Glycoside hydrolases (GHS) can be grouped into two broad classes, exo- and endo-enzymes, according to how they degrade polysaccharides. Endo-GHs cleave glycosidic bonds randomly in the interior of the polysaccharide chain, whereas exo-GHs cleave mono-, di-, or oligosaccharide units from one end of the chain. Branched polysaccharides have more nonreducing ends than reducing ends, and most currently identified exo-GHs are ones acting on the nonreducing ends of polysaccharides.

Rex (reducing end xylose-releasing exo-oligoxylanase) from Bacillus halodurans C-125 (gene product of BH2105) is one of the few exo-type enzymes that act on the reducing end (1). Unlike other so-called “reducing end-specific” exo-GHs, such as MalZ (maltodextrin glucosidase) from Escherichia coli K12 (2), an amylolytic enzyme from Thermotoga maritima (TM1835) (3), oligoxyloglucan reducing end-specific cellobiohydrolase (4), and reducing end-specific processive cellobiohydrolases (CBHs) (5), Rex recognizes the xylose unit of the reducing end in a highly strict manner, even discriminating the β-anomeric hydroxyl configuration from the α-anomer or 1-deoxyxylose. To our knowledge, therefore, Rex is the only complete exo-GH that releases a monosaccharide from the reducing end. The enzyme is thought to play a key role in the intracellular xylan metabolism of B. halodurans by cleaving xylooligosaccharides. Considering the substrate specificity for xylooligosaccharides of various lengths, the enzyme is presumed to have three subsites (−2, −1, and +1). Because Rex is an inverting enzyme that produces the α-anomer at the reducing end, spontaneous mutarotation of the substrate is required for subsequent processing in vivo. It remains unclear whether there are any factors supporting the mutarotation of xylooligosaccharides in vivo or if there are any biological reasons for such an inefficient processing mechanism.

Rex belongs to the GH-8 family according to the CAZY classification (available on the World Wide Web at afmb.cnrs-mrs.fr/CAZY) (6). GH-8 and GH-48 are grouped to form clan GH-M, sharing similar (α/α)6 barrel fold (7). Within the GH-8 family, the crystal structures of three extracellular endo-GHs, CelA (endoglucanase from Clostridium thermocellum) (8), pXyl (a cold-adapted xylanase from the Antarctic bacterium Pseudoalteromonas haloplanktis) (9), and ChoK (chitosanase from Bacillus sp. K17) (10), have been determined. In particular, the subsites of CelA (−3 to +3) have definitely been determined with the complex structure with cellopentaose at atomic resolution (11). Adachi et al. (10) proposed dividing the GH-8 family into three subfamilies (GH-8a, -8b, and -8c), depending on the position of the catalytic base residue. CelA, pXyl, and Rex are grouped into the GH-8a subfamily, and ChoK is grouped into GH-8b. GH-8a enzymes have proton donor and catalytic base residues at the N termini of the α4 and α6 helices within the (α/α)6 barrel, respectively. The two catalytic residues of Rex (Glu116 as a proton donor and Asp263 as a catalytic base) have already been confirmed by site-directed mutagenesis (1). Interestingly, the catalytic base of GH-8a enzymes (Asp) is replaced by Asn (or Ser) in the GH-8b and -8c enzymes. The crystal structure of ChoK revealed that the catalytic base of GH-8b is located in the long loop inserted between α2 and α6 (10). We describe here crystal structures of Rex and show the structural basis for its strict substrate specificity, especially for a xylose unit at the reducing end.
**EXPERIMENTAL PROCEDURES**

