Characterization of a GH36 β-L-Arabinopyranosidase in *Bifidobacterium adolescentis*

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Abstract: β-L-Arabinopyranosidases are classified into the glycoside hydrolase family 27 (GH27) and GH97, but not into GH36. In this study, we first characterized the GH36 β-L-arabinopyranosidase BAD_1528 from *Bifidobacterium adolescentis* JCM1275. The recombinant BAD_1528 expressed in *Escherichia coli* had a hydrolytic activity toward p-nitrophenyl (pNP)-β-L-arabinopyranoside (Arap) and a weak activity toward pNP-α-D-galactopyranoside (Gal). The enzyme liberated L-arabinose efficiently not from any oligosaccharides or polysaccharides containing Arap-β1,3-linkages, but from the disaccharide Arap-β1,3-Ara. However, we were unable to confirm the in vitro fermentability of Arap-β1,3-Ara in *B. adolescentis* strains. The enzyme also had a transglycosylation activity toward 1-alkanols and saccharides as acceptors.

Key words: β-L-arabinopyranosidase, glycoside hydrolase family 36, arabinogalactan protein, *Bifidobacterium adolescentis*, transglycosylation

INTRODUCTION

L-Arabinose is a widely occurring sugar in plant cell wall polysaccharides and proteoglycans such as arabinan, arabinoxylan, pectic arabinoxylan, arabinogalactan, and gum arabic, which is substituted in GH4, GH27, GH36, GH57, GH97, and GH110. In *B. adolescentis* strains, the enzyme also had a transglycosylation activity toward 1-alkanols and saccharides as acceptors.

Streptomyces avermitilis,6 Fusarium oxysporum,7 Chitinophaga pinensis,8 Geobacillus stearothermophilus,9 and *A. thaliana*.10 We also cloned and characterized GH27 β-L-arabinopyranosidase (BLJ1823) from *Bifidobacterium longum* subsp. *longum*.11 GH97 β-L-arabinopyranosidase has recently been cloned from *Bacteroides thetaiotamomycron*.12 Because of the structure similarity between β-L-Arap and α-D-galactopyranosyl (α-Gal), α-D-galactopyranosidase (EC 3.2.1.22) can potentially possess a β-L-arabinopyranosidase activity. In fact, almost all β-L-arabinopyranosidases belonging to GH27 and GH97 have α-D-galactopyranosidase activity.

On the other hand, α-D-galactopyranosidases are included in GH4, GH27, GH36, GH97, GH79, and GH110. In particular, GH36 α-D-galactopyranosidases have been characterized in several bacteria, fungi, and plants. However, the weak β-L-arabinopyranosidase activity (the *K*_<sub>c</sub>) for pNP-β-Arap is 500-fold higher than that for pNP-α-Gal was reported only in the GH36 from the archaean *Sulfobus solfataricus*.13 GH36 α-D-galactopyranosidases have been cloned and characterized in several bifidobacterial species such as *B. longum*,14 *B. adolescentis*,15 *B. bifidum*,16 and *B. breve*.17 These enzymes also have transglycosylation activities for the synthesis of neo-glycoconjugates. *B. adolescentis* JCM1275 encodes BAD_1528, which is another member of GH36 with 24 % identity of a characterized GH36 α-D-galactopyranosidase BAD_1576. In this study, we characterized BAD_1528 from *B. adolescentis* JCM1275 as a novel GH36 β-L-arabinopyranosidase.
USA). Larch AG was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Wheat flour arabinoxylan and sugar-beet arabinan were obtained from Megazyme ( Wicklow, Ireland). These polysaccharides were used after purification by ethanol precipitation. β-Ara-1,3-α-Ara-1,3,β-Gal-1,6-β-Gal-1,6-Gal (Arab-β1,3-Araf-Gal,) was prepared from larch AG as previously described. All other chemicals were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Arab-β1,3-L-arabinitol (Arab-β1,3-ART) was prepared from Arab-β1,3-Ara by re-duction with sodium borohydride.

