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# Detailed Modes of Action and Biochemical Characterization of *endo*-Arabinanase from *Bacillus licheniformis* DSM13

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**An *endo*-arabinanase (BLABNase) gene from *Bacillus licheniformis* DSM13 was cloned and expressed in *Escherichia coli*, and the biochemical properties of its encoded enzyme were characterized. The BLABNase gene consists of a single open reading frame of 987 nucleotides that encodes 328 amino acids with a predicted molecular mass of about 36 kDa. BLABNase exhibited the highest activity against debranched α-(1,5)-arabinan in 50 mM sodium acetate buffer (pH 6.0) at 55°C. Enzymatic characterization revealed that BLABNase hydrolyzes debranched or linear arabinans with a much higher activity than branched arabinan from sugar beet. Enzymatic hydrolysis pattern analyses demonstrated BLABNase to be a typical *endo*-(1,5)-α-L-arabinanase (EC 3.2.1.99) that randomly cleaves the internal α-(1,5)-linked L-arabinofuranosyl residues of a branchless arabinan backbone to release arabinotriose mainly, although a small amount of arabino-oligosaccharide intermediates is also liberated. Our results indicated that BLABNase acts preferentially along with the oligosaccharides longer than arabinopentaose, thus enabling the enzymatic production of various arabino-oligosaccharides.**

***Keywords***: *Bacillus licheniformis* DSM13, *endo*-(1,5)-α-L-arabinanase, gene expression, enzymatic hydrolysis patterns, arabino-oligosaccharides production

## Introduction

L-Arabinose has been considered as a physiologically functional low-calorie sweetener because of its inhibitory action against digestion and absorption of sucrose in the blood stream (Seri *et al*., 1996; Hizukuri, 1999). Although L-arabinose is widely present in various hemicellulosic biomasses such as arabinoxylan, sugar beet arabinan is considered to be the best source for L-arabinose production because of its homopolymeric structure. Arabinan, a pectic polysaccharide consists of a backbone of α-(1,5)-linked L-arabinofuranosyl residues, some of which are substituted with α-(1,2)- or α-(1,3)-linked arabinofuranosides. Degradation of arabinan polymer to arabinose sugars is driven by the synergistic action of two major enzymes, α-L-arabinofuranosidase (AFase; EC 3.2.1.55) and *endo*-(1,5)-α-L-arabinanase (ABNase; EC 3.2.1.99) (Kim, 2008). AFases specifically catalyze the hydrolysis of terminal non-reducing L-arabinofuranosyl residues from various arabinose-containing polysaccharides (Numan and Bhosle, 2006). The resulting debranched arabinan backbone could be efficiently hydrolyzed by *endo*-acting ABNases, thus generating a variety of arabino-oligosaccharides.

In contrast to extensive studies of AFases, only a few ABNases from different genus of *Bacillus* (Takao *et al*., 2002; Leal and de Sá-Nogueira, 2004; Seo *et al*., 2010), *Pseudomonas* (McKie *et al*., 1997), and *Aspergillus* (Ramon *et al*., 1993) have been reported till date because of their limited distribution in nature. ABNases, belonging to the glycoside hydrolase (GH) family 43 (http://www.cazy.org), possess a monomeric three-dimensional structure with a typical cylindrical β-propeller topology (Nurizzo *et al*., 2002; Yamaguchi *et al*., 2005; Alhassid *et al*., 2009). While mesophilic ABNases (molecular mass of 30–45 kDa) were shown to exhibit the highest activity at pH 5.5–7.0 and 40–60°C, thermophilic ABNases from *B. thermodenitrificans* (Takao *et al*., 2002) and *Caldicellulosiruptor saccharolyticus* (Hong *et al*., 2009) were characterized to have optimal reaction temperatures at 70 and 75°C, respectively. Furthermore, a novel inverting α-(1,5)-arabinanase from *Cellvibrio japonicus* (formerly known as *Pseudomonas fluorescens* subsp. *cellulosa*) was shown to hydrolyze linear arabinans into arabinotriose, via an *exo*-acting reaction, apart from *endo*-acting catalytic ability (Proctor *et al*., 2005).

Arabinose-containing oligosaccharides derived from arabinoxylans and arabinans have been identified as potential prebiotics (Grootaert *et al*., 2007), and the synergistic enzymatic production of L-arabinose from arabinan has been recently reported (Lim *et al*., 2011). To date, nevertheless, a detailed mode of action of ABNases has not been studied well. This necessitates extensive research on the enzymatic activity of ABNases in order to efficiently produce L-arabinose and prebiotic arabino-oligosaccharides from natural arabinan polymers.

