Note

Molecular Identification of a Cold-adapted Endo-arabinanase of *Penicillium chrysogenum*

(Received February 28, 2005 ; Accepted July 8, 2005)

Tatsuji Sakamoto,^{1,*} Hideshi Ihara,² Asako Shibano,² Hisao Nagahiro¹ and Haruhiko Kawasaki¹

¹*Division of Applied Life Sciences, Graduate School of Life and Environmental Sciences, Osaka Prefecture University (1-1, Gakuen-cho, Osaka 599-8531, Japan)*

²*Department of Biological Science, Graduate School of Science, Osaka Prefecture University (1-1, Gakuen-cho, Osaka 599-8531, Japan)*

Abstract: A cDNA encoding a cold-adapted endo-arabinanase (Abnc) was isolated by *in vitro* cloning from *Penicillium chrysogenum* 31B. The coding sequence of the *abnc* gene was 960 bp in length which encodes a 320-amino acid polypeptide. The deduced amino acid sequence of Abnc exhibited 66% and 65% identities to the deduced amino acid sequence of the mesophilic endo-arabinanases of *Aspergillus niger* and *A. aculeatus*, respectively, which have been grouped into GH family 43. The presumed catalytic acid and base in enzymes belonging to GH family 43 were conserved as Glu¹⁹⁹ and Asp³⁴ in the deduced amino acid sequence of Abnc. Optimum temperature and thermostability of the recombinant enzyme, which was expressed in *Escherichia coli*, were similar to that of the *P. chrysogenum* Abnc. The degrading activity of the recombinant Abnc towards a series of arabinooligosaccharides was higher on more than arabinohexaose. The enzyme did not hydrolyze arabinotriose.

Key words: endo-arabinanase, cold-adapted enzyme, nucleotide sequence, overexpression, *Penicillium chrysogenum*

Pectin and hemicellulose are important components of the primary cell walls of higher plants. They are heteropolysaccharides composed of uronic acids as well as sugar residues such as arabinose, galactose, or xylose, forming linear or branched polymers. L-Arabinan, which has a backbone consisting of α -1,5-linked arabinofuranose units with side-chains of arabinofuranose units at C2 and/or C3, is rich in sugar beet pulp.¹⁾ α -L-Arabinofuranosidases (EC 3.2.1.55; AFases) that hydrolyze synthetic *p*-nitrophenyl- α -L-arabinofuranoside are classified into two types depending on their mode of action by Beldman *et al.*²⁾ Type-A AFases preferentially degrade α -1,5-L-arabinofurano-oligosaccharides to arabinose and are inactive with polymers.^{3,4)} In contrast, Type-B AFases can debranch L-arabinose residues from side-chains of polymers.^{5,6)} Debranched arabinan, which was obtained from L-arabinan by treatment with type-B AFases, is the best substrate for endo-1,5- α -L-arabinanases (EC 3.2.1.99).^{6,7)}

We have previously shown that four different arabinan-degrading enzymes are produced by *Penicillium chrysogenum* 31B, which was isolated from rotten sugar beet.^{8–10)} One of the enzymes, Abnc, catalyzes the hydrolysis of debranched arabinan in an endo manner.¹⁰⁾ The enzyme exhibits optimum enzyme activity at lower temperatures from 30 to 40°C compared to *Aspergillus niger*⁶⁾ and *A. aculeatus* endo-arabinanases^{11,12)} whose optimum temperature of enzyme activity was in the range 50 to 60°C. Furthermore, Abnc is much more thermally labile than *A. niger* endo-arabinanase. So far, only two fungal genes that

encode endo-arabinanases have been isolated and sequenced, one from *A. niger*¹³⁾ and the other from *A. aculeatus*.¹²⁾ Information on nucleotide sequence of the gene encoding Abnc should help to elucidate the mechanism of cold adaptation of Abnc at the molecular level. This paper reports the nucleotide sequence for the gene that encodes Abnc of *P. chrysogenum* 31B (*abnc*) and its overexpression in *Escherichia coli*.

The N-terminal amino acid sequence of Abnc has been determined to be YANPGAXSGAXNIHDPTLIQDADG, where X indicates an undetermined residue.¹⁰⁾ Internal amino acid sequence of the enzyme was determined by sequencing N-terminus of a peptide proteolytically digested with lysylendopeptidase according to the method of Ihara *et al.*¹⁴⁾ The sequence of a major fragment, F1, was VGDLYHVVYYSV. Based on the amino acid sequences, three primers were designed as follows: araA (5'-TAYGCIAAYCCIGGNGCNTGY), araB (5'-GCITG YAAYATHCAYGAYCCN), and ara4 (5'-RTARTAIAC RTGRTADAGRTCNC). The region between the N-terminus of the enzyme and the F1 peptide was PCR amplified using the genomic DNA of *P. chrysogenum* 31B as the template with two sets of primers, araA/ara4 for first PCR and araB/ara4 for second PCR. Based on the nucleotide sequence of the obtained PCR product (*ca.* 300 bp) that termed CA-1, four primers were designed as follows: C1R (5'-GTCACCCACTTTTTCACAT), C2R (5'-CCGTCGGCGTCCTGGATCAA), C1F (5'-TTGATCCA GGACGCCGACGG), and C2F (5'-ATGTGCAAAAAG TGGGTGAC). The 5' and 3' regions of CA-1 were PCR-amplified from an *Xba* I cassette-ligated genomic DNA by cassette ligation-mediated PCR as described previously.¹⁵⁾ For amplification of the 5' region, two sets of