**Expression and purification of recombinant BAD_1528.** The genomic DNA of *B. adolescentis* JCM1275 was used for the PCR amplification of the BAD_1528 gene. The for-ward (5’- AGGAGATATACCATGATCGGCGTG- TATCT-3’) and reverse (5’- GTGTGTTGCCGTCGAGCGGCGAAGATGC-3’) primers were designed from nucleotides 1–17 and 2387–2403, respectively. The underlined letters represent the nu-cleo-tides complementary to the template. The amplicon was subsequently cloned into the pET-23d vector (Novagen Inc., Madison, WI, USA) using an In-Fusion HD cloning kit (Clontech Laboratories Inc., Palo Alto, CA, USA). This nucleotide sequence reported in this paper has been depos-ited in the DDBJ/GenBank/EMBL Data Bank with acces-sion number LC374389. *E. coli* SoluBL21 cells (Genlantis, Inc., San Diego, CA, USA) were transformed with the plasmid and subsequently grown at 37 °C using the Overnight Express Autoinduction System (Novagen). The cell culture was subsequently centrifuged, and the resultant pellet was then resuspended in the BugBuster protein extraction re-agent (Novagen). The His-tagged BAD_1528 protein was purified with a column containing the TALON metal affini-ty resin (Clontech). The 10 mM imidazole fraction contain-ing the eluted protein was desalted and concentrated using an ultrafiltration membrane with a 10 kDa cutoff (Millipore Co., Billerica, MA, USA).

**Enzyme assays.** The hydrolytic activity of the BAD_1528 enzyme was assayed using pNP-β-Ara as the standard substrate. The 200 μL reaction mixture containing BAD_1528 and 1 mM substrate in 50 mM sodium acetate buffer (pH 5.0) was pre-incubated at 40 °C for 2 h with 100 mU/mL of BAD_1528 in 40 μL of 50 mM sodium acetate buffer (pH 5.0) with 10 % methanol, ethanol, or 1-propanol as the acceptor. Subsequently, the reaction products were analyzed by TLC. The transglycosylation product in the presence of methanol was purified with activated carbon (Autoprep fiberAC, Showa Denko K. K., Tokyo, Japan) and analyzed by HPAEC-PAD. For the transglycosylation reactions in the presence of monosaccharides and disaccharides, 2.5 mM pNP-β-Ara was incubated at 40 °C for 5 h with 100 mM of BAD_1528 in 1,000 μL of 50 mM sodium acetate buffer (pH 5.0) with 300 mM glucose, galactose, L-arabinose, or sucrose as the acceptor. The reaction products were purified with activated carbon and analyzed by HPAEC-PAD. The transglycosylation reactions in the presence of monosaccharides and disaccharides, 2.5 mM pNP-β-Ara was incubated at 40 °C for 5 h with 100 mM of BAD_1528 in 800 μL of 50 mM sodium acetate buffer (pH 5.0). The reaction product was purified by HPLC with a Cosmosil PBr column (10 mm internal diam-eter × 250 mm; Nacalai Tesque Inc., Kyoto, Japan) and analyzed by HPAEC-PAD. The transglycosylation reactions in the presence of monosaccharides and disaccharides, 2.5 mM pNP-β-Ara was incubated at 40 °C for 5 h with 100 mM of BAD_1528 in 800 μL of 50 mM sodium acetate buffer (pH 5.0). The reaction product was purified by HPLC with a Cosmosil PBr column (10 mm internal diam-eter × 250 mm; Nacalai Tesque Inc., Kyoto, Japan) at 30 °C. The column was eluted at a flow rate of 4.7 mL/min using the following gradient: 0–2 min, 100 % eluent A (0.1 M NaOH); 2–30 min, 0–100 % eluent B (0.5 M sodium acetate and 0.1 M NaOH); and 30–35 min, 100 % eluent B.

The pH dependence of enzyme activity was determined using pNP-β-Ara as the substrate between pH 3.5 and 7.0 using the following buffers: 50 mM sodium acetate (pH 3.5–6.0) and 50 mM MES (pH 5.5–7.0). The temperature on enzyme activity was determined using 50 mM sodium acetate buffer (pH 5.0) at 25–50 °C.

**Kinetic analysis.** The kinetic parameters for BAD_1528 were determined using 0.5–8.0 mM pNP-β-Ara, pNP-α-Gal, and Arab-β1,3-Ara. The reactions with each pNP sub-strate were measured as pNP concentrations by measuring the absorbance of the reaction mixture at 400 nm, and that with Arab-β1,3-Ara was measured as L-arabinose concentra-tion by HPAEC-PAD as described above.