In the present study, therefore, we describe the isolation and expression of an *endo*-(1,5)-α-L-arabinanase (BLABNase) gene from *Bacillus licheniformis* DSM13. In addition to characterizing the biochemical properties in detail, our study

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elucidates the hydrolytic action of BLABNase against arabino-oligosaccharides or arabinans.

## Materials and Methods

### Bacterial strain and chemicals

*Bacillus licheniformis* DSM13 (KCTC 1918 and ATCC 14580) was obtained from Korean Collection for Type Cultures (KCTC; Korea) and grown in nutrient broth (0.3% beef extract and 0.5% peptone) at 37°C. Restriction endonucleases were purchased from Roche Applied Science (Germany) and Takara Biomedical (Japan). AccuPrep plasmid extraction kit, PCR purification kit, and oligonucleotide primers were obtained from Bioneer (Korea). Arabino-oligosaccharide standards, sugar beet (native and branched) arabinan, debranched arabinan, and linear arabinan were procured from Megazyme International (Ireland). Oat spelt xylan was obtained from Sigma-Aldrich (USA). Other reagents and chemicals used in this study were purchased from Duchefa Biochemie (The Netherlands), Sigma-Aldrich, Merck (Germany), Junsei Chemical (Japan).

### Gene amplification and cloning

A set of oligonucleotide primers, BLABN-N (5′-TTTTGGATCCCATATGTTAAAGACATCGAAATTT-3′) BLABN-C (5′-TTTTCTCGAGATACTTCGGCCAGCCTTT-3′), were designed to amplify the BLABNase gene from *B. licheniformis* DSM13. PCR amplification was performed using a Px2 thermal cycler (Thermo-Hybaid, UK) as follows: an initial denaturation step at 98°C for 30 sec, followed by 30 cycles consisting of denaturation at 98°C for 10 sec, annealing at 55°C for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The resulting 0.9 kb DNA fragment was digested with *Bam*HI and *Xho*I, cloned into a plasmid vector, pET-21a(+) (Novagen, Germany), and designated as pETBLABN. The entire nucleotide sequence was verified by SolGent (Korea) using an ABI PRISM 3730XL DNA analyzer (Applied Biosystems, USA).

### Gene expression and enzyme purification

The resulting plasmid, pETBLABN, was introduced into *Escherichia coli* BL21 (DE3) cells by calcium chloride transformation method. Recombinant *E. coli* harboring pETBLABN was cultivated in Luria-Bertani (LB) broth containing 100 µg/ml of ampicillin at 37°C. When the optical density at 600 nm reached 0.6, the gene expression was induced by adding 0.1 mM IPTG (isopropyl-β-D-thiogalactopyranoside). After an additional cultivation of 12 h, cells were harvested and disrupted by sonication (VCX750, Sonics & Materials, USA). Finally, the recombinant BLABNase with a C-terminal six histidines-tag was purified to an apparent homogeneity using the AKTA Prime purification platform with a HisTrap-FF column (GE Healthcare, Sweden). The purity and molecular mass of the enzyme were determined by 12% SDS-PAGE analysis. The protein concentration was measured using the BCA$^{TM}$ protein assay kit (Pierce Biotechnology, USA) with bovine serum albumin (BSA) as the standard. The N-terminal amino acid sequence of the recombinant protein was analyzed by Korea Basic Science Institute (Korea) using a Procise 491 HT protein sequencer (Applied Biosystems).

### Enzyme activity assay

Purified BLABNase was incubated with a specific concentration of each substrate in 50 mM sodium acetate buffer (pH 6.0) at 55°C for an appropriate duration. The 3,5-dinitrosalicylic acid (DNS) method for reducing sugar (Miller, 1959) was employed for determining the hydrolytic activity against 1% of sugar beet, debranched, and linear arabinans, and 5% of oat spelt xylan. One unit of the hydrolyzing activity towards polymeric substrates corresponds to the amount of enzyme producing 1 µmol equivalent of L-arabinose per min under optimal reaction conditions. The activity assay was carried out using *p*-nitrophenyl arabinofuranoside (0.5 mM, Sigma-Aldrich) as the substrate. The reaction mixture was incubated at 55°C for 10 min, and the quantity of *p*-nitrophenol liberated was measured by the absorbance at 405 nm. One unit of enzyme activity was defined as the amount of enzyme that liberated 1 µmol *p*-nitrophenol per min un-

