The three-dimensional structure (3D structure) of Xyn11A, a family 11 xylanase from Bacillus firmus K-1, was obtained through homology modeling. To study the substrate-binding site of Xyn11A, six xylooligosaccharides, xylobiose to xylheptaose (X2–X7), were docked into the active site of Xyn11A by molecular docking. Based on the docked energy and estimated free energy of binding combined with modeled enzyme-substrate complexes, the substrate-binding site of Xyn11A probably contained six subsites, defined as −3, −2, −1, +1, +2, and +3. Focus on possible stacking interaction presented seven aromatic residues, that played an important role in six subsites of Xyn11A such as Tyr165 (−3), Trp9 and Tyr69 (−2), Tyr80 (−1), Tyr65 (+1), Tyr88 (+2) and Tyr173 (+3). The bond-cleavage positions showed that X2 and X3 did not bind at the cleft (subsites −1 and +1) of Xyn11A. Related to the experiment, the end products of larchwood xylan hydrolysis by purified Xyn11A were X2 and X3. X2 and X3 acted as the end product inhibitors of Xyn11A.

Key words: Bacillus firmus; family 11 xylanase; homology modeling; molecular docking; substrate-binding site

Endoxylanases (EC 3.2.1.8), which cleave β-1,4 glycosidic bonds of the linear polyxyllose chains of xylan to short-chain xylooligosaccharides, are classified into plant cell-wall hydrolases.1 According to amino acid sequence similarities, the xylanases are generally classified to glycosyl hydrolase families 10 and 11,2,3 and a few of the xylanases belong to families 5, 8, and 43 (http://afmb.cnrs-mrs.fr/CAZY/). The 3D structures of family 11 xylanases present one single (catalytic) domain with a β-jelly roll fold conformation.4 The study of substrate-binding sites is necessary for understanding the substrate specificity and catalytic mechanism of the enzymes.5 The substrate-binding sites of glycosyl hydrolases often comprise aromatic residues, mostly tyrosine and tryptophan, which can pack against the sugar units as well as the amino acid residues, that form hydrogen bonds with the hydroxyl groups of substrates.5

Molecular docking for investigating the substrate-binding site of Xyn11A in this study was performed using Autodock version 3.0.5.6 The Autodock is a well-known computational docking program for studying the interaction of protein-substrate complexes.6 It is a fully automated docking method of predicting the interaction of flexible substrates and rigid protein targets of known three-dimensional structures, which performs a rapid energy evaluation through a precalculated grid of affinity potentials with a Lamarckian genetic algorithm as the search method.6 Autodock has been used successfully in docking studies of proteins and carbohydrates such as that of family 8 xylanase from Pseudalteromonas haloplanktis TAH3a with xylotriose8 and cellobiohydrolase from Trichoderma reesei and endoglucanase from Themnobifida fusca with cellobio-losaccharides.9 Previous study revealed that autodock could predict binding modes of protein-substrate complex similar to experimentally observed modes.10

X-Ray diffraction is a commonly experimental strategy, used to study the substrate-binding sites of enzymes. The binding sites of a few family 11 xylanases have been studied via this method, e.g., the X-ray structure of the inactive Bacillus circulans xylanase mutant (E172C) complexed with xylotetraose in which only two xylose units bound to the binding site at subsites −1 and −2.12 Subsequently, the X-ray structures of this xylanase and Bacillus agaradhaerens xylanase covalently complexed with 2-F-xylobiosyl were determined for studying glycosyl-enzyme intermediate via focusing on two subsites (−1 and −2).13 Alternatively, the computational strategy is a possibly useful method for studying the binding sites of enzymes. Currently, this method is popular and developing rapidly. This method has been used to study substrate-binding sites of family 11 xylanases, e.g., the substrate-binding sites of XYNI and XYNII from Trichoderma reesei were studied via docking different xyloligomer models into the active sites of both xylanases.14 Similarly, the binding sites of xylanase XylI from Streptomyces sp. 38 and xylanase XynC from Aspergillus kawachii were studied through energy minimization of the geometries of modeled enzyme-xylooligose
complexes obtained using the previously X-ray complexed structures as references. Moreover, the binding site of Thermomonospora fusca xylanase focusing on only aromatic residues was roughly predicted by the Insight II program. To date, the substrate-binding sites of some family 11 xylanases have been studied; however, their informations were less clear. In this study, we used homology modeling to build the 3D structure of Xyn11A, a family 11 xylanase from B. firmus K-1 (formerly known as Bacillus sp. strain K-1). The modeled structure was then used to investigate the number of subsites and possible amino acid residues presented on the substrate-binding site of Xyn11A by using the molecular docking technique. The end products from xylan hydrolysis by the enzyme and the inhibition effect of the end products on enzyme activity are also reported.