**Transglycosylation activities.** The transglycosylation reac-tions were performed using pNP-β-Ara as the donor and 1-alkanols, monosaccharides, disaccharide, and pNP-β- Ara as acceptors. For the transglycosylation reactions in the presence of 1-alkanols, 2.5 mM pNP-β-Ara was incubated at 40 °C for 2 h with 100 mU/mL of BAD_1528 in 40 μL of 50 mM sodium acetate buffer (pH 5.0) with 10 % methanol, ethanol, or 1-propanol as the acceptor. Subse-quently, the reaction products were analyzed by TLC. The transglycosylation product in the presence of methanol was purified with activated carbon (Autoprep fiberAC, Showa Denko K. K., Tokyo, Japan) and analyzed by HPAEC-PAD. The transglycosylation reactions in the presence of monosaccharides and disaccharides, 2.5 mM pNP-β-Ara was incubated at 40 °C for 5 h with 100 mM of BAD_1528 in 800 μL of 50 mM sodium acetate buffer (pH 5.0). The reaction product was purified by HPLC with a Cosmosil PBr column (10 mm internal diam-eter × 250 mm; Nacalai Tesque Inc., Kyoto, Japan) at 254 nm. Subsequently, the fractions containing the transglycosyla-
tion product were collected and analyzed by TLC, HPAEC-PAD, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics GmbH, Leipzig, Germany).

Assays of bacterial enzyme activities and in vitro fermentability of Arap-β1,3-Ara. B. adolescentis JCM1275 and JCM15918 were cultured at 37 °C under anaerobic conditions using the AnaeroPack system (Mitsubishi Gas Chemical Company, Inc., Tokyo, Japan) in a modified MRS broth containing 2 % glucose or galactose as the carbohydrate source and 0.05 % L-cysteine hydrochloride. The cell cultures were centrifuged at 10,000 rpm for 10 min, and the resultant pellets were washed with 50 mM sodium acetate buffer (pH 5.5). Afterwards, the pellets were sonicated with a Branson Sonifier 250 (Branson Ultrasonics Corporation, Danbury, CT, USA). The cell lysates were centrifuged at 15,000 rpm for 10 min, and the supernatants were incubated with pNP-β-Arap or Arap-β1,3-Ara at 37 °C for 16 h and then analyzed by TLC and HPAEC-PAD as described above.

The in vitro fermentabilities of Arap-β1,3-Ara were examined on peptone-yeast extract-Fildes (PYF) medium containing 0.2 % Arap-β1,3-Ara. The growth profiles of B. adolescentis JCM1275 and JCM15918 were monitored by measuring the optimal density at 600 nm and also the pH profile.

RESULTS
Sequence analysis and phylogenetic relationship of BAD_1528.
BAD_1528 lacks the putative signal peptide and terminal transmembrane domain, according to the signalP 4.1 and InterPro servers, suggesting that the enzyme localizes to the intracellular compartment. The genes homologous to BAD_1528 were conserved in several bifidobacterial species such as B. animalis subsp. lactis (59 %), B. catenulatum (81 %), B. pseudocatenulatum (81 %), B. dentium (75 %), B. kashiwanohense (81 %), and B. pseudolongum (58 %). The catalytic region of BAD_1528 were phylogenetically independent of other characterized GH36 α-D-galactopyranosidases (21–27 % identities) and was located far (<20 % identities) from the GH27 enzymes having β-L-arabinopyranosidase/α-D-galactopyranosidase activities (Fig. 1A). BAD_1528 formed a gene cluster with several L-arabinosidase candidates belonging to GH27, GH43, GH51, and GH127 (Fig. 1B). B. adolescentis JCM15918, BBMN23, and 22L conserves these L-arabinosidase candidates except GH27 β-L-arabinopyranosidase. On the other hand, B. longum did not conserved BAD_1528 homologous gene, and other bifidobacterial species conserved BAD_1528 homologous gene and part of L-arabinosidase candidates.

Preparation and characterizaiton of recombinant BAD_1528.
The recombinant BAD_1528 protein was expressed at 37 °C as a soluble protein. SDS-PAGE showed that the purified recombinant BAD_1528 protein migrated as a single band with an apparent molecular mass of 89 kDa (Fig. 2), which was in agreement with its calculated molecular mass of 88,803 Da.