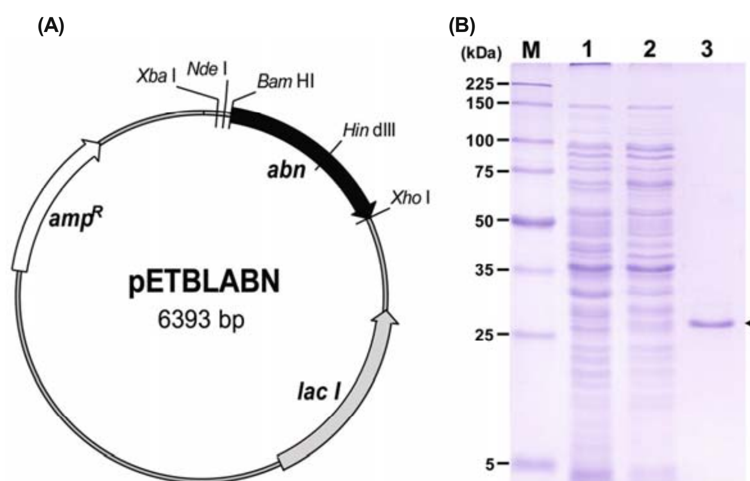


**Fig. 1. Gene expression and purification of BLABNase.** (A) Restriction enzyme map of pETBLABN (B) SDS-PAGE analysis. Lanes: M, protein molecular weight marker; 1, negative control from *E. coli* harboring pET-21a(+); 2, crude extract from *E. coli* harboring pETBLABN; 3, BLABNase purified by Ni-NTA chromatography (arrow head).

der optimal assay conditions.

### Analysis of hydrolysis products

Thin layer chromatography (TLC) and high-performance anion exchange chromatography (HPAEC) were applied to the analysis of hydrolysis products. An appropriate amount of BLABNase was allowed to react with 1% of each of the substrates under optimal conditions. For TLC analysis, the resultant products were separated on a $60F_{254}$ silica gel plate (Merck) using ethyl acetate: acetic acid: and water (2:1:1) as the solvent system. Detection was achieved by dipping the plate in a developing solution (0.3% *N*-1-naphthyl-ethylene-diamine and 5% $H_2SO_4$ in methanol), and subsequently heating it at 110°C for 10 min. HPAEC analysis was performed using a Bio-LC ICS-3000 system (Dionex, USA) coupled to a CarboPac PA1 column (0.4×25 cm, Dionex), and detection was performed using an pulsed electrochemical detector (ED40, Dionex). Samples were eluted with a linear gradient from 100% buffer A (150 mM NaOH in water; Fisher Scientific, USA) to 50% buffer B (600 mM of sodium acetate in buffer A, Sigma-Aldrich) for over 50 min. The flow rate of the mobile phase was maintained at 1.0 ml/min.

## Results and Discussion

### Gene cloning and primary structure analysis

A putative open reading frame encoding BLABNase (GenBank accession No. AE017333.1) was identified in the genome of *Bacillus licheniformis* DSM13 (Veith *et al.*, 2004) using BLAST analyses. The target gene was PCR-amplified and cloned into an inducible expression vector pET-21a(+), which was designated as pETBLABN (Fig. 1A). The BLABNase gene consists of 987 bp nucleotides that encode a protein of 328 amino acids with a predicted molecular mass of 36,358 Da. The deduced amino acid sequence of the BLABNase gene shares a significant sequence identity with the GH 43 ABNases from *B. subtilis* 168T (75%; Leal and de Sá-Nogueira, 2004), *B. thermodenitrificans* (54%; Takao *et al.*, 2002), and *Cellvibrio japonicus* (41%; McKie *et al.*, 1997). The catalytic residues of *B. thermodenitrificans* ABNase were identified on the basis of the three-dimensional structure (Takao *et al.*, 2002). The amino acid sequence alignment between BLABNase and *B. thermodenitrificans* ABNase revealed that BLABNase possesses three putative catalytic amino acid residues (Asp50, Asp168, and Glu219) that are highly conserved among GH 43 ABNases (data not shown).

