Characterization of a Novel β-L-Arabinofuranosidase in Bifidobacterium longum

FUNCTIONAL ELUCIDATION OF A DUF1680 PROTEIN FAMILY MEMBER

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Background: β-L-Arabinofuranosyl linkages are found in many plant biopolymers, but the degradation enzyme has never been found.

Results: A novel β-L-arabinofuranosidase was found in Bifidobacterium longum.

Conclusion: β-L-Arabinofuranosidase plays a key role in Bifidobacterium longum for β-L-arabinooligosaccharide usage.

Significance: The members of the DUF1680 family might be used for the degradation of plant biopolymers.

Pfam DUF1680 (PF07944) is an uncharacterized protein family conserved in many species of bacteria, actinomycetes, fungi, and plants. Previously, we cloned and characterized the hypBA2 gene as a β-L-arabinobiosidase in Bifidobacterium longum JCM 1217. In this study, we cloned a DUF1680 family member, the hypBA1 gene, which constitutes a gene cluster with hypBA2. HypBA1 is a novel β-L-arabinofuranosidase that liberates l-arabinose from the l-arabinofuranose (Araf)-β1,2-Araf disaccharide. HypBA1 also transglycosylates 1-alkanols with retention of the anomeric configuration. Mutagenesis and azide rescue experiments indicated that Glu-338 is a critical residue for catalytic activity. This study provides the first characterization of a DUF1680 family member, which defines a new family of glycoside hydrolases, the glycoside hydrolase family 127.

β-L-Arabinofuranosyl linkages with 1–4 arabinofuranosides are found in the sugar chains of extensin and solanaceous lectins (1, 2). Extensins and solanaceous lectins are members of the hydroxyproline (Hyp)2-rich glycoproteins that are widely observed in plant cell wall fractions. Furthermore, terminal β-L-arabinofuranosyl residues have been found in arabinogalactan protein from the pollen of timothy grass (3), rhamnogalacturonan-II (4–6), olive arabinan (7), arabinoglucan from Angelica sinensis (8), and tomato arabinoxylloglucan (9). However, despite the broad distribution of β-L-arabinofuranosyl residues in plant cells, the degradative enzyme β-L-arabinofuranosidase (EC 3.2.1.185)3 has never been found.

Recently, we cloned a hypBA2 gene that encodes a novel β-L-arabinobiosidase from Bifidobacterium longum JCM 1217 on the basis of the sequence of BL0421 from B. longum NCC2705, which belongs to the glycoside hydrolase (GH) family 121 (10). The enzyme releases Araf-β1,2-Araf disaccharide (β-Ara2) from Araf-β1,2-Araf-β-Araf-β-Hyp (Ara2–Hyp). Because released β-Ara2 should be hydrolyzed by its own enzyme for assimilation, we predicted that B. longum has a gene encoding β-L-arabinofuranosidase. BL0422 is part of a gene cluster with BL0421 and BL0420 and contains a domain of unknown function (DUF) 1680 family in the Pfam database (PF07944), which is a large family annotated as putative glycosyl hydrolases of unknown function.

In this study, we cloned the gene of a BL0422 ortholog from B. longum JCM 1217 and characterized the recombinant protein as a novel β-L-arabinofuranosidase. This is the first report of the characterization of a DUF1680 family member.

EXPERIMENTAL PROCEDURES

Materials—Extensin, potato lectin, Hyp-linked β-L-arabinooligosaccharides, β-Ara2, and Araf-β1,2-Araf-β-OMe (Ara2–Me) were prepared as described previously (10). Dansylated Hyp-linked β-L-arabinooligosaccharides were prepared as described by Gray (11). p-Nitrophenyl (pNP) substrates were obtained from Sigma. l-Arabinose was obtained from Wako Chemicals. The chemical structures of substrates are shown in Fig. 1. HypBA2-Δ486 was expressed and purified as described previously (10).