The substrate specificities of BAD_1528 were identified using synthetic pNP substrates. BAD_1528 hydrolyzed pNP-β-Arap and weakly hydrolyzed pNP-α-Gal, but not other pNP substrates (Fig. 3). The optimal temperature and pH for pNP-β-Arap were 40 °C and 5.0, respectively (Fig. 4). Several disaccharides, oligosaccharides, and polysaccharides were used to identify the specificities of BAD_1528. Arap-β1,3-Ara was best substrate for the enzyme, except for pNP substrates (Fig. 5A and Table 1). BAD_1528 had weak activities for Arap-β1,3-ART and Gal-α1,3-Ara (Figs. 5B and C, respectively). These specific activities were 0.0820 and 0.177 % of those for Arap-β1,3-Ara, respectively (Table 2). On the other hand, the hydrolysis of Arap-β1,3-Araf3Gal was below the detection limit after 16 h (Fig. 5D). The enzyme also had no activities for all polysaccharides including gum arabic, larch AG, arabinoxylan, and arabian (data not shown). The kcat (1.48 mM) for pNP-β-Arap was 5.9-fold and 4.0-fold lower than those for Arap-β1,3-Ara and pNP-α-Gal, respectively, and the kcat (81.5 s−1) for pNP-β-Arap was 11-fold and 34-fold higher than those for Arap-β1,3-Ara and pNP-α-Gal, respectively (Table 1). Consequently, the kcat/Km values for Arap-β1,3-Ara and pNP-α-Gal were 62-fold and 135-fold lower than those for pNP-β-Arap, respectively.

Transglycosylation activity of recombinant BAD_1528.
When 10 % methanol and ethanol were used as the acceptors, the transglycosylation products were detected by TLC (Fig. 6A). On the other hand, the transglycosylation product was not confirmed with 10 % l-propanol as an acceptor. The purified transglyosylation product to methanol was partially hydrolyzed to L-arabinose by BAD_1528 treatment (Fig. 6B), which indicates that the transglycosylation product is methyl-β-Arap with the retention of the anomeric configuration.

Next, glucose, galactose, L-arabinose, sucrose, and pNP-β-Arap were used as the acceptors for the transglycosylation reactions. The arabinopyranosyl moiety was slightly transferred to all the acceptors (data not shown), and the partial purified products were hydrolyzed by BAD_1528 treatment (Fig. 7), which indicate that the arabinopyranosyl moieties were linked by the β-anomeric form. These data suggest that BAD_1528 has broad acceptor specificities, and that the arabinopyranosyl unit is not exclusively attached by β1,3-linkage. Especially, purified Arap-β-Arap-β-pNP was detected at 13.3 min on HPAEC-PAD (Fig. 7E), which is different from the peak corresponding to pNP-β-Arap (retention time: 15.0 min). MALDI-TOF MS revealed a molecular ion peak at m/z 405.05 for Arap-β-Arap-β-pNP (calc. m/z 403.11). Arap-β-β-Arap-β-pNP had a high specificity toward BAD_1528, which was 56.6 % of that for Arap-β1,3-Ara (Table 2). Almost all GH36 α-D-galactopyranosidases have transglycosylation activities for the synthesis of α-galactosyl neo-glycoconjugates. Therefore, BAD_1528 has a potential for the production of β-L-arabinopyranosyl neo-glycoconjugates.
Bacterial enzyme activities and in vitro fermentability of Arap-β1,3-Ara.

When cell lysates of *B. adolescentis* JCM1275 and JCM15918 grown in the medium containing glucose and galactose were used as the enzyme source, β-L-arabinopyranosidase activities were observed using pNP-β-Ara (Fig. 8). Moreover, weak activities for Ara-β1,3-Ara were detected by TLC (Fig. 8), and the released L-arabinose was

Phylogenetic relationships and gene clusters of BAD_1528.