### Expression of BLABNase gene in *E. coli*

Upon addition of 0.1 mM IPTG, BLABNase gene was expressed in the recombinant *E. coli* BL21 (DE3) cells harboring pETBLABN and its expression levels reached 221.5 U/L of culture broth when incubated at 37°C for 14 h. Although the recombinant BLABNase tagged with six histidine residues at its C-terminus was poorly expressed in *E. coli*, it could be successfully purified to an apparent homogeneity via Ni-NTA column chromatography. The molecular mass of the isolated BLABNase was approximately 30 kDa, as estimated using SDS-PAGE (Fig. 1B). Analysis of the amino

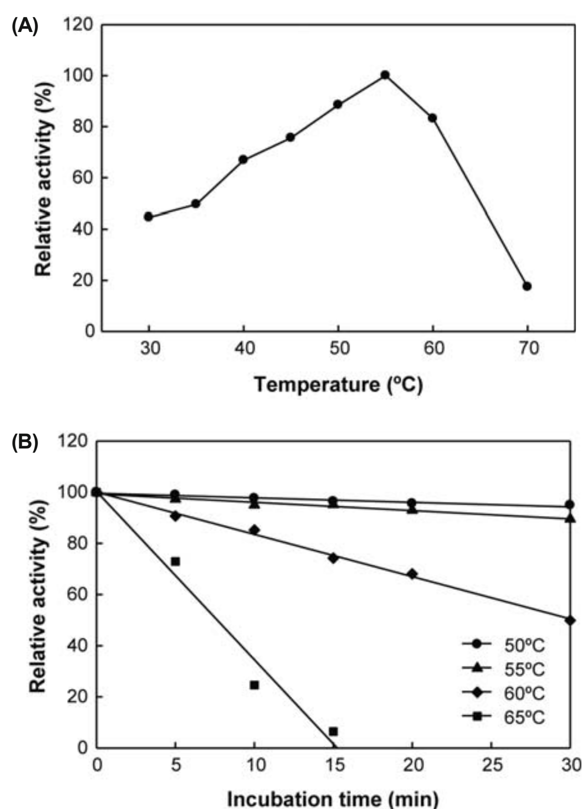


**Fig. 2. Effect of reaction temperature on BLABNase (A) activity and (B) stability.** Enzyme activity on 1% debranched arabinan was measured after the reaction in 50 mM sodium-acetate (pH 6.0) at each temperature by DNS reducing sugar assay.

acid sequence revealed that the purified BLABNase had five consecutive amino acids, Ala-Phe-Trp-Asp-Thr, at its N-terminus, where the first alanine residue corresponds to the 38th amino acid of the deduced sequence of BLABNase. Corroborating this, the proteolytic cleavage site as predicted by the SignalP program ver. 3.0 (Bendtsen *et al.*, 2004; http://www.cbs.dtu.dk/services/SignalP/) coincides exactly between Ala37 and Ala38 of BLABNase. A similar N-terminal signal peptide cleavage site has also been reported in *B. subtilis endo*-(1,5)-α-L-arabinanase (AbnA), where the recombinant protein was secreted into the periplasmic space and correctly processed by the *E. coli* signal peptidase (Leal and de Sá-Nogueira, 2004), thus implying that the *Bacillus* signal peptide is fully functional in the *E. coli* system. Our findings therefore confirmed the mature BLABNase protein to consist of 291 amino acids.

### Enzymatic characterization of BLABNase

The optimal pH and temperature for BLABNase were determined for enzymatic characterization. The hydrolytic activity of BLABNase towards debranched arabinan was found to be the highest at 55°C (Fig. 2A). The effect of temperature on the enzyme stability was also determined and compared with that of the other ABNases. The half-life of BLABNase at 50, 55, and 60°C were determined to be