Crystallography—Expression and purification of the wild-type and E70A mutant enzymes were previously reported (1). Both types of enzyme were crystallized under the conditions described elsewhere (12). The xylose complex (WT-xylose) was prepared by co-crystallization using a reservoir solution containing 10 mM xylose. The xylose complex of the E70A mutant enzyme (E70A-xylose) was prepared by co-crystallization using a reservoir solution containing 10 mM xylolose, and the crystals that grew in 6 days were used for data collection. Diffraction data were collected using a charge-coupled device camera on the BL-5A station at the Photon Factory and the NW-12 station at the Photon Factory AR, High Energy Accelerator Research Organization (KEK), Tsukuba, Japan. The crystals were flash-cooled in a stream of liquid nitrogen at 100 K. Diffraction images were indexed, integrated, and scaled using the HKL2000 program suite (13). Initial phases of the native structure were obtained by the molecular replacement method, using the structure of psychrophilic endo-β-1,4-xylanase from *P. haloplanktis* (Protein Data Bank code 1H14) as a search model. Molecular replacement was performed with MOLREP (14) in the CCP4 program suite (15). The program ARP/wARP (16) was used for automatic model building. Visual inspection of the models was performed using XtalView (17). Crystallographic refinement was carried out using CNS1.1 (18). The data collection and refinement statistics are summarized in Table I. The complex structures were solved by starting from the refined native structure. The figures were prepared using SPOCK (19), Raster3D (20), MOLSCRIPT (21), and XtalView.

**RESULTS**

Crystallography—The unliganded crystal structure of the wild-type Rex (WT-native) was solved by means of molecular replacement, using the crystal structure of pXyl. The complex structure of the wild-type Rex with xylose (WT-xylose) was determined by a co-crystallization method. In the WT-xylose structure, the electron density of a xylose molecule was found at subsite +1, following the definition of the subsites of CelA.

### TABLE I

| Crystallographic data collection and refinement statistics | WT-native | WT-xylose | E70A-xylose |
|----------------------------------------------------------|-----------|-----------|-------------|
| Data collection statistics                               |           |           |             |
| X-ray source                                             | PF BL-5A  | PF BL-5A  | PF-AR NW-12 |
| Wavelength (Å)                                           | 1.000     | 1.000     | 1.000       |
| Space group                                              | P2₁,2₁,2₁| P2₁,2₁,2₁| P2₁,2₁,2₁   |
| Unit-cell parameters                                     |           |           |             |
| a (Å)                                                    | 52.69     | 52.30     | 53.30       |
| b (Å)                                                    | 86.02     | 85.42     | 86.61       |
| c (Å)                                                    | 87.92     | 87.44     | 87.71       |
| Resolution (Å)                                           | 62.02 to 1.35 (1.40 to 1.35) | 62.86 to 2.20 (2.28 to 2.20) | 62.02 to 1.45 (1.50 to 1.45) |
| Measured reflections                                     | 1,797,205 | 687,303   | 891,274     |
| Unique reflections                                       | 87,069    | 20,272    | 72,191      |
| Completeness (%)                                         | 98.4 (98.4) | 99.8 (99.3) | 99.2 (97.9) |
| Mean I/σ(I)                                             | 21.1 (4.7) | 24.1 (6.4) | 26.5 (3.9)  |
| Rmerge (%)                                               | 3.0 (2.5)  | 8.4 (2.4)  | 5.3 (2.5)   |
| Refinement statistics                                    |           |           |             |
| Resolution (Å)                                           | 25.19–1.35 | 44.88–2.20 | 31.39–1.45  |
| R/ Rfree (%)                                             | 18.0/19.9 | 17.1/21.1 | 17.1/18.0   |
| No. of waters                                            | 583       | 302       | 495         |
| Average B-factors (Å²)                                   |           |           |             |
| Protein                                                  | 13.84     | 21.47     | 12.44       |
| Waters                                                   | 28.84     | 31.06     | 30.38       |
| Ni²⁺                                                    | 10.20     | 17.54     | 8.99        |
| Glycerol (packing site)                                  | 16.79     | 23.37     | 13.02       |
| Ligand 1                                                 | 30.62 (xylose) | 10.72 (xylose) | 21.27 (glycerol) |
| Ligand 2                                                 |           |           |             |
| Root mean square deviation from ideal values             |           |           |             |
| Bond lengths (Å)                                         | 0.005     | 0.006     | 0.005       |
| Bond angles (degrees)                                    | 1.2       | 1.2       | 1.3         |