A, The phylogenetic tree of BAD_1528 with homologous proteins was constructed by the neighbor-joining method using the aligned sequences with the MUSCLE program implemented in MEGA7 software. The locus tag or the characterized enzymatic activities are shown with the abbreviations of the organisms and the GenBank accession numbers. The protein characterized in this study is enclosed in the dash box. The abbreviations of the enzymatic activities and the organisms are as follows: AGA, α-D-galactopyranosidase; BAL, β-L-arabinopyranosidase; Bad, *B. adolescentis*; Bal, *B. animalis* subsp. *lactis*; Bbi, *B. bifidum*; Bbr, *B. breve*; Bc, *B. catenulatum*; Bd, *B. dentium*; Bk, *B. kashiwanohense*; Bl, *B. longum*; Bll, *B. longum* subsp. *longum*; Bpc, *B. pseudocatenulatum*; Bpl, *B. pseudolongum*; Cj, *Clostridium josui*; Cp, *Chitinophaga pinensis*; Fo, *Fusarium oxysporum*; Gs, *Geobacillus stearothermophilus*; La, *Lactobacillus acidophilus*; Rg, *Ruminococcus gnavus*; Sa, *Streptomyces avermitilis*. B, The gene clusters encoding L-arabinosidases in *Bifidobacterium* strains. Genes encoding sugar transporters and transcriptional regulators are depicted in gray and black arrows, respectively. Light gray bars indicate orthologous regions. The white arrows indicate glycoside hydrolase candidates or hypothetical proteins. The numbers of predicted glycosidase hydrolases or the predicted enzymatic activities are shown in the white arrows with locus tags. DNase, TatD DNase family protein.

**Bacterial enzyme activities and in vitro fermentability of Arap-β1,3-Ara.**

When cell lysates of *B. adolescentis* JCM1275 and JCM15918 grown in the medium containing glucose and galactose were used as the enzyme source, β-L-arabinopyranosidase activities were observed using pNP-β-Ara (Fig. 8). Moreover, weak activities for Arap-β1,3-Ara were detected by TLC (Fig. 8), and the released L-arabinose was
detected by HPAEC-PAD (data not shown). Although we predicted that *B. adolescentis* JCM1275 and JCM15918 possess Ara\(^\text{p} \text{-} \beta_1,3\text{-Ara}\) assimilation ability, we observed no distinct differences in growth between culture mediums without any carbohydrate source and with Ara\(^\text{p} \text{-} \beta_1,3\text{-Ara}\) (data not shown). Moreover, no decrease in the amount of Ara\(^\text{p} \text{-} \beta_1,3\text{-Ara}\) in the culture medium was detected by HPAEC-PAD (data not shown). These data suggested that Ara\(^\text{p} \text{-} \beta_1,3\text{-Ara}\) is not degraded or metabolized in *B. adolescentis* strains used in this study.

**DISCUSSION**

In the present study, we clarified BAD\(_{1528}\) to be the first characterized GH36 \(\beta\)-L-arabinopyranosidase that exhibits a hydrolytic activity toward Ara\(^\text{p} \text{-} \beta_1,3\text{-Ara}\) derived from a natural substrate. The enzyme showed little or no releasing activities toward oligosaccharides or polysaccharides containing Ara\(^\text{p} \text{-} \beta_1,3\text{-Ara}\)-structures and had a weak activity for Ara\(^\text{p} \text{-} \beta_1,3\text{-ART}\). Because the pyranose ring form is thermodynamically more stable than the furanose form, Ara\(^\text{p} \text{-} \beta_1,3\text{-Ara}\) is thought to be present in the form of pyranose as Ara\(^\text{p} \text{-} \beta_1,3\text{-Arap}\) in aqueous solution.

**Fig. 2.** SDS-PAGE analysis of recombinant BAD\(_{1528}\).

Purified BAD\(_{1528}\) was electrophoresed on a 10 % polyacrylamide gel and stained with Coomassie Brilliant Blue R-250. Lanes 1 and 3, molecular size markers; lane 2, purified enzyme. The arrow indicates the band that corresponds to BAD\(_{1528}\).