Materials and Methods

Homology modeling of Xyn11A. The amino acid sequence of Xyn11A, belonging to family 11 glycosyl hydrolase, was reported by Chang et al. The X-ray structure of family 11 xylanase from B. circulans, XlnA, obtained from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb; PDB code 1BVV) was used as the template structure for building the 3D structure of Xyn11A. The structure and sequence data were operated using the homology module of Insight II, a well-known homology modeling program. Because sequence alignment is a crucial technique in homology modeling, the sequences of Xyn11A and template were aligned by the homology module to identify the blocks that are likely to contain structurally conserved regions. All atomic coordinates of the residues in those blocks were transferred from the template to build the modeled structure of Xyn11A. However, for the mismatch residues, only the atomic coordinates of Cα were transferred from template while the residual atomic coordinates were generated by using a library.

Energy minimization. All hydrogen atoms were added to the homology-modeled structure of Xyn11A by builder module and then were energy-minimized by discover module of Insight II. The minimization was performed with a 5 Å-water layer in dimensions of 60 × 60 × 60 Å in a water box. First, the steepest descent algorithm was used for 1,000 steps to remove close van der Waals contacts, followed by the more efficient conjugate gradient algorithm until a tolerance of 0.01 kcal/mol/Å in the gradient was reached. After minimization, the layer water was removed. Validation of the model was carried out using Ramachandran plot calculations computed with the Procheck program.

Molecular docking. Xylooligosaccharides preparation. The 3D structures of six soluble xylooligosaccharides (X1–X5) were constructed and then energy-minimized using geometry optimization by builder module of Insight II. These resulting molecules served as starting structures for the docking experiments. Automated docking simulation. Autodock program version 3.0.5 was used for automated docking simulation of xylooligosaccharides to the active site of the Xyn11A structure. The atomic partial charges of generated xylooligosaccharides and the enzyme were added using the Gasteiger method. All xylooligosaccharides were assigned active torsions by autotors module of Autodock program. Autodock uses a grid-based method for energy evaluations in which the grid points contain precalculated affinities for the different atom types of the xylooligosaccharides. The sizes of the grid points of each xylooligosaccharide were set in range of 65–110 Å for each x, y, and z direction equally spaced at 0.375 Å, yielding a grid box that covered the entire active site of Xyn11A and each xylooligosaccharide

The flexible xylooligosaccharides were docked into the active site of Xyn11A using a Lamarckian Genetic Algorithm. The simulation consisted of 100 docking runs, and the number of generations in each run was 10,000 (at maximum of 1,000,000 energy evaluations). After docking, all structures generated for the single compound were assigned to clusters on the basis of a tolerance of 2 Å for all atoms root mean square deviation (RMSD)’s from the lowest-energy structure. The hydrogen bonds were calculated by the Insight II program and the distances for forming these bonds were not over 3.5 Å. The stacking interactions between sugar rings of xylose units and aromatic rings of xylooligosaccharides could be determined via measurement of the distance between the sugar rings and aromatic rings that were not over 5.0 Å

Enzyme production. Alkaliphilic B. firmus K-1, isolated from the waste treatment plant of a pulp and paper manufacturer, was grown in Ville’s mineral salts medium, containing corn husk (1%, w/v) as a carbon source. The pH of the medium was initially adjusted to 10.5 with Na2CO3 (20%, w/v) after autoclaving. The inoculated culture was incubated in a rotary incubator (200 rpm) at 37 °C for 2 d. After that, the cells were removed by centrifugation (9,500 × g for 10 min) at 4 °C. The culture supernatant was used as crude enzyme.

Xylanase assay. The xylanase activity was measured by determining the amount of reducing sugar released from larchwood xylan (NBS Biologicals, L., Huntingdon, England). The reaction mixture consisted of 1% xylan (w/v) in 25 mM sodium phosphate buffer (pH 7.0) and enzyme to give a final volume of 0.25 ml. After incubation for 10 min at 50 °C, the increase in the amount of reducing sugar was determined by the Somogyi-Nelson method.