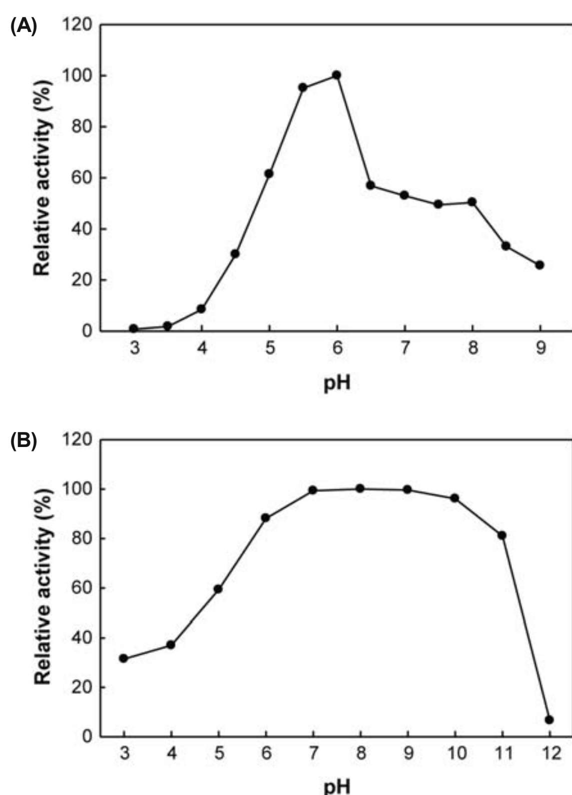


**Fig. 3. Effect of pH on BLABNase (A) activity and (B) stability.** Enzyme activity was determined against debranched arabinan by using the DNS reducing sugar assay. A variety of reaction buffers were tested as follows: sodium citrate (pH 3.0–4.0); sodium acetate (pH 4.5–6.0); sodium phosphate (pH 6.5–7.5); sodium borate (pH 8.0–9.0).

280, 152, and 30 min, respectively (Fig. 2B). Intriguingly, BLABNase was found to be unstable and it rapidly got inactivated in the absence of any substrate at temperatures above, albeit its highest activity at 55°C. The finding of our study is in agreement with several previous reports on ABNases. Recently, Seo *et al.* (2010) reported that the ABNase from *B. licheniformis* KS12 displayed the highest activity at 35°C, and further demonstrated that only 5% of activity remains after 30 min of incubation at 45°C. In accordance to the present study, it was also observed that the thermostability of *B. subtilis* ABNase significantly reduced in the absence of the arabinan substrate (Leal and de Sá-Nogueira, 2004). The ABNase from *B. thermodenitrificans*, unlike other *Bacillus* species, exhibited the highest optimal reaction temperature at 70°C with a half-life of 4 h at 75°C (Takao *et al.*, 2002). Recently, Hong *et al.* (2009) cloned the most thermophilic ABNase gene from *Caldicellulosiruptor saccharolyticus*, and characterized the enzymatic properties. Further, the dimeric *C. saccharolyticus* ABNase was shown to exhibit the highest activity at 75°C and possess a half-life of 93 h.

**Table 1.** **Specific activity of BLABNase against various substrates**

| Substrates | Specific activity (U/mg) |
|---|---|
| Debranched arabinan | 71.69±1.97 |
| Linear arabinan | 64.52±3.25 |
| Sugar beet (branched) arabinan | 0.13±0.01 |
| Oat spelt (arabino) xylan | ND[a] |
| *p*-Nitrophenyl arabinofuranoside | ND |

[a] Not detected

In the present study, in order to determine the optimal pH, the enzymatic activity was measured in various buffer solutions ranging from pH 3.0 to 9.0. BLABNase displayed the highest activity towards debranched arabinan in 50 mM sodium acetate buffer, pH optimum of 6.0 (Fig. 3A). However, its activity was found to be markedly reduced at pH 5.0 or below and at pH 6.5 or above, respectively. On the contrary, the enzyme was quite stable over a broad pH range (6.0–11.0) (Fig. 3B). Thus, our results confirm the optimal pH and temperature of BLABNase to be 6.0 and 55°C in the presence of debranched arabinan. Notably, the specific activity of BLABNase against debranched arabinan was measured to be 71.69 U/mg, which is comparable to the other ABNases isolated from *B. subtilis* (366 U/mg; Leal and de Sá-Nogueira, 2004), *B. licheniformis* (27 U/mg; Seo *et al.*, 2010), and *C. saccharolyticus* (12 U/mg; Hong *et al.*, 2009).

In addition, in the present study, we examined the hydrolyzing activity of BLABNase on a variety of substrates including sugar beet (branched) arabinan, debranched arabinan, and arabinoxylan (Table 1). Debranched arabinan was found to be the best substrate for BLABNase. On the contrary, the enzyme displayed no activity towards oat spelt arabinoxylan, arabinogalactan, gum arabic, and *p*-nitrophenyl-α-L-arabinofuranoside. Interestingly, BLABNase could hydrolyze debranched and linear arabinan with over 500 times higher activity when compared to sugar beet (branched) arabinan, thus implying that the enzyme exhibited specificity for the α-(1,5)-linked backbone of the branchless arabinan polymer, but not for the α-(1,2)- or α-(1,3)-linked branched arabinofuranosyl backbone. It was observed that BLABNase could not cleave *p*-nitrophenyl-α-L-arabinofuranoside, thus proving that it is a typical *endo*-arabinanase without *exo*-arabinofuranosidase activity. Similarly, *B. thermodenitrificans* and *B. subtilis* ABNases exhibited higher hydrolyzing activity against the debranched arabinan compared to the sugar beet arabinan (Takao *et al.*, 2002; Leal and de Sá-Nogueira, 2004). On the contrary, a novel ABNase originated from *B. subtilis* was shown to possess a broad substrate specificity towards linear α-(1,5)-L-arabinan, sugar beet arabinan, and