**Fig. 3.** TLC analysis of BAD\(_{1528}\) reactions with the \(\text{pNP}\)-substrates.

The \(\text{pNP}\)-substrates were incubated in the absence (lane a) or presence (lane b) of the recombinant enzyme at 40 °C for 16 h. \(\text{pNP-\(\alpha\)-Arap}\) (lane 3), \(\text{pNP-\(\beta\)-Arap}\) (lane 4), \(\text{pNP-\(\alpha\)-Gal}\) (lane 5), \(\text{pNP-\(\beta\)-Gal}\) (lane 6), \(\text{pNP-\(\alpha\)-Xyl}\) (lane 7), \(\text{pNP-\(\beta\)-Xyl}\) (lane 8), \(\text{pNP-\(\alpha\)-Glc}\) (lane 9), and \(\text{pNP-\(\beta\)-Glc}\) (lane 10) were used as substrates. Lane 1, L-arabinose standard; lane 2, galactose standard.

**Fig. 4.** Effects of pH and temperature on the activity of BAD\(_{1528}\).

A, pH dependence of BAD\(_{1528}\) activity in various buffers at 40 °C for 20 min. Enzyme activities are expressed as a percentage of the activity in acetate buffer at pH 5.0. B, Temperature dependence of BAD\(_{1528}\) activity at pH 5.0 for 20 min. The enzymatic activities are expressed as the percentage of the activity at 40 °C. Buffers: sodium acetate (closed square) and MES (open circle).

**Fig. 5.** HPAEC-PAD analysis of BAD\(_{1528}\) reactions with disaccharides and oligosaccharides.

Ara\(^\text{p} \text{-} \beta_1,3\text{-Ara}\) (A), Ara\(^\text{p} \text{-} \beta_1,3\text{-ART}\) (B), Gal-\(\alpha_1,3\text{-Ara}\) (C), and Ara\(^\text{p} \text{-} \beta_1,3\text{-Ara}\)/Gal\(_3\) (D) were incubated with (b) or without (a) BAD\(_{1528}\) at 40 °C for 16 h.

**Table 1.** Kinetic parameters of BAD\(_{1528}\).

| Substrate     | \(K\_m\) (mM) | \(k\text{cat}\) (s\(^{-1}\)) | \(k\text{cat}/K\_m\) (s\(^{-1}\)mM\(^{-1}\)) |
|---------------|---------------|-------------------------------|----------------------------------|
| \(\text{pNP-\(\beta\)-Arap}\) | 1.48±0.04 | 81.5±0.51 | 54.9 |
| Ara\(^\text{p} \text{-} \beta_1,3\text{-Ara}\) | 8.69±1.9 | 7.66±1.6 | 0.881 |
| \(\text{pNP-\(\alpha\)-Gal}\) | 5.96±0.03 | 2.43±0.01 | 0.408 |
BAD_1528 is a novel exo-β-L-arabinopyranosidase that recognizes the Ara\(^p\)-β1,3-Ara\(^p\) structure. It is considered that Ara\(^p\)-β1,3-Ara degradative enzymes may be present in almost all bifidobacterial species, because the homologous genes (59.3–81.3 % identities with BAD_1528) were conserved in several bifidobacterial species shown in Fig. 1B.

Although Ara\(^p\)-β1,3-Ara can be prepared artificially by partial acid hydrolysis, its existence in nature has never been reported. Recently, we have confirmed the Ara\(^p\)-β1,3-Ara releasing activity in B. longum JCM7052 (unpublished data). To the best of our knowledge, AGPs are the sole source of Ara\(^p\)-β1,3-Ara production. We also characterized an AGP degradative enzyme and predicted the AGP metabolic pathway in B. longum.

### Table 2. Substrate specificities of BAD_1528.

| Substrate\(^a\) | Specific activity (Units/mg) | Relative activity\(^b\) (%) |
|-----------------|-----------------------------|-----------------------------|
| pNP-β-Arap      | 46.8                        | 2480                        |
| Arap-β1,3-Ara\(^a\) | 1.89                      | 100                         |
| Arap-β-Arap-β-pNP\(^h\) | 1.07                   | 56.6                        |
| Arap-β1,3-ART   | 0.00155                     | 0.0820                      |
| Arap-β1,3-Ara/Gal\(^h\) | 0.903                 | 47.8                        |
| pNP-α-Gal      | 0.00335                     | 0.177                       |

\(^a\) The substrate concentrations of pNP-β-Ara, Ara-β1,3-Ara, Arap-β-Ara-β-pNP, Arap-β1,3-ART, Arap-β1,3-Ara/Gal\(^h\), pNP-α-Gal, and Gal-α1,3-Ara were 8.0, 5.0, 0.77, 0.25, 0.25, 8.0, and 0.44 mM, respectively. \(^b\) Relative activity is expressed as the percentage of the activity toward Arap-β1,3-Ara. The specific activity was calculated on the value for released L-arabinose by one-half.