* Rmerge = \( \sum_{h} \sum_{i} I(h,i) - \langle I(h) \rangle \sum_{i} I(h,i) \), where \( I(h,i) \) is the intensity of the \( i \)th measurement of reflection \( h \) and \( \langle I(h) \rangle \) is the average value over multiple measurements.

* Calculated using a test data set; 5% of total data randomly selected from the observed reflections.
The density peaks were relatively low and the temperature factors were relatively high (Table I), the density was clear in shape, so we could confidently determine the orientation and conformation of the xylose molecule. Further attempts to obtain other types of complex structures (i.e. co-crystallization of the wild-type Rex in 10 mM xylobiose or a mixture of 5 mM xylose and 5 mM xylobiose) resulted in a similar density to that in the case of WT-xylose (a xylose molecule was bound at subsite +1; data not shown). When the wild-type Rex crystals were soaked in 10 mM xylotriose for a short time (10 s), density peaks for sugar units ranging from subsite −2 to +1 were observed. However, the density at subsite −1 was ambiguous and appeared to reflect a mixture of several conformations, probably involving a fraction of the cleaved substrate. The second type of complex structure was obtained by co-crystallizing the inactive E70A mutant with xylotriose (E70A-xylobiose). In the substrate binding cleft of E70A-xylobiose, the clear density of a xylobiose unit at subsites −2 and −1, as well as a glycerol at subsite +1, was observed (Fig. 1b). There are two possibilities that explain the discrepancy between the co-crystallized reagent (xylotriose) and the density observed for xylobiose: (a) xylotriose had been cleaved during the crystal formation (6 days), although the hydrolytic activity of the E70A mutant is 10^4 orders lower than that of the wild-type; and (b) xylotriose is bound at subsites −3 to −1, but a xylose moiety at the nonreducing end could not be detected due to high mobility or disorder.

All three structures contain a metal ion and a glycerol, both of which bind at a crystal packing interface far from the active site (Fig. 2b). The metal ion was assigned as a nickel ion (Ni^2+) because a Ni^2+-nitrilotriacetic acid-agarose column was used for purification, and no other candidate was included in the reservoir solution. Refined temperature factors of the nickel ion in the three structures were within 8.9−17.6 Å^2 (Table I), and the refined F_0 − F_ maps were almost flat (data not shown). The nickel ion was tetrahedrally coordinated by Gln27, Gln30, Asp253, and His259 (symmetry-related residues are indicated by primes). The glycerol molecule was held by the main-chain atoms of Gln192, Tyr247, and Asp248 and the side chain of Trp123. Glycerol was absolutely required for crystallization (12). A polypeptide chain extending from Glu6 and Pro381 was modeled in WT-xylose and E70A-xylobiose, whereas Glu45 and Thr46 were not included in WT-native because of local disorder.

Overall Structure—The structure of Rex comprises a disordered (α/α)_6 barrel similar to that of pXyl, whereas CelA has a less disordered (α/α)_6 barrel with a circular cross-section (Fig. 2). The root mean square deviations as to pXyl and CelA are 1.8 Å for 357 residues and 2.3 Å for 335 residues, respectively. There are four free cysteine residues located inside the molecule. This characteristic is usual for intracellular enzymes. On the other hand, two cysteine residues of pXyl, which is an extracellular enzyme, form a disulfide bond (9). The molecular surfaces of the three GH-8a enzymes are shown in Fig. 3. The substrate-binding cleft of CelA is clearly larger than those of the other two enzymes. Interestingly, subsite +2 of Rex is blocked by a barrier at the upper side of the cleft (described below). In contrast, the other two endo-GHs have a long cleft spanning the molecule, which can accommodate a long polysaccharide chain.

