%. All ions were added as their sodium salts, except for Pb acetate.

The xylanase Xyl2495 retained almost 100% of its initial activity when pre-incubated without the substrate at 60°C for 3 h. At 70°C, its half-life was approximately 1 h, and quick inactivation was observed at 80°C (Fig. 4). Approximately the same thermostability characteristics were observed for recombinant xylanase Xyl2090, in spite of the much higher temperature optimum of this enzyme. Surprisingly, Xyl2091 exhibited the lowest thermostability, losing 50% activity upon incubation at 60°C for 2.7 h; at 70°C, the enzyme activity was completely lost after 20 min. Unlike Xyl2495, Xyl2091 is a multidomain protein and possibly its complex structure is less resistant to elevated temperature. However, Xyl2091 and Xyl2090 appeared to be more stable at high temperatures in the course of xylan hydrolysis (Fig. 4). The half-life time of Xyl2090 at 80°C was about 1 h. For Xyl2091, no decrease of activity was observed up to 3 h at 60°C, while at 70°C the half-life of the enzyme was about 45 min. Probably, the presence of the substrate and/or hydrolysis products increases the stability of xylanases at high temperatures. Both Xyl2091 and Xyl2090 contain CBM domains that could facilitate binding of these enzymes to xylans.

The effects of metal ions on the enzyme activity were also analyzed (Table 2). The results revealed that the enzyme activity was significantly inhibited by divalent cations Hg<sup>2+</sup>, Cd<sup>2+</sup>, Zn<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup>, and Pb<sup>2+</sup>. The effects of Mg<sup>2+</sup>, Ca<sup>2+</sup>, and monovalent cations (Cs<sup>+</sup>, Rb<sup>+</sup>, Li<sup>+</sup>, Na<sup>+</sup>, and K<sup>+</sup>) on the enzyme activity were insignificant.

Experiments were carried out to study the specificity of recombinant xylanases to various polysaccharide substrates and pNP-derivatives (Table 1). All three enzymes showed activity on birchwood and beechwood xylans. Xyl2495 was the most active against both xylan substrates, and the two other enzymes exhibited much lower specific activities. Microcrystalline and carboxymethyl celluloses, locust bean gum, guar gum, p-nitrophenyl-β-D-glucopyranoside, p-nitrophenyl-β-D-galactopyranoside, and p-nitrophenyl-β-D-mannopyranoside did not serve as substrates for any of the endoxylanases, indicating the narrow specificity of these enzymes. Trace activity of Xyl2091 and Xyl2495 was detected against p-nitrophenyl-β-D-xylopyranoside.

The kinetics of enzymatic reactions were characterized by recording the effect of varying concentrations of birchwood xylan and plotting a Hanes–Woolf curve (Table 1). The $K_m$ values of the enzymes for birchwood xylan (10.5 mg/ml for Xyl2090, 3.0 mg/ml for Xyl2091, and 1.05 mg/ml for Xyl2495) are within the range for microbial xylanases (0.14–14 mg/ml).

### Analysis of Hydrolysis Products

Birchwood xylan and X<b>5</b>–X<b>5</b> xyl-o-oligosaccharides were incubated with recombinant xylanases, and the samples were then collected at different time intervals and analyzed by thin-layer chromatography. Analysis of xylan hydrolysis products revealed the production of xylobiose and xylose by Xyl2091 and Xyl2495, whereas only products longer than X<sub>6</sub> oligomers were detected in the case of Xyl2090 (Fig. 5). Consistently, no activity against X<sub>6</sub>–X<sub>5</sub> xyl-o-oligosaccharides was observed for Xyl2090 (Fig. 6). The other two xylanases digested xylpentaoese, xylotetraose, and xylotriose to produce xylobiose and xylose (Fig. 6). The absence of β-xylsidase activity for all recombinant xylanases was confirmed by their inability to hydrolyze xylobiose (Fig. 6).