Expression and Purification of Recombinant HypBA1—The genomic DNA of B. longum JCM 1217 was extracted using a FastPure DNA kit (Takara) and then used for PCR amplification of the gene for the BL0422 ortholog, hypBA1. The forward (5′-AACAGGATATACATATGGAACGTTACCATT-CCT-3′) and reverse (5′-GGGCGCGCTGACACAGGCTAAT-3′) primers were designed from nucleotides 4–22 and 1959–1974, respectively, of BL0422 from B. longum JCM 1217.
longum NCC2705 to generate a C-terminal His₆-tagged recombinant protein. The PCR amplification product of hypBA1 was cloned into the pET-23b vector (Novagen) with the In-Fusion Advantage PCR cloning kit (Clontech). The full-length hypBA1 gene was sequenced on an ABI 3100 DNA sequencer with a Big-Dye Terminator 3.1 cycle sequencing kit (Applied Biosystems). The resulting pET23b-hypBA1 plasmid was transformed into Escherichia coli BL21 (DE3) cells, which were then grown at 20 °C by using the Overnight Express Autoinduction System (Novagen). Subsequently, the cell cultures were centrifuged, and the resultant pellet was resuspended in BugBuster protein extraction reagent (Novagen). The His-tagged proteins were purified on TALON metal affinity resin (Clontech), desalted by dialysis with a cellulose membrane (Wako), and concentrated using a 10-kDa ultrafiltration membrane (Millipore).

**Enzyme Assays**—The hydrolytic activity of the HypBA1 enzyme was assayed using dansylated cis-Ara₂-Hyp-DNS as a substrate. The 40-μl reaction mixture contained 50 mM sodium acetate buffer (pH 4.5), 25 μM substrate, 5 mM tris(2-carboxyethyl)phosphine (TCEP), and 0.17 milliunits ml⁻¹ of the HypBA1 enzyme. One unit of enzyme activity was defined as the amount of enzyme required to produce 1 μmol of cis-Ara-Hyp-DNS per min. After incubating the reaction mixture at 37 °C, the reaction was stopped by adding 10 μl of 5% trichloroacetic acid and then analyzed by HPLC. The sample was applied to a Cosmosil 5C18-AR-II (2.5 μm, Nacalai) column at 30 °C with a mobile phase of methanol and 20 mM sodium phosphate (pH 2.5) (60:40, v/v) and a constant flow rate (1.0 ml min⁻¹). The elution was monitored by a fluorescence detector (FP-202, Jasco) with excitation and emission wavelengths of 365 and 530 nm, respectively. For TLC analysis of dansylated substrates, the spots on the plates were developed with a 3:1:1 mixture (v/v/v) of 1-butanol/acetic acid/water and then visualized with UV light.

**FIGURE 1. Chemical structures of the β-L-arabinooligosaccharides used in this study.** The arrows indicate the cleavage sites for HypBA1.
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Substrate Specificity of HypBA1—Arafβ-Hyp (Ara-Hyp), Ara2-Hyp, Ara3-Hyp, Araf-α,1,3-Araf-B1,2-Araf-B1,2-Araf-β-Hyp (Ara5-Hyp), Ara2-Me, β-Ara2, and pNP substrates were incubated at 37 °C for 16 h with 0.17 milliunits/mL of HypBA1 enzyme in 100 μL of 50 mM sodium acetate buffer (pH 4.5). The reaction was stopped by boiling for 3 min. For TLC analysis, oligosaccharides were spotted on a Silica Gel 60 aluminum plate (Merck) using a 2:1:1 solvent mixture (v/v/v) of ethyl acetate/acetic acid/water. The sugars were visualized by spraying an orcinol-sulfate reagent onto the plate (12). For high performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) analysis, oligosaccharides were analyzed with a CarboPac PA-1 column. The column performance anion-exchange chromatography with pulsed amperometric detection

The enzymatic activity for dansylated cis-Ara2-Hyp-DNS for 16 h at 37 °C and then analyzed by HPLC as described above.