Complex Structures—WT-native and the two complex structures (WT-xylose and E70A-xylobiose) of Rex were almost identical (root mean square deviation for Co atoms <0.26 Å and for all atoms <0.44 Å between all pairs of the three Rex structures). There was no large conformational change on substrate binding like on other GH-8a enzymes. For example, substrate binding on CelA induces only small structural changes in the protein, mostly slight reorientations of aromatic and polar side chains in contact with the substrate (11). However, substrate binding on Rex induced slight movements in two regions (Thr62−Asn64 and Gly355−Arg357) around subsite −1, accompanied by movement of the adjacent Asn356 residue. The side chain of Arg357 swings to form a specific interaction with the xylose at subsite +1, accompanied by movement of the adjacent Asn356 residue. The side chain of Arg357 was ordered in WT-xylose, whereas it was disordered in the other two structures.

The xylose molecule in WT-xylose takes on the 4C1 conformation, and all of the oxygen atoms (O-1 to O-5) form direct or water-mediated hydrogen bonds with protein atoms (Fig. 5a). His319 forms one of the two direct hydrogen bonds present between xylose and the Rex protein, which recognizes the β-hydroxyl group at the O-1 position. A stacking interaction of Tyr360 with the β-face of the xylose also occurs. This residue can sterically interfere binding of an α-anomer. When an α-glucopyranose molecule was superimposed onto the β-xylose, the α-anomeric hydroxyl oxygen atom was located at distances of 2.7 and 2.5 Å from the Oβ and Oγ atoms of Tyr360, respectively (see Supplemental Material). Actually, electron density for only the β-anomer was observed, probably because of these interactions.
In the E70A-xylobiose complex, the xylobiose molecule takes on the \(^4C_1\) conformation at both of the two sugar rings and the \(\beta\)-anomeric configuration at the reducing end. The glycerol molecule is located at almost the same position as subsite +1 and forms a water-mediated hydrogen bond with the xylobiose (Fig. 5b). Although the positions of the three hydroxyl oxygen atoms of the glycerol are slightly different from those of xylose at subsite +1, they interact with several residues at subsite +1 and cause movement of the main chain in the two regions described above. All of the oxygen atoms form direct or water-mediated hydrogen bonds with the protein, and the xylose ring at subsite -2 of the xylobiose is stacked with Trp\(^{112}\) at the \(\beta\)-face.

Subsites—Fig. 4b shows composite superimpositioning of the subsites of the three GH-8a enzymes: the WT-xylose structure of Rex combined with xylobiose in the E70A-xylobiose structure (gray), the pXyl D144N mutant structure (Protein Data Bank code 1H14) combined with a xylose molecule at subsite +4 in the complex structure (Protein Data Bank code 1H12) (green), and the CelA E95Q mutant structure complexed with cellopentaose (Protein Data Bank code 1KWF) (yellow). Both the cellopentaose molecule (subsites -3 to +2; ball-and-stick model) and the partially bound product molecule (cellotriose at subsites +1 to +3) are shown. Xylose and xylobiose in Rex approximately overlap with the glucose units at subsites -2 to +1 in CelA.