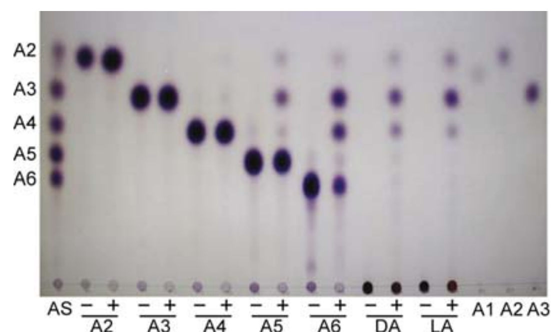


**Fig. 4. TLC analysis of the hydrolysis patterns of BLABNase on various substrates.** AS, arabino-oligosaccharides standards; A1–A6, arabinose to arabinohexaose; DA, debranched arabinan; LA, linear arabinan; reaction products with (+) and without (-) BLABNase.

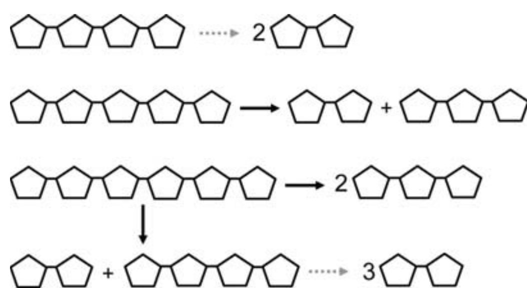

**Fig. 5. Proposed hydrolytic mode of action of BLABNase against various arabino-oligosaccharides.** The major and minor hydrolytic reactions are drawn by solid black and dashed gray arrows, respectively.

pectin (Inácio and de Sá-Nogueira, 2008). Taken together, our findings highlight a proposed five bladed β-propeller model with a unique 'velcro'-like structure, which had been revealed on the basis of the crystal sructure of *B. thermodenitrificans* ABNase, specifically for the enzymes that displayed extremely low activity towards branched arabinan substrates (Yamaguchi *et al.*, 2005).

### Hydrolytic mode of action of BLABNase

In order to investigate the mode of action of BLABNase, various α-(1,5)-L-linked arabino-oligosaccharides or arabinans were allowed to react with the enzyme, and the resultant products were analyzed by TLC and HPAEC. As arabinobiose migrates slightly faster than arabinose on a TLC plate (Fig. 4), the identified arabino-oligosaccharide compounds were validated using HPAEC analyses. It was observed that small amounts (1 U) of BLABNase could not hydrolyze the short chain arabino-oligosaccharides such as arabinobiose, arabinotriose, and arabinotetraose, although small parts of arabinopentaose were cleaved to arabinobiose and arabinotriose. Arabinobiose, arabinotriose, and arabinotetraose were detected from the reaction with arabinohexaose. In contrast, arabinobiose was generated as a major product along with small amounts of arabinose and arabinotriose, when an excess amount (10 U) of the enzyme was reacted with arabinotetraose (data not shown). Notably, in the presence of debranched or linear arabinan substrates, BLABNase generated arabinose, arabinobiose, arabinotriose, and arabinotetraose as the major end products. The resultant hydrolysis patterns of BLABNase against arabino-oligosaccharides are schematically summarized in Fig. 5.