Bacterial Strains and Culture Conditions—The bifidobacteria strains grown in Gifu anaerobic medium (GAM) broth (Nissui) were as follows: B. longum JCM 1217 and JCM 7054; B. longum subsp. infantis JCM 1222; B. pseudolongum JCM 1205; B. adolescentis JCM 1275; B. breve JCM 1192, and B. bifidum JCM 1254. The in vitro fermentation ability of β-Ara2 was tested using B. longum JCM 1217 and B. adolescentis JCM 1275 in peptone-yeast extract-Fildes (PYF) medium (13) containing 0.25% β-Ara2, glucose, or l-arabinose. The bacteria were cultured for 3 days at 37 °C under anaerobic conditions. The bacterial growth was judged from the decreased pH of the culture solution (14).

Assays of Bacterial Enzyme Activities—The cell cultures were centrifuged at 17,000 × g for 20 min, and the resultant pellets were washed with 50 mM Tris-HCl buffer (pH 6.8). Afterward, they were resuspended in 50 mM Tris-HCl buffer (pH 6.8) and sonicated with a Sonifier 250 (Branson). The cell lysates were incubated with 25 μM cis-Ara2-Hyp-DNS for 16 h at 37 °C and then analyzed by HPLC.

RESULTS

Expression and Purification of HypBA1—HypBA1 consisted of 658 amino acid residues exhibiting 98.9% identity with that of BL0422 and coincided with that of BL11J_0211 from B. longum JCM 1217, for which the complete genome sequence is available (15). The recombinant HypBA1 protein was expressed at 20 °C as a soluble protein. SDS-PAGE showed that the purified recombinant HypBA1 protein migrated as a single band with an apparent molecular mass of 74 kDa (Fig. 2), which was in agreement with its calculated molecular mass of 74,329 Da. The final yield of the purified enzyme was 140 mg/liter of culture.

Substrate Specificity and General Properties of HypBA1—The enzymatic activity for dansylated cis-Ara2-Hyp-DNS was detected in the presence of β-mercaptoethanol, dithiothreitol, or TCEP but not in the absence of reducing agents (Fig. 3). Several β-l-arabinooligosaccharides and synthetic pNP substrates were used to identify the substrate specificities for HypBA1 in the presence of TCEP. The enzyme released l-arabinose from Ara-Hyp, Ara2-Hyp, Ara3-Hyp, and Ara2-Me, but it did not act on pNP-α-l-arabinopyranoside, pNP-α-l-arabinofuranoside, pNP-β-l-arabinopyranoside, or Ara4-Hyp (Fig. 4). HypBA1 also released l-arabinose from β-Ara2 (Fig. 5B). The suitable temperatures and pH values for cis-Ara2-Hyp-DNS were determined at 35–40 °C and 4.5, respectively (Fig. 6). The

### Table 1

<table>
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<th>Name</th>
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<td>E322A_Forward_Primer</td>
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<td>5’-CGTACCTACGTGCTTACCTACG-3’</td>
</tr>
<tr>
<td>E338A_Forward_Primer</td>
<td>5’-CAGCTGTTACGGTACACGTGCTTACCTACG-3’</td>
</tr>
<tr>
<td>E338A_Reverse_Primer</td>
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<td>E322A_Forward_Primer</td>
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</tr>
<tr>
<td>E322A_Reverse_Primer</td>
<td>5’-GGTTGACAGTCTCTGCGCGCGCGCTTACCG-3’</td>
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</table>
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specific activity of the purified enzyme was 2.1 units·mg⁻¹ protein. The kinetic parameters for β-Ara₂, cis-Ara₂-Hyp-DNS, and cis-Ara-Hyp-DNS are summarized in Table 2. The $K_m$ and $k_{cat}$ values for β-Ara₂ and cis-Ara₂-Hyp-DNS were within the same range, but the $k_{cat}$ value for cis-Ara-Hyp-DNS was 480-fold lower than that of cis-Ara₂-Hyp-DNS. Consequently, the $k_{cat}/K_m$ ratio of cis-Ara-Hyp-DNS was 670-fold lower than that of cis-Ara₂-Hyp-DNS. HPAEC-PAD analysis showed that L-arabinose was released from Ara₂-Hyp and then the liberated Ara-Hyp gradually hydrolyzed to L-arabinose and Hyp (Fig. 5A). Likewise, both cis- and trans-Ara₂-Hyp-DNS also hydrolyzed to Ara-Hyp-DNS, which then hydrolyzed to Hyp-DNS (Fig. 7). Under the conditions in which Ara₂-Hyp could be degraded by HypBA2 and HypBA1 (Fig. 8A, lane 4), the reactivities for the glycoproteins were tested. Liberated sugars were detected from carrot extensin and potato lectin by HypBA2 but not by HypBA1 (Fig. 8). Furthermore, HypBA1 did not act on pNP-galacto-, gluco-, and xylo-pyranosides. The substrate specificity is summarized in Table 3. These results suggested that HypBA1 reacts with the liberated β-L-arabinooligosaccharides. Consequently, we classified the enzyme as an exo-acting β-L-arabinofuranosidase. The cleavage sites for HypBA1 are shown in Fig. 1.