At subsite -3 of CelA, Trp\(^{205}\) forms a stacking interaction, and the side chain of Arg\(^{204}\) forms a hydrogen bond with the O-3 atom. However, there is no subsite here in pXyl and Rex. In Rex, the Trp is substituted by Ile\(^{188}\), and there is no corresponding residue with the Arg, losing specific interactions to a sugar moiety at this site. Rex exhibits significantly higher \(K_m\) for xyooligosaccharides of longer than xylotetraose (1). There is, however, no steric hindrance at subsite -3, and the subsite is highly accessible to solvent. We could not find any convincing structural factor that can make the binding effect on this subsite negative. Perhaps the side chain of Glu\(^{190}\), which is solvent-exposed and somewhat disordered in the crystal structure, may interfere with the sugar binding in an extended conformation (Fig. 4b). At subsite -2, the bound xylose/glucose groups of Rex and CelA almost completely overlap. The stacking Trp residue (Trp\(^{112}/\text{Trp}\(^{114}/\text{Trp}\(^{132}\)) in Rex, pXyl, and CelA) is conserved in GH-8a enzymes. The O-2, O-3, and O-5 atoms at subsite -2 form water-mediated hydrogen bonds with Tyr\(^{244}\) and Tyr\(^{198}\) (Fig. 5b).

A significant kink is observed between sugar rings at subsites -1 and +1, the two sugar rings being twisted to become almost perpendicular to each other. Catalytically important residues are rather concentrated around this region. Glu\(^{70}\) (Glu\(^{78}/\text{Glu}\(^{94}\) in pXyl and CelA) has been identified as the proton donor (1). The catalytic base residue (Asp\(^{263}/\text{Asp}\(^{281}/\text{Asp}\(^{278}\)) in Rex, pXyl, and CelA) holds a water molecule through hydrogen bonds together with a conserved Tyr residue (Tyr\(^{198}/\text{Tyr}\(^{203}/\text{Tyr}\(^{215}\)). The water is thought to correspond to the nucleophilic water, which is activated by the catalytic base residue (11). In the CelA-cellopentaose complex structure, the third catalytically important residue (Asp\(^{128}/\text{Asp}\(^{144}/\text{Asp}\(^{152}\)) forms bifurcated hydrogen bonds with the O-2 and O-3 atoms of the glucose unit at subsite -1 with the \(2,5\)-B conformation (11). The interaction is critical to stabilize the sugar ring in a

**Fig. 3.** Molecular surfaces of Rex (a), pXyl (b), and CelA (c), showing the substrate binding cleft. Positive and negative potentials are shown in blue and red, respectively. Ligand oligosaccharides are shown as a ball-and-stick model. c, a part of the cellopentaose molecule (subsites -3 to -1 of -3 to +2) and the cellotriose molecule (subsites +1 to +3) are shown.
strained boat conformation, which is thought to be a prerequisite for the inverting hydrolytic mechanism through a transition state with the oxocarbenium ion, the planarity of the atoms C-5, O-5, C-1, and C-2 facilitating the formation of a partial double bond between O-5 and C-1 (11). In the E70A-xylobiose structure, Asp128 does not form any interaction with the ligand. However, the side chain of Asp128 is positioned similarly with that of Asp152 of CelA, and it would also stabilize the xylose ring at subsite H11002 if the ring takes on a boat conformation. In summary, the catalytic mechanism of Rex seems to be basically conserved with other GH-8a enzymes.

At subsite H11001, the xylose molecule in Rex overlaps better with a glucose unit of the product cellotriose molecule in CelA, rather than that of the uncleaved cellobiase molecule. For CelA, the product molecule at subsites H11001 to H11002 is thought to represent a possible first step during which the leaving group rotates slightly and shifts away from the reaction center, the stacking interactions being preserved (11). The xylose molecule in WT-xylose of Rex also seems to correspond to a product molecule being released. Subsite H11001 of Rex is unique compared with that of other GH-8a enzymes, because the residues at this subsite (Asp61, Asn64, Arg68, Ser262, His319, and Arg357) are not conserved in CelA and pXyl. However, the stacking Tyr residue (Tyr360/Tyr381/Tyr372 in Rex, pXyl, and CelA) is conserved and fixes the sugar unit at an approximate position.