One unit (U) of enzyme activity was defined as the amount of enzyme which released 1 μmole of reducing sugar in 1 min under the above conditions.

Protein determination. Protein was assayed by the method of Lowry et al. with bovine serum albumin as a standard. The protein content eluted from a column was measured by monitoring the optical density at 280 nm.

Preparation of corn husk. Dried corn husk was cut by scissors to a small size, then ground in a blender and sieved to 80–100 mesh size. After they were washed several times with warm distilled water to remove residual sugars, the ground corn husk was dried at 50 °C and kept for later use.

Purification of enzyme. The purification method was modified from the method of Tacharaapakoon et al. The culture supernatant was used as a source of Xyn11A. The corn husk suspended in 100 mM phosphate buffer (pH 7.0) was packed in a column (2 cm × 10 cm) and equilibrated with the same buffer. Twenty-five mg protein of the culture supernatant was applied on the column. After washing the enzyme-corn husk complex several times with the same buffer until unbound protein was removed absolutely, the xylanase was eluted by 1% (v/v) triethylamine (Sigma-Aldrich, St. Louis, MO) with a flow rate of 2 ml/min. One-mI fractions were collected by a fraction collector. The active xylanase fractions were collected and dialyzed against 10 mM phosphate buffer (pH 7.0) at 4 °C. After that, the enzyme was concentrated by freeze drying before it was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE).

SDS–PAGE. SDS–PAGE was performed on a 12% polyacrylamide gel by the method of Laemmli. The proteins were stained with Coomassie brilliant blue R-250. The molecular weight standards were used from a molecular weight calibration kit (Pierce, Rockford, IL).

Preparation of soluble xylan. The preparation of soluble xylan was performed by the method of Ghangas et al. Ten g of commercial larchwood xylan was suspended in 200 ml of distilled water and adjusted to pH 10 by 1 M NaOH. Then the mixture was stirred for 1 h at room temperature, and centrifuged for 10 min at 10,000 × g. After centrifugation, the supernatant was neutralized with 1 M acetic acid, and then freeze dried.
End product inhibition. The high concentration of the end products (2%, w/v of X₇ and X₆ mixture from xylan hydrolysis by Xyn11A) were preincubated with the purified enzyme (1.0 U) and shaken continually at 4 °C for 30 min. After that, (1%, w/v) soluble larchwood xylan suspension in 50 mM phosphate buffer (pH 7.0) was added (1:1 by volume) to the mixture of the enzyme and end products. Then they were incubated and shaken consecutively at 50 °C for 15 min and 24 h. The inhibition effect of the end products on the enzyme activity was detected by TLC.

Results and Discussion

3D structure generation

The 3D structure of Xyn11A was generated by the homology module of Insight II based on the X-ray structure of family 11 xylanase from B. circulans (code 1BVV), which showed the highest identity percentage (77.84%) of amino acid sequences. The RMSD for the backbone atoms of two whole structures was 0.33 Å. The small value of RMSD could be interpreted to mean that the two structures share common homology. To validate the modeled structure of Xyn11A, a Ramachandran plot of Xyn11A and template were drawn by the Procheck program. The spot distribution in the Ramachandran plot of the Xyn11A structure was similar to the X-ray structure of the B. circulans xylanase. The results showed that the homology-modeled structure of Xyn11A is reasonable and acceptable.

The homology-modeled structure of Xyn11A is shown in Fig. 1. The overall structure of Xyn11A resembled a closed right hand. It consisted of 14 β-sheets and one α-helix. The most predominant feature of the structure was a long open cleft containing the active site. In the active site, two conserve glutamic acid residues (Glu78 and Glu171), located on either side of the cleft, played pivotal role in catalysis of the degradation of xylan.

Number of subsites prediction base on the docked energy and estimated free energy of binding

The autodock program calculated the binding energies such as docked energy and estimated the free energy for each run depending on its energy function for the final docked structures. The docked energy of binding of Xyn11A-xylooligosaccharides complexes was calculated from a combination of the final intermolecular energy and the final internal energy of xylooligosaccharides, while estimated free energy of binding of those complexes was computed from a combination of the final intermolecular energy and the torsional free energy.