Transglycosylation Activity of HypBA1—When 1-alkanols were used as the acceptors, the transglycosylation products were detected on TLC (Fig. 9A). The purified transglycosylation product (methyl L-arabinofuranoside) was hydrolyzed to L-arabinose by the HypBA1 treatment (Fig. 9B), which indicates that the methanol was linked by the β-anomeric form. The structure of this product was determined by $^1$H and $^{13}$C NMR (Fig. 10 and Table 4). The $^1$H NMR spectrum showed the anomeric proton as a doublet at 4.74 ppm with coupling constant $J_{1,2} = 4.8$ Hz. Furthermore, the $^{13}$C NMR spectra revealed that the transglycosylation product was found to be consistent with
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A methyl β-L-arabinofuranoside (Ara-Me) (16). These data indicated that HypBA1 is a retaining enzyme.

Sequence Analysis of HypBA1—HypBA1 consisted of 658 amino acids that included DUF1680 without other sequence motifs (Fig. 11). HypBA1 was 38–98% identical to other DUF1680 members from bifidobacteria (Fig. 11 and supplemental Fig. S1). Duplicated DUF1680 members were found in the sequences of almost all Bifidobacterium species. HypBA1 (BLLJ_0211) constitutes a gene cluster with HypBA2 (BLLJ_0212) and a GH43 family member (BLLJ_0213) (Fig. 11). The gene cluster was conserved in B. longum NCC2705, B. longum subsp. infantis 157F, and Bifidobacterium catenulatum DSM 16992. In addition, the gene cluster without the GH43 family member was conserved in Bifidobacterium catenulatum DSM 20438 and Bifidobacterium dentium ATCC 27678.

Critical Amino Acid Residues of HypBA1—The candidate acidic amino acid residues were selected for site-directed mutagenesis studies based on multiple alignments and the HMM logo of the DUF1680 family in the Pfam database (17). Alanine substitutions were introduced at the positions of Glu-322, Glu-338, and Glu-366, which are highly conserved among the HypBA1 homologs (indicated as asterisks in supplemental Fig. S1). The mutant enzymes were purified for the determination of specific activities. The E322A and E338A mutant enzymes were recovered in the soluble fractions with BugBuster. The E338A mutant enzyme exhibited a significant decrease in activity (0.0013%), and the E322A mutant showed 1.5% of the activity relative to the wild-type enzyme (Table 5). The E366A mutant enzyme was insoluble, and only a small amount of protein was recovered. Nonetheless, it exhibited 16% relative activity compared with the wild-type enzyme. The effect of external nucleophile on the activity of the E338A mutant was further investigated by using different concentrations of sodium azide. The activity of the mutant was rescued by the addition of azide (Fig. 12). In the presence of 200 mM sodium azide, the enzymatic activity was 33-fold greater than in the absence of external nucleophile. We also confirmed azide rescue by β-Ara2 as a substrate, whereas the glycosyl azide product was not observed on HPAEC-PAD and TLC (data not shown).