The most notable difference between Rex and the other two GH-8a enzymes is the blockage of subsite H11002 by the kink in the loop before Ser317-Pro320 (Fig. 4b). His319 is directly hydrogen-bonded with the β-hydroxyl of the xylose at subsite H11001, contributing to the discrimination of the anomers at the reducing end. Leu318 blocks subsite H11002 with its long side chain together with His319. A proline residue (Pro320) is present only in Rex, and the main chain trace bends at an almost right angle at this position. The bent loop structure seems to be intrinsically stable, because there is no conformational difference between the unliganded and liganded structures. On the other hand, in pXyl and CelA, this loop is located away from the substrate. Instead, a Tyr residue in the loop before Ser317-Pro320 (Tyr378/Tyr369 in pXyl and CelA) forms subsite H11003.

Specificity for Xylosides—The order of preference of Rex for xylo-/glucoooligosaccharides is XXX/GXX/XXG/GXX (where G represents glucose and X is xylose), and no detectable activity is observed for XGG, GGX, and XGX (1). This indicates that subsite H11001 strictly requires a xyloside, and the other two subsites also exhibit a certain level of selectivity. At the putative C-6 position of subsite H11002 in Rex, there is a long side chain of the Arg68 residue that interferes with binding of a glucose unit. Arg at
this position is conserved in pXyl (Arg<sup>76</sup>), whereas CelA has the Val<sup>93</sup> residue. Instead, a guanidium group of Arg<sup>84</sup> comes from another position in CelA. Therefore, the selectivity for xylose/glucose at subsite /H<sub>1</sub> seems to be caused by these Arg residues.

On the other hand, the selectivity at subsites /H<sub>2</sub> and /H<sub>1</sub>i is not structurally obvious. At subsite /H<sub>2</sub>, the main chain atom of Pro<sup>125</sup>-Ala<sup>126</sup> and the side chain of Ile<sup>188</sup> appear to sterically clash against an O-6 group. At subsite /H<sub>1</sub>i, the catalytic base residue (Asp<sup>263</sup>) appears to cause a steric hindrance. Actually, the catalytic base of CelA (Asp<sup>278</sup>) is rotated at its carboxyl end to avoid a steric clash. The residues in the distal area from the catalytic base are not conserved in CelA and the other two GH-8a enzymes. Molecular surface representation also shows that this area of CelA comprises a relatively wide open cleft (Fig. 3).

**Kinetic Analysis of H319A Mutant**—In order to investigate the importance of the His<sup>319</sup> residue in the discrimination of the anomers at the reducing end, we examined the enzymatic activity of the H319A mutant on X<sub>3</sub> and X<sub>3-de</sub> substrates as well as the kinetic parameters of X<sub>3</sub> hydrolysis (Table II). The H319A mutant exhibited significant decrease in the specificity to the anomeric hydroxyl group (activity ratio for X<sub>3</sub>/X<sub>3-de</sub> = 1.6), compared with the wild type (activity ratio for X<sub>3</sub>/X<sub>3-de</sub> = 31). The mutation caused a 14.3-fold increase in the $K_m$ value and a 7.8-fold decrease in the $k_{cat}$ value for X<sub>3</sub> hydrolysis. As shown in Fig. 6, however, the H319A mutant produced $\beta$-anomer of X<sub>1</sub> and $\alpha$-anomer of X<sub>2</sub> from X<sub>3</sub> in the reaction for 1 min. Furthermore the $\alpha$-anomer of X<sub>3</sub> was the predominant anomer remaining in the reaction. These results indicate that the H319A mutant still discriminates the $\beta$-anomer from the $\alpha$-anomer at the reducing end like the wild-type enzyme does, whereas the catalytic efficiency for the $\beta$-anomer is drastically decreased. The effect of the H319A mutation is consistent with the structural feature of Rex. Steric crash at the side chain of Tyr<sup>360</sup> remains in the H319A mutant, interfering with the binding of $\alpha$-anomer. However, loss of the direct hydrogen bond of His<sup>319</sup> leads to a drastic decrease in the activity toward the X<sub>3</sub> substrate. The remaining two water-mediated hydrogen bonds held by Asn<sup>64</sup>, Arg<sup>357</sup>, and Asp<sup>61</sup> (Fig. 5a) may be responsible for the slight preference of the H319A mutant for X<sub>3</sub> against X<sub>3-de</sub>.