Clusters containing the highest frequency of docked substrates binding at same position were first selected. In the highest frequency cluster, the orientation and conformation of docked xylooligosaccharides (X₇-X₇) presenting the lowest energies of docked energy and estimated free energy of binding as well as rotating the reducing end in the same direction was carried out to predict the number of subsites in the substrate-binding site and further to study the binding mechanism of Xyn11A and its substrates. As shown in Fig. 2, the trends of docked energy and estimated free energy were in correspondence. They showed steady decreases from X₂ to X₆. However, both energies of docked X₇ (−14.45 and −13.75 kcal/mol respectively) equaled docked X₆ (−14.27 and −13.50 kcal/mol respectively). These results reveal that when the size of xylooligosaccharides increased, the binding energies of the enzyme-substrates interaction became more negative until the chain of substrates fitted the full number of subsites, causing the structure of enzyme-substrate complexes formed to be more stable. The binding energies reached a plateau in the completely fitted substrate (six subsites). However, the binding energies of the longer-chain substrate (X₇), exceeded the number of subsites and changed slightly. Plotting the binding energies versus the size of the substrates gave a straight line, until the full number of subsites was similar to the free energy of binding of two

![Fig. 1. Ribbon Diagram of the Homology-Modeled Structure of Xyn11A.](image1)

![Fig. 2. The Lowest Energies of Docked Energy (●) and Estimated Free Energy (▲) of Xylooligosaccharides Binding to Xyn11A.](image2)
family 10 xylanases from *Geobacillus stearothermophilus* T-6 with different xylooligosaccharides. These results indicate that substrate-binding site of Xyn11A might contains six subsites.

From the results of automated docking simulation, all docked xylooligosaccharides (X$_2$–X$_6$) bound to the active site of Xyn11A by rotating the reducing end (anomic carbon) to the right whereas the non-reducing end was turned to the left in the case of Glu171 and Glu78 locating at top and bottom respectively (Fig. 3). Based on the direction of the reducing end/non-reducing end as well as the location of two catalytic residues, which have been used to define the subsites of other family 11 xylanases, combined with superposition of modeled Xyn11A-xylooligosaccharides complexes and 1BCX (the X-ray structure of mutant 1BVV complexed with xylotetraose) (data not shown), the six subsites of Xyn11A were defined as $-3$, $-2$, $-1$, +1, +2, and +3. Until recently, although the numbers of subsites of family 11 xylanases have been studied, their entire numbers of subsites still have not been cleared, e.g., the binding site of *A. kawachii* XynC probably contained five subsites while *Streptomyces* sp. 38 XylI had six subsites. Moreover, the binding sites of two family 11 xylanases, XYNI and XYNII, from the same microorganism, *T. reesei*, were clearly different. XYNI had three subsites while XYNII contained five subsites. These results reveal that X$_3$ and X$_5$ might be the end products for xylan hydrolysis by Xyn11A. To confirm these results, Xyn11A was purified by affinity chromatography using a corn husk column. A single protein band appeared on SDS–PAGE and had a molecular weight of 23 kDa (Fig. 5A, lane 1). Hydrolysis products of soluble larchwood xylan (low substituted xylan) by the purified Xyn11A were analyzed by TLC, as shown in Fig. 5B. At the initial incubation time, Xyn11A liberated a series of short chain xylooligosaccharides larger than X$_1$ (Fig. 5B, lane 3). The complete hydrolysis of the xylan by high activity of Xyn11A at long time incubation showed only X$_2$ and X$_3$ (Fig. 5B, lane 4). These results reveal that Xyn11A liberated X$_2$ and X$_3$ as the end products similar to family 11 xylanases from *Thermomyces lanuginosus* ATCC 44008, *Clostridium stercorarium* (Xyn11A), *Penicillium griseofulvum* (PgXynA), and *Penicillium funiculosum* (PfXynC). In contrast, family 11 xylanases from *Bacillus halodurans* C-1 (XylI) liberated a series of xylooligosaccharides larger than xylotriose while *Aspergillus niger* (AnxA) released X$_3$ as the main hydrolysis product. These results suggest that the end products obtained from xylan hydrolysis by the members of family 11 xylanase are different, probably

**Fig. 3.** The Conformation of the Lowest-Energy of X$_2$–X$_6$ Bound to Active Site of Xyn11A.

The Glu171 and Glu78 are located at top and bottom, respectively.

**Fig. 4.** Schematic Representation of the Pattern of Six Xylooligosaccharides (X$_2$–X$_3$) Bound to Subsites of Substrate-Binding Site of Xyn11A.

The predicted bond-cleavage positions are indicated as filled triangles.
depending on modes of action of the enzymes due to differences in their substrate-binding sites.