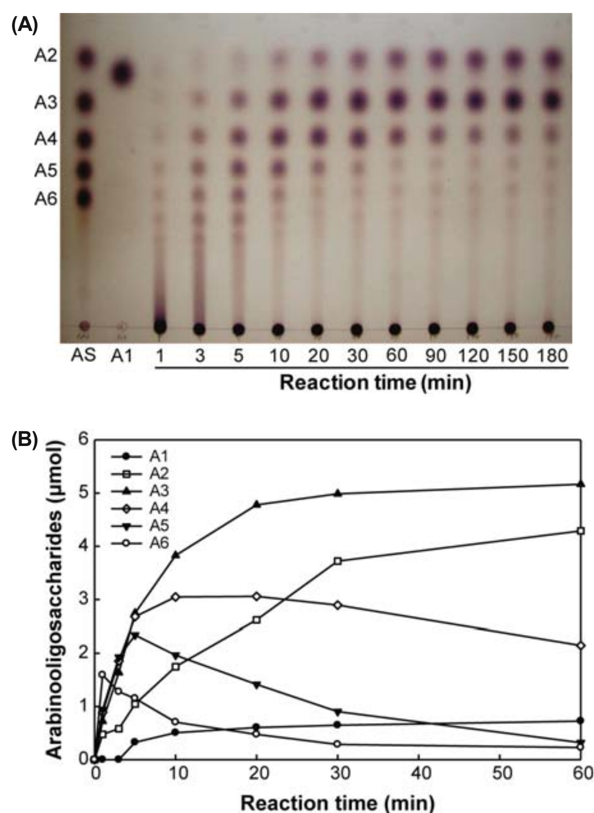


**Fig. 6. Time-course analyses of debranched arabinan hydrolysates by BLABNase via (A) TLC and (B) HPAEC.** AS, arabino-oligosaccharides standards; A1–A6, arabinose to arabinohexaose.

To examine the production of various arabino-oligosaccharide intermediate compounds, the time-course profile of the debranched arabinan hydrolysis was studied using TLC and HPAEC analyses (Fig. 6). At the beginning of the reaction, BLABNase mainly produced a series of arabino-oligosaccharides longer than arabinohexaose. However, as the reaction proceeded, the long chain arabino-oligosaccharides were gradually degraded into those shorter than arabinohexaose. The degradation patterns of arabino-oligosaccharides shorter than arabinohexaose were found to coincide exactly with the scheme in Fig. 5. Intriguingly, when an excess amount of the enzyme was added, arabinobiose and arabinotriose got accumulated and they were finally observed to be the end products, in addition to the generation of a small amount of arabinose. McKie *et al.* (1997) reported *Cellvibrio japonicus exo*-arabinanase to be an *exo*-acting hydrolase that released arabinotriose exclusively from linear α-(1,5)-arabinans. Another study demonstrated that the unique *exo*-type mode of action of the enzyme was achieved through subtle changes that took place in its open active-center cleft (Nurizzo *et al.*, 2002). The same study further showed that *exo*-(1,5)-α-L-arabinanase cleaved carboxymethyl (CM)-arabinan, debranched arabinan, as well as linear arabinan, while being inactive towards sugar beet arabinan, wheat arabinoxylan, and *p*-nitrophenyl-α-L-arabinofuranoside, thus indicating the enzyme completely hydrolyzed debranched arabinan and synthetic arabino-oligosaccharides to arabinose. Collectively, these results suggested that the arabinanases do not produce any arabino-oligosaccharide, which is longer than arabinotriose, due to their unique *exo*-activity. Strengthening these results, our study therefore emphasizes that a variety of arabino-oligosaccharides could be obtained from the arabinan polymer in the presence of BLABNase by controlling its dosage and/or the reaction time.

In conclusion, our results revealed BLABNase to be a typical *endo*-(1,5)-α-L-arabinanase that specifically hydrolyzes branchless arabinan polymers to produce mainly arabinobiose and arabinotriose, in addition to small amounts of various arabino-oligosaccharide intermediates. Further, our

study highlights the utility of BLABNase in the enzymatic processes that catalyze the efficient production of prebiotic arabino-oligosaccharides or L-arabinose, as functional food materials.

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## References

**Alhassid, A., Ben-David, A., Tabachnikov, O., Libster, D., Naveh, E., Zolotnitsky, G., Shoham, Y., and Shoham, G.** 2009. Crystal structure of an inverting GH 43 1,5-α-L-arabinanase from *Geobacillus stearothermophilus* complexed with its substrate. *Biochem. J.* **422**, 73–82.

**Bendtsen, J.D., Nielsen, H., von Heijne, G., and Brunak, S.** 2004. Improved prediction of signal peptides: SignalP 3.0. *J. Mol. Biol.* **340**, 783–795.

**Grootaert, C., Delcour, J.A., Courtin, C.M., Broekaert, W.F., Verstraete, W., and Van de Wiele, T.** 2007. Microbial metabolism and prebiotic potency of arabinoxylan oligosaccharides in the human intestine. *Trends Food Sci. Technol.* **18**, 64–71.

**Hizukuri, S.** 1999. Nutritional and physiological functions and uses of L-arabinose. *J. Appl. Glycosci.* **46**, 159–165.

**Hong, M.R., Park, C.S., and Oh, D.K.** 2009. Characterization of a thermostable *endo*-1,5-alpha-L-arabinanase from *Caldicellulosiruptor saccharolyticus*. *Biotechnol. Lett.* **31**, 1439–1443.