**DISCUSSION** To date, the crystal structures of two types of reducing end-specific CBHs (Cel7A and Cel48A) have been determined, and similar mechanisms for processing of the substrate are proposed (7, 23). Both types of CBHs have a long tunnel formed by extra loops or domains covering a cleft of the core domain, and a long polysaccharide chain can penetrate into it. The two subsites at the reducing end are relatively open. After releasing
the cleaved disaccharide (cellbiose), the polysaccharide chain slides for the next processing position. The tunnels of Cel7A from Hypocrea jecorina (formerly known as CBH I from Trichoderma reesei) is formed by extra loops covering the cleft of the basal β-jelly roll fold, in comparison with other endo-type GH-7 cellulases having an open cleft (23). The tunnels of GH-48 CBHs from Clostridia (Cel48A; known as CelF and CelS) are formed by an extra β-domain on a (α/α)β barrel core, which is structurally similar to those of GH-8 enzymes (7, 24).

It is not clear to what extent GH-74 oligoxygenoglucean reducing end-specific cellobiohydrolase is specific for the reducing end sugars of substrates, because no complex structures are yet available. However, an insertion/deletion of a loop comprising about 10 amino acid residues has been implied to be the determinant for the exo- and endo-types of GH-74 enzymes (25).

Two GH-13 enzymes (maltodextrin glucosidase from E. coli K12 and an amyloytic enzyme from T. maritima) are also known to be reducing end-specific monomer-forming exo-glycosidases (2, 3). However, they can also release various xylooligosaccharides to incorporate into periplasm. The xylooligosaccharides are not specific for the group released from the reducing end-specific cellobiohydrolase is specific for the reducing end xyloside (25). Therefore, these enzymes are not specific for the exo- and endo-types of GH-74 enzymes (25).

As clearly seen in Fig. 4a, the O-2 and O-3 atoms of the xylose at subsite −2 point into solvent, whereas those atoms at subsites −1 and +1 are blocked, suggesting that Rex can hydrolyze xylooligosaccharides decorated at xylose n − 2 (when n is the released xylose at the reducing end). Recently, it was shown that the major decorated xylooligosaccharides products generated by GH-10 xylosanes contain arabino- or glucuronic side chains at the nonreducing end (26–28). The xylooligolytic mechanism of Cellulibrio japonicus, a mesophilic bacterium, has been proposed according to the localization and the specificities of the related enzymes (28). In the mechanism, xylan is initially solubilized by the three secreted endoxylanases (Xyn10A, Xyn11A, and Xyn11B), followed by processing with the cell-bound endoxylanase (Xyn10C), α-arabinofuranosidase (Abf1), and α-gluconuronicidase (GlcA67) into short undecorated xylooligosaccharides to incorporate into periplasm. The xylooligosaccharides are degraded in periplasm by the specific endoxylanase (Xyn10D) and transported into cytoplasm as cellbiose. Finally, the cellbiose was converted into cytole in the cytoplasm by intracellular β-xylosidases. The xylanolytic system of B. halodurans, an alkalophilic bacterium, is supposed to be different from that of C. jecorina. In the system, xylan is degraded into xylooligosaccharides having an α-arabinofuranosyl or α-glucuronyl residue at their nonreducing ends by the two extracellular alkalophilic endoxylanases (29, 30), and the resultant oligosaccharides are directly transported into the cytoplasm (1). The reducing end specificity of Rex might be useful to hydrolyze such oligosaccharides by the concurrent action with the intracellular α-arabinofuranosidases and α-glucuronidase (1), to produce smaller undecorated xylooligosaccharides, which are suitable substrates for β-xylosidases.

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