**Effect of the end products on inhibition of the enzyme**

The effect of Xyn11A inhibition by its end products, X2 and X3, was determined using TLC, as shown in Fig. 5C. Due to the end products inhibition, the soluble xylan was not hydrolyzed by the enzyme. Therefore, only X2 and X3 were observed during incubation while non-digested soluble xylan appeared as the smear band below (Fig. 5C, lanes 2–3). These results indicate that X2 and X3 acted as the end product inhibitors of Xyn11A. Many researchers studied the effect of X2, the end product inhibitor, on the activity of xylanase, e.g., family 11 xylanase from *Thermomonospora fusca* was inhibited by xylobiose\(^1\) while xylobiose reduced the activity of family 10 xylanase from *Thermomonospora fusca* BD25.\(^2\) However, until now, no published paper reported the inhibition effect of Xyn11A. The stacking interaction and hydrogen bond are very important for studying subsites in substrate binding sites before the enzymatic hydrolysis reaction. Hence, the stacking interaction and hydrogen bond are focused for determination the potential amino acid residues, that formed bonds with xylose units at individual subsites (−3 to +3) of Xyn11A. Due to Xyn11A consisting of six subsites, the modeled Xyn11A-X\(\text{C}_0\) complex was represented. The

<table>
<thead>
<tr>
<th>Subsite</th>
<th>Amino acid residues forming bonds with xylose molecules in each subsite</th>
<th>Hydrogen bond</th>
</tr>
</thead>
<tbody>
<tr>
<td>−3 Xyl 1</td>
<td>Tyr165 (4.85) OH2 OH Tyr165 (2.89)</td>
<td>OH Tyr165 (2.89)</td>
</tr>
<tr>
<td>−2 Xyl 2</td>
<td>Trp9 (3.26) OH2 OH Tyr69 (1.79)</td>
<td>OH Tyr69 (1.79)</td>
</tr>
<tr>
<td>−1 Xyl 3</td>
<td>Tyr80 (4.41) OH2 NH2 Arg112 (1.77)</td>
<td>NH Arg112 (1.79)</td>
</tr>
<tr>
<td>+1 Xyl 4</td>
<td>Tyr65 (2.38) OH2 OH Tyr65 (2.32)</td>
<td>OH Tyr65 (2.32)</td>
</tr>
<tr>
<td>+2 Xyl 5</td>
<td>Tyr88 (4.01) OH2 OH Tyr88 (2.00)</td>
<td>OH Tyr88 (2.00)</td>
</tr>
<tr>
<td>+3 Xyl 6</td>
<td>Tyr173 (3.47)</td>
<td>OH Tyr173 (2.18)</td>
</tr>
</tbody>
</table>

*~Labeled number at hydroxyl group indicates the position of carbon atom in each xylose ring.*

*Endocyclic oxygen.*

*No hydrogen bond.*

**Potential amino acids in each subsite of Xyn11A**

The stacking interaction and hydrogen bond are very important for studying subsites in substrate binding sites of xylanases because both bonds are implicated in the binding mechanism between xylanases and xylans before the enzymatic hydrolysis reaction. Hence, the stacking interaction and hydrogen bond are focused for determination the potential amino acid residues, that formed bonds with xylose units at individual subsites (−3 to +3) of Xyn11A. Due to Xyn11A consisting of six subsites, the modeled Xyn11A-X\(\text{C}_0\) complex was represented. The neighboring amino acids possibly implicated in stacking interactions and/or hydrogen bonds with the bound X\(\text{C}_0\) at individual subsites are depicted in Fig. 6 and summarized in Table 1. Most of the aromatic residues in each subsite potentially formed both stacking interactions and hydrogen bonds except Trp9 and Tyr173 at subsite −2 and +3 respectively, which formed only stacking interaction. For hydrogen bonds in the non-reducing end direction, the xylose moiety at subsite −1 formed hydrogen bonds between its hydroxyl groups at the OH3 position to the side chains of Tyr80 and carbonyl group of Pro116 and two positions at the OH2 to side chains of Arg112. The hydroxyl groups of xylose moiety at subsite −2 formed hydrogen bonds with side chains of Glu7 (at OH3) and Tyr69 (at OH2). Only Tyr165 formed hydrogen bond to xylose moiety (at OH2) at subsite −3. For
Fig. 6. The Binding Interaction of Xyn11A and Xylohexaose from the Automated Docking Simulation.