**Inácio, J.M. and de Sá-Nogueira, I.** 2008. Characterization of abn2 (yxiA), encoding a *Bacillus subtilis* GH43 arabinanase, Abn2, and its role in arabino-polysaccharide degradation. *J. Bacteriol.* **190**, 4272–4280.

**Kim, T.J.** 2008. Microbial *exo*- and *endo*-arabinosyl hydrolases: Structure, function, and application in L-arabinose production, pp. 229–257. *In* Park, K.H. (ed.) Carbohydrate-Active Enzymes. CRC Press, Woodhead Publishing Ltd., Cambridge, England.

**Leal, T.F. and de Sá-Nogueira, I.** 2004. Purification, characterization and functional analysis of an *endo*-arabinanase (AbnA) from *Bacillus subtilis*. *FEMS Microbiol. Lett.* **241**, 41–48.

**Lim, Y.R., Yeom, S.J., Kim, Y.S., and Oh, D.K.** 2011. Synergistic production of L-arabinose from arabinan by the combined use of thermostable *endo*- and *exo*-arabinanases from *Caldicellulosiruptor saccharolyticus*. *Bioresour. Technol.* **102**, 4277–4280.

**McKie, V.A., Black, G.W., Millward-Sadler, S.J., Hazlewood, G.P., Laurie, J.I., and Gilbert, H.J.** 1997. Arabinanase A from *Pseudomonas fluorescens* subsp. *cellulosa* exhibits both an *endo*- and an *exo*-mode of action. *Biochem. J.* **323**, 547–555.

**Miller, G.L.** 1959. Use of dinitrosalicyclic acid reagent for determination of reducing sugars. *Anal. Chem.* **31**, 426–428.

**Numan, M.T. and Bhosle, N.B.** 2006. α-L-Arabinofuranosidases: the potential applications in biotechnology. *J. Ind. Microbiol. Biotechnol.* **33**, 247–260.

**Nurizzo, D., Turkenburg, J.P., Charnock, S.J., Roberts, S.M., Dodson, E.J., McKie, V.A., Taylor, E.J., Gilbert, H.J., and Davies, G.J.** 2002. *Cellvibrio japonicus* α-L-arabinanase 43A has a novel five-blade β-propeller fold. *Nat. Struct. Biol.* **9**, 665–668.

**Proctor, M.R., Taylor, E.J., Nurizzo, D., Turkenburg, J.P., Lloyd, R.M., Vardakou, M., Davies, G.J., and Gilbert, H.J.** 2005. Tailored catalysis for plant cell-wall degradation: Redesigning the *exo*/*endo* preference of *Cellvibrio japonicus* arabinanase 43A. *Proc. Natl. Acad. Sci. USA* **102**, 2697–2702.

**Ramon, D., vd Veen, P., and Visser, J.** 1993. Arabinan degrading enzymes from *Aspergillus nidulans*: induction and purification. *FEMS Microbiol. Lett.* **113**, 15–22.

**Seo, E.S., Lim, Y.R., Kim, Y.S., Park, C.S., and Oh, D.K.** 2010. Characterization of a recombinant *endo*-1,5-α-L-arabinanase from the isolated bacterium *Bacillus licheniformis*. *Biotechnol. Bioproc. Eng.* **15**, 590–594.

**Seri, K., Sanai, K., Matsuo, N., Kawakubo, K., Xue, C., and Inoue, S.** 1996. L-Arabinose selectively inhibits intestinal sucrase in an uncompetitive manner and suppresses glycemic response after sucrose ingestion in animals. *Metabolism* **45**, 1368–1374.

**Takao, M., Akiyama, K., and Sakai, T.** 2002. Purification and characterization of thermostable *endo*-1,5-α-L-arabinase from a strain of *Bacillus thermodenitrificans*. *Appl. Environ. Microbiol.* **68**, 1639–1646.

**Veith, B., Herzberg, C., Steckel, S., Feesche, J., Maurer, K.H., Ehrenreich, P., Bäumer, S., Henne, A., Liesegang, H., Merkl, R., and *et al.*** 2004. The complete genome sequence of *Bacillus licheniformis* DSM13, an organism with great industrial potential. *J. Mol. Microbiol. Biotechnol.* **7**, 204–211.

**Yamaguchi, A., Tada, T., Wada, K., Nakaniwa, T., Kitatani, T., Sogabe, Y., Takao, M., Sakai, T., and Nishimura, K.** 2005. Structural basis for thermostability of *endo*-1,5-α-L-arabinanase from *Bacillus thermodenitrificans* TS-3. *J. Biochem.* **137**, 587–592.