In Vitro Fermentability of β-Ara2 by B. longum—First, lysates of bifidobacterial cells grown in GAM were used as the enzyme...
source. β-L-Arabinofuranosidase activity was found in the cell lysate of B. longum JCM 1217 and B. longum JCM 7054 but not in that of B. adolescentis JCM 1275, B. breve JCM 1192, B. bifidum JCM 1254, B. pseudolongum JCM 1205, or B. longum subsp. infantis JCM 1222 (Fig. 13A). Moreover, enzymatic activity was not observed in the culture medium or in the bacterial cell suspensions for all Bifidobacterium strains described above (data not shown). The PYF medium containing 0.25% β-L-Ara₂ was utilized as a carbohydrate source by B. longum JCM.
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1217 but not by B. adolescentis JCM 1275 (Table 6). In addition, β-Ara\textsubscript{2} in the PYF medium was utilized by the fermentation of B. longum JCM 1217 (Fig. 13B). Furthermore, β-L-arabinofuranosidase activity was found in the cell lysate of B. longum JCM 1217 grown on β-Ara\textsubscript{2} but not in the lysate of cultures grown in media containing glucose and L-arabinose (Fig. 13C). These data suggested that β-Ara\textsubscript{2} is metabolized by β-L-arabinofuranosidase in B. longum.

**DISCUSSION**

The DUF1680 family has 597 members distributed among 315 species of enteric bacteria (i.e. Bifidobacterium, Bacteroides, Salmonella, Clostridium, and Escherichia), plant-pathogenic Xanthomonas, actinomycetes, fungi, and plants, as shown in the Pfam database. The members of this family are hypothetical proteins of unknown function and have no sequence similarity with other glycoside hydrolase families. In this study, we cloned the gene encoding a member of the DUF1680 family and characterized its product as a novel β-L-arabinofuranosidase. Therefore, we propose that the enzyme be assigned to a new family of glycoside hydrolases, the glycoside hydrolase family 127.

**FIGURE 12. Effect of azide concentration on the activity of HypBA1 E338A mutant.** Error bars show the standard deviation of triplicate measurements.

**TABLE 4**

Assignments of signals in \textsuperscript{1}H and \textsuperscript{13}C NMR spectra of methyl β-L-arabinofuranoside

<table>
<thead>
<tr>
<th>Assignment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Me</th>
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<tr>
<td>\textsuperscript{1}H (6)</td>
<td>4.74</td>
<td>3.99</td>
<td>3.85</td>
<td>3.73</td>
<td>3.61</td>
<td>3.46</td>
</tr>
<tr>
<td>\textsuperscript{13}C (6)</td>
<td>102.72</td>
<td>76.86</td>
<td>75.03</td>
<td>82.54</td>
<td>63.65</td>
<td>55.65</td>
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**TABLE 5**

The specific activities of HypBA1 mutants

<table>
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<th>Specific activity* (milliliters mg\textsuperscript{-1})</th>
<th>Percentage of specific activity</th>
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<tr>
<td>Wild type</td>
<td>2100</td>
<td>100</td>
</tr>
<tr>
<td>E338A</td>
<td>0.028</td>
<td>0.0013</td>
</tr>
<tr>
<td>E366A</td>
<td>340</td>
<td>16</td>
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</table>

* Enzymatic activities were determined using cis-isomer of Ara\textsubscript{2}-HyD-DNS.

**FIGURE 11. Phylogenetic relationships between HypBA1 homologs in Bifidobacterium strains.** The black boxes indicate the DUF1680 conserved region (middle). The lengths of the sequences are shown on the right side of each schematic sequence. The organisms, locus tag, and GenBank\textsuperscript{19} accession numbers are as follows: BL1, B. longum subsp. longum JCM 1217, 1 (BL1J_0211 and BAJ65881), 2 (BL1J_1826 and BAJ67491), 3 (BL1J_1848 and BAJ67512), 4 (BL1J_0089 and BAJ65759); BL1, B. longum NCC2705, 1 (BL0422 and AAN24259), 2 (BL0174 and AAN24029); BL, B. longum subsp. infantis 157F, 1 (BLIF_0192 and BAJ70339), 2 (BLIF_1895 and BAJ70209); BB, B. pseudocatenulatum DSM 20438, 1 (BIFPSEUDO_02879 and EGG71985), 2 (BIFPSEUDO_02839 and EGG71945); BC, B. catenulatum DSM 16992, 1 (BIFCAT_0050 and EEB20621), 2 (BIFCAT_00247 and EEB22303), 3 (BIFCAT_01782 and EEB20699); BD, B. dentium ATCC 27678, 1 (BIFDEN_01462 and EDT45627), 2 (BIFDEN_00978 and EDT45157); BAd, B. adolescentis ATCC 15703 (BAD\textsubscript{1} //529 and BAF40310); BAn, B. animalis subsp. lactis AD011 (BLA\textsubscript{1} //513 and ACL29795); BB, B. breve DSM 20213 (BIFBRE_03130 and EFE89858). The protein characterized in this study is enclosed in the box. The phylogenetic tree was constructed with the neighbor-joining method using MEGAS software (left). Comparison of the gene clusters containing GH127, GH121, and GH43 members in Bifidobacterium strains (right). aa, amino acids.
**TABLE 6**