A. The active site of the modeled complex of the Xyn11A to xylohexaose. The substrate (in balls and sticks) and the neighboring amino acid residues (in sticks) of Xyn11A are shown. B. Schematic representation of Xyn11A-X6 complex showed possible hydrogen bond (---) and stacking interaction (++) in the individual subsites. R symbol indicates the reducing end.

Table 2. Comparison of the Amino Acid Residues in Each Subsite of Xyn11A and Other Family 11 Xylanases

<table>
<thead>
<tr>
<th>Family 11 xylanases</th>
<th>Strategy</th>
<th>Amino acid residues in individual subsites</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xyn11A from B. firmus K-1</td>
<td>Computational method</td>
<td>Y165, Q7, W9, Y69, Y80, R112, W65, Q126, Y88, Y173</td>
<td>This study</td>
</tr>
<tr>
<td>XYNI from T. reesei</td>
<td>X-Ray crystallography/Computational method</td>
<td>Y9, W166</td>
<td>Torronen and Rouvinen, 1995</td>
</tr>
<tr>
<td>XYNII from T. reesei</td>
<td>X-Ray crystallography/Computational method</td>
<td>W18, Y179, Y96</td>
<td>Torronen and Rouvinen, 1995</td>
</tr>
<tr>
<td>Xyl11A from T. fusca</td>
<td>X-Ray crystallography/Computational method</td>
<td>Y9, F14, Y168, Y76, Y87, W135, Y115</td>
<td>Georl et al., 2000</td>
</tr>
<tr>
<td>Xyl1 from Streptomyces sp. 38</td>
<td>Computational method</td>
<td>S18, Y179, W20, R121, Y89, R121, Y166, P116</td>
<td>Esteves et al., 2004</td>
</tr>
<tr>
<td>XynC from A. kawachii</td>
<td>Computational method</td>
<td>Q8, Y172, Y10, P119, Y81, R115</td>
<td>Esteves et al., 2004</td>
</tr>
<tr>
<td>XlnA from B. circulans</td>
<td>X-Ray crystallography</td>
<td>W9, Y69, W80, R112, Y166, P116</td>
<td>Wakarchuck et al., 1994</td>
</tr>
<tr>
<td>Xyl11 from B. agaradherens</td>
<td>X-Ray crystallography</td>
<td>E17, W19, Y85, R129, R49, Y85, P133</td>
<td>Sabini et al., 1999</td>
</tr>
</tbody>
</table>

*aStrategy for study of the substrate binding site.
—, Not identified.
opposite direction, reducing end, the xylose units formed hydrogen bondings at OH2 position to side chains of Tyr65 and at O5 position to Gln126 at subsite +1 and two positions at OH2 and OH3 to side chain of Tyr88 at subsite +2. However, hydrogen bond was not found at subsite +3.

The potential amino acid residues in individual subsites of Xyn11A was compared to other family 11 xylanases whose binding sites have been studied (Table 2). All residues at subsite −1 of Xyn11A resemble the same subsite of X-ray complexed structures of XlnA from B. circulans (2) and Xyn11 from B. agaradhaerens. (13) At subsite −2, aromatic ring of Trp9 of Xyn11A formed stacking interaction with xylose ring corresponding to XYNII from T. reesei, (14) XlnA from B. circulans, (15) and Xyl11 from B. agaradhaerens, (16) whereas XYNI from T. reesei (18) is replaced by tyrosine. Moreover, Gln7 at subsite −2 of Xyn11A is equivalent to Ser18 in Xy11 from Streptomyces sp. 38 and Gin8 in XynC from A. kawachii. (17) This residue also equals Glu17 in Xyn11 from B. agaradhaerens. (12) Tyr80 and Tyr69, at subsites −1 and −2 respectively, showing extremely critical roles in the substrate binding of XlnA from B. circulans, (15) are involved in substrate binding at the same subsites of Xyn11A and Xy11 from B. agaradhaerens. (13) Although Tyr166 occupied at subsite −2 of XlnA from B. circulans, displaying a smaller role in the binding site. (15) It was found at subsite −3 of Xyn11A (Tyr165). On the other hand, subsite +1 (Tyr65 and Gln126), subsite +2 (Tyr88), and subsite +3 (Tyr173) of Xyn11A were not similar to the substrate-binding sites of other family 11 xylanases (see Table 2). These results suggest that various family 11 xylanases from different microorganisms have distinct substrate-binding sites, as shown by the difference in the amino acid compositions in each subsite of those xylanases.

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