**Fig. 6.** Transglycosylation activity of BAD_1528 in the presence of 1-alkanols.

A, BAD_1528 was incubated with pNP-β-Arap in the presence of either 10 % methanol (lane 2), ethanol (lane 3), or 1-propanol (lane 4) at 40 °C for 2 h. Lane 1, pNP-β-Arap standard. Me, methyl; Et, ethyl. B, Purified Arap-β-OMe was incubated with (b) or without (a) BAD_1528 at 40 °C for 16 h.

BAD_1528 is a novel exo-β-L-arabinopyranosidase that recognizes the Arap-β1,3-Arap structure. It is considered that Arap-β1,3-Ara degradative enzymes may be present in almost all bifidobacterial species, because the homologous genes (59.3–81.3 % identities with BAD_1528) were conserved in several bifidobacterial species shown in Fig. 1B. Although Arap-β1,3-Ara can be prepared artificially by partial acid hydrolysis, its existence in nature has never been reported. Recently, we have confirmed the Arap-β1,3-Ara releasing activity in B. longum JCM7052 (unpublished data). To the best of our knowledge, AGPs are the sole source of Arap-β1,3-Ara production. We also characterized an AGP degradative enzyme and predicted the AGP metabolic pathway in B. longum. Interestingly, B. longum

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lose residues, and AXHm23 (BAD 0423), which releases arabinosyl residues from single-substituted xylose residues. Furthermore, it has GH1 α-L-arabinofuranosidase specific for α1,5-arabino-oligosaccharides (BAD 0156). We also characterized neighboring genes of BAD 1528: BAD 1525 as GH27 α-D-galactopyranosidase/β-L-arabinopyranosidase, BAD 1527 as GH43 α-L-arabinofurano- idase, and BAD 1529 as GH127 β-L-arabinofuranosidase (unpublished data). The conservation of these L-arabinosi- dases in B. adolescentis is meaningful from the following viewpoints. L-Arabinose can be used by some bifidobacteria- rial species as a carbohydrate source, although substrate preference varies by species. In this study, B. adolescentis strains did not grow on Arap-β1,3-Ara as the carbohydrate source, in spite of the presence of intracellular β-L-arabinobio- pyranosidase activity, which indicates that Arap-β1,3-Ara is not good carbohydrate source for B. adolescentis similarly to L-arabinose. Pastell et al. reported that α-L-arabinofuranosidases are needed for the elimination of L-arabinose residues on xylooligosaccharides, and the releasing L-arabi- nose may be used by other bifidobacterial species such as B. longum. As a result, various L-arabinosidases in B. adolescentis may play important roles in bifidobacterial symbiosis by providing L-arabinose that can be used as an energy source to other bifidobacterial species. In addition, these enzymes are predicted to be accessory enzymes for the intracellular degradation of plant oligosaccharides. There are several reports that α-L-arabinofuranosidases have synergistic effects with β-xylanases for the arabinoxylan degradation. Therefore, releasing L-arabinose from oligosaccharides is important for facilitating access of other enzymes to the substrates.

In this study, we confirmed β-L-arabinopyranosidase ac- tivity in two strains of B. adolescentis (JCM1275 and JCM15918). B. adolescentis JCM1275 encodes three β-L-arabinopyranosidase candidates, i.e., one GH27 (BAD 1525) and two GH36s (BAD 1528 and BAD 1576), whereas JCM15918 encodes only two GH36s (BSTER 1815 and BSTER 1892). Because β-L-arabinopyranosidase activities were detected in both JCM1275 and JCM15918, GH36 enzymes may be responsible for the β- L-arabinopyranosidase activity. However, it is not certain whether or not BAD 1528 and the ortholog BSTER 1892 are expressed in each strain. BAD 1576 or the homologous enzymes encoding α-D-galactopyranosidases have the po- tential to have the β-L-arabinopyranosidase activity, al- though it has not been reported yet. Therefore, ad- ditional functional characterization and transcriptional analysis of BAD 1528 and BAD 1576 may be necessary.

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