<table>
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<th>Carbon source</th>
<th>B. longum JCM 1217</th>
<th>B. adolescentis JCM 1275</th>
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<tr>
<td>Glucose</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>l-Arabinose</td>
<td>++</td>
<td>−</td>
</tr>
<tr>
<td>β-Ara₂</td>
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β-Ara₂ was a suitable substrate for HypBA1 as well as Ara₃-Hyp and Ara₂-Hyp, which contain the Araβ₁,2-Ara₂/Ara₃ structure at the nonreducing terminal. In extensins, β-l-arabinofuranosylsaccharides are in close existence on repetitive Ser-Hyp₄ motifs and contribute to protease resistance. It is thought that Hyp-linked β-l-arabinofuranosylsaccharides do not occur naturally in the normal environment. Furthermore, HypBA1 did not directly release l-arabinose from extensin or potato lectin (Fig. 8). In addition, we showed that β-Ara₂ was used as a carbohydrate source for B. longum, with enzymatic activity detected in the cell lysate (Table 6 and Fig. 13). Interestingly, the enzymatic activity was not detected in cells grown in the presence of l-arabinose or glucose. The amino acid sequence of HypBA1 lacks both a signal secretory and a transmembrane domain. Collectively, these results indicate that HypBA1 is an intracellular enzyme that degrades HypBA2-released β-Ara₂, as schematically summarized in Figs. 14 and 15.

Previously, we characterized an endo-α-N-acetylgalactosaminidase (BLIJ_0168) from B. longum JCM 1217, which releases Gal-β₁,3-GalNAc (GNB) disaccharide from core-1 mucin-type O-glycans (18). Kitaoka and co-workers (19, 20) proposed a metabolic pathway for GNB from core-1 mucin-type O-glycans and Gal-β₁,3-GlcNAc (LNB) from human milk oligosaccharides based on the characterization of the genes encoded in the GNB/LNB operon (BLIJ_1620-BLLJ_1626) of B. longum. Fushinobu and co-workers (21, 22) characterized the GNB/LNB-binding protein (BLIJ_1626) of an ATP-binding cassette-type sugar transport system in the GNB/LNB pathway. As shown in Fig. 15, the contiguous genes of the hypBA1 gene (BLIJ_0211) have also been annotated as encoding subunits of a putative ATP-binding cassette-type sugar transport system (23) as follows: a solute-binding protein (BLIJ_0208) and two transmembrane subunits (BLIJ_0209 and Blij_0210). Blij_0208 exhibits 28% identity with the GNB/LNB-binding protein, and Blij_0209 and Blij_0210 also have >27% identity with the GNB/LNB transmembrane subunits (BLIJ_1624 and Blij_1625). Furthermore, the neighboring gene (BLIJ_0207) is predicted to be a LacI-type transcriptional regulator. Thus, we expect that Blij_0207 will regulate the gene cluster containing the β-Ara₂ transport system (BLIJ_0208-BLLJ_0210) and the β-l-arabinofuranosylsaccharides degradation enzymes (BLIJ_0211-BLLJ_0213) by internalizing β-Ara₂.
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The β-L-arabinofuranosidase metabolic pathway in *B. longum* is predicted as shown in Fig. 15. First, a GH43 family member (BLLJ_0211) releases L-arabinose from extensin (Ara4-Hyp) and then HypBA2 (BLLJ_0212) releases β-Ara2 (Ara3-Hyp to Ara2-Hyp) on the bifidobacterial cell surface. Next, the released L-arabinose and β-Ara2 are internalized into the bifidobacterial cell by uncharacterized transport system and predicted β-Ara2 transport system (BLLJ_0208-BLLJ_0210), respectively. Then, HypBA1 (BLLJ_0211) degrades β-Ara2 to L-arabinose. Furthermore, the L-arabinose metabolic enzymes for the conversion to D-xylulose 5-phosphate, which have been characterized in *Corynebacterium glutamicum* ATCC 31831 (24), exhibit 50–59% identity with those of *B. longum* JCM 1217 as follows: L-arabinose isomerase (BLLJ_0342), L-ribulokinase (BLLJ_0340) and L-ribulose 5-phosphate 4-epimerase (BLLJ_0341). As a result, HypBA1 plays a key role in *B. longum* for β-L-arabinofuranosidases usage as a carbohydrate and energy source.