### Discussion

The three xylanases of <i>M. roseus</i> characterized in this study appeared to have quite different biochemical characteristics. Xyl2495 appeared to be the most active, having the specific activity against birchwood xylan of 1,920 U/mg, which is higher than that of bacterial xylanases from <i>Geobacillus</i> and <i>Thermotoga</i> species [2, 14, 16, 19, 20], and commercial fungal...
enzymes from *Trichoderma viride* (100–300 U/mg). This enzyme has a wide pH range and retains 80–30% of the maximal activity under alkaline conditions (pH 8–10). Although the enzyme has the temperature optimum at 40°C and is particularly active at low temperatures, exhibiting more than 30% of the maximal activity even at 0°C, its activity at 60°C (about 20% of the maximal) is still higher than that of most known thermostable xylanases, and no thermonactivation of the enzyme was observed during prolonged incubation at 60°C.

The other two endoxylanases, Xyl2090 and Xyl2091, have much lower specific activities (41 U/mg and 75 U/mg, respectively) but optimal temperatures above 60°C. In *M. roseus*, these enzymes are probably linked to the cell surface. The xylanase Xyl2090 exhibits the highest activity at 80°C, higher than fungal and most bacterial xylanases but lower than xylanases from hyperthermophilic bacteria of the genus *Thermotoga* (95–102°C) [16, 24]. Analysis of the substrate specificity of these three *M. roseus* xylanases showed that they hydrolyze xylan exclusively, with no activity against carboxymethyl and microcrystalline cellulose.

Such cellulase-free xylanases are particularly useful for bleaching of paper pulp, since it would selectively remove hemicellulose components with minimal damage to cellulose.
Analysis of the xylan hydrolysis products revealed the production of different ranges of xylo-oligosaccharides, reflecting different activities of the three enzymes. The xylanase Xyl2090 appeared to generate only xylo-oligosaccharides longer than X₂ and exhibited no activity towards X₂-X₅ oligomers. The closest functionally characterized homolog of Xyl2090, xylanase Xyn30A of B. licheniformis SVDI, also showed low or no activity with X₂–X₅ xylo-oligosaccharides [15]. On the contrary, xylose and xylobiose were the major products of birchwood xylan hydrolysis by the recombinant enzyme Xyn10A from M. roseus, whereas Xyl2091 and Xyl2495 hydrolyze them to xylose and xylobiose; the latter could be split by several intracellular β-xylanases and extracellular β-xylosidases, indicating that Xyl2091 and Xyl2495 lack β-xylosidase activity. The low molecular weight xylo-oligosaccharides can be used in a variety of fields, including pharmaceuticals, prebiotics for fish and poultry industries, agricultural purposes, and food applications [1, 18], and the desired end-products can be obtained by varying the xylan hydrolysis time.

Three endoxylanases encoded in the M. roseus genome, Xyl12090, Xyl12091, and Xyl2495, exhibit different biochemical characteristics. M. roseus is able to grow in a rather wide temperature range, from 35°C to 60°C, with the optimum at 52–55°C. The presence of the three endoxylanases with different temperature optima, covering the whole organism’s temperature growth range, and presumably different localization, ensures efficient xylan hydrolysis. The highly processive endoxylanase Xyl2495, containing only the N-terminal signal peptide and GH10 catalytic domain, is likely secreted from the cell and acts as a free enzyme, able to hydrolyze xylan under moderate temperatures. The other two endoxylanases additionally contain carbohydrate-binding modules that could facilitate their specific binding to xylans, and Por sorting domains. Probably, in M. roseus, these enzymes are linked to the cell surface, as observed for Por domain-containing xylanase Xyn10A from R. marinus [8] and cellulases from M. roseus [7]. Such mode of action could explain the lower specific activities observed in vitro for recombinant Xyl12090 and Xyl12091 relative to Xyl2495. In M. roseus, the cell-linked xylanase Xyl12090 probably degrades xylans to relatively long xylo-oligosaccharides, whereas Xyl12091 and Xyl2495 hydrolyze them to xylose and xylobiose; the latter could be split by several intracellular and extracellular β-xylosidases.

Acknowledgments

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References