Recently, Fukuda et al. (15) reported that *B. longum* has an advanced ability for fructose uptake and acetate production, with the released acetate improving the intestinal defense mediated by epithelial cells. In addition to fructose, L-arabinose is a naturally found common carbohydrate and is found as a component of biopolymers such as hemicellulose and pectin. *B. longum* JCM 1217 encodes a number of candidates for the α-L-arabinofuranosidase gene, 11 members of the GH43 gene family, and 4 members of the GH51 gene family. Several reports indicate that *B. longum* has the ability to grow on L-arabinose and α-L-arabinofuranosaccharides (14, 23, 25–27). We showed that *B. longum* also uses β-Ara2 as a carbohydrate source (Table 6). Several α- and β-L-arabinofuranosaccharide degradation enzymes in *B. longum* might be involved in L-arabinose acquisition from plant polymers in the large intestine.

HypBA1 was identified as a retaining glycoside hydrolase, as described above. Hydrolysis by retaining glycoside hydrolases proceeds through a double-displacement mechanism with two catalytic residues. The catalytic residues typically utilized are either aspartate or glutamate residues. In the chemical rescue study, E338A mutant was rescued by the addition of azide, which suggests that Glu-338 is a catalytic residue for HypBA1. However, no glycosyl azide product was formed in the reaction mixture. A water molecule activated by azide ion might be reactivated E338A mutant without glycosyl azide production, as shown in GH43 β-xylanase and GH14 β-amylase (28, 29).

*B. longum* JCM 1217 encodes four members of the DUF1680 family (BLLJ_0211, BLLJ_0212, BLLJ_0213, and BLLJ_0214), whereas *B. longum* NCC2705 encodes two members (BL0422 and BL0174) (Fig. 11). BL0422 constitutes a conserved gene cluster with the GH121 β-L-arabinobiosidase gene and the GH43-encoding gene as well as BLLJ_0211. BL0174 (98.8% identity with BLLJ_0216) is flanked by a gene cluster with five GH43 members and one α-galactosidase (BL0176–BL0190), whereas BLLJ_0216 is flanked by a small gene cluster without GH43 members (BLLJ_0182–BLLJ_0184). Interestingly, BLLJ_0184 constitutes a gene cluster with five duplicated GH43 members (BLLJ_0185–BLLJ_0184), in which the cluster is replaced by insertion sequences in *B. longum* NCC2705.

Hydroxyproline-rich glycoproteins that contain β-L-arabinofuranosides are widely distributed in land plants, mosses, ferns, and green algae (30). Furthermore, terminal β-L-arabinofuranosides are found in many plant biopolymers (3–8) and in yessotoxin from the dinoflagellate algae *Protoceratium reticulatum* (31, 32). Because DUF1680 family members are conserved in many species of bacteria, actinomycetes, fungi, and plants, they are thought to play a role in the effective degradation of plant biopolymers as well as hydroxyproline-rich glycoproteins.

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REFERENCES


Characterization of a Novel β-L-Arabinofuranosidase