*Corresponding author (Tel. +81-72-254-9456, Fax. +81-72-254-9921, E-mail : sakamoto@biochem.osakafu-u.ac.jp). T. Sakamoto and H. Ihara are equal first authors.

primers (C1/C1R for first PCR and C2/C2R for second PCR) were used. Similarly, two sets of primers (C1/C1F for first PCR and C2/C2F for second PCR) were used for amplification of the 3' regions. The C1 and C2 primers were obtained from Takara Bio Inc. (Japan). Together, the sequences of CA-1 and the PCR fragments of 5' and 3' regions yielded a 3217-bp sequence. Nucleotide sequence data obtained here are available in the DDBJ/EMBL/GenBank databases under the accession number AB121666. The coding sequence (CDS) of the *abnc* gene is shown in Fig. 1. To clone the full-length *abnc* cDNA, a set of PCR primers was designed based on the sequencing data of genomic DNA. The sense primer (cold-A) was 5'-CACCTCCATCTATTTCTGCT. The antisense primer (cold-stop/Xba) was 5'-GCTCTAGATTACACCGAAGGCCAGC having an *Xba* I site at its 5' end. Total RNA was extracted from a 13 day-old culture of *P. chrysogenum* 31B as described previously.¹⁶⁾ Single stranded cDNA was synthesized from the total RNA with oligo-dT primer. The DNA fragment was PCR amplified with the primers using the cDNA of *P. chrysogenum* 31B as the template. The amplified fragment was sequenced, demonstrating that the CDS was interrupted by three introns, A, B, and C having lengths of 98, 61, and 49 bp, respectively (Fig. 1). The splice junctions follow the GT/AG rule. The CDS of the *abnc* gene contained 960 bp which encodes a protein of 320 amino acids. The deduced 24-amino acid sequence from residue 20 to residue 43 corresponds to that of the N-terminus of the purified *P. chrysogenum* Abnc. The calculated molecular mass of the polypeptide consisting of residues 20 to 320 was 32.3 kDa, which is in good agreement with that of Abnc. The amino acid sequence of the proteolytic hydrolysate (F1)

```

1 ATGAACCTACAATATTCTTACTTGGCCCTGCTCAGGCTGCGCCGTTTATGTTAC
  M N L Q Y S F T L A L L Q A A A V Y G Y
61 GCGAATCCCGAGCTTGTTCGGGTGCTGCAATCCATGATCCTACTTTGATCCAGGAC
  A N P G A C S G A C N I H D P T L I O D
121 GCGACGGAACGTACTATCGCTTCTCCACGGGCGGTGGCATTCTCTGCTTCTGCTTCA
  A D G T Y Y R F S T G G G I S Y A S A S
181 TCCATTGAGGGCCCTGGACAGGTGGTATCCGATTGCCAGCGGATCCTCGATCGAC
  S I E G P W T G R G S V L P S G S I D
241 AATCCGGAAGAATGATCCTTGGGTACTTATTTCTTCTTCTGAGTGAAGTGCAC
  N S G K N D P W
301 CTCCATTATTTATCTTCCGCGCTGCTATTCAATAAATAGGCGCTGAACAATCCTT
  .....intron A.....
361 AGGCCCTGATGTGCAAAAAGTGGGTGACCTTACCACTCTACTCCGATCGACCT
  .A P D V Q K V G D L Y H V Y Y S V S T F
421 TCAGAACCCAAATCTCTCCATTGGCTTGGCACTCCGAAACCATGGAAGGGTACTT
  E T Q I S S I G L A T S E T M E E G T W
481 GGAATGACAGGGGTCGATTGGAGTTGACTCCAAAGAGGGTGACAGTACACCGGATCG
  T D R G S I G V D S K E G D Q Y N A I D
541 ATGCAAACTCTGATCGATGCAACCAATACATGATCTTTGGATCATTCTGGCAGA
  A N L L I D G N T N Y M I F G S F W Q N
601 ACATTTTCAAAGTTACATAAATGGGACGCGACGCTTCTGACATCGACACCGGTTAATG
  I F Q V T L N G D A T S S T S T P V N V
661 TCGCGTTTGACCCGGCTGGTACACATCCGGTTGAGGGAGCTTACCTGTCAAAATACGGGG
  A F D P A G T H P V E G A Y L Y K Y G D
721 ATTATTACTCTATTCTACTCATGGGCGAGCTGCTGCAAGTATGATCAGGATGATGCCG
  Y Y Y L F Y S W G S C C K Y D Q G M P A
781 CGGACGGGAAGAGTACAAGTCAAGTTTGTGGTCAAGCAGTCCGACTGAGGGCTTTG
  A G E E Y K I K V C R S S S P T E G F
841 TGAGTTATCTTTTCTCTTCCAGTTATGCAATCTGTAAGGCGCTTGGCTAATGACGAG
  .....intron B.....
901 GTTGATGCGAACGGTGTTCATGCAAGGAGGCGGTGGACACCTGCTCTGGAAGCCAT
  V D A N G V A C T E G G G T V V L E S H
961 GGTATGTCTATGCACTGGTGGACAGTAAGTGGCTCAATCTCTCTTCAAGATTATTC
  G N V Y G P G G Q .....intron C.....
1021 CACTAACCATCGTAGGGGTGTCTACACCGATCCGAAAGCTTGGCCCTTCTTACTACCA
  .....G V Y T D P K L G P I L Y Y H
1081 CTACGTTGATACGACCATTTGGTACGCTGACTCCAGAACTGTTGGATGGAACGTCAT
  Y V D T T I G Y A D S Q K L F G W N V I
1141 TGATTTCTCGAGCGGCTGGCCTTCGGTGA
  D F T S S G W P S V *

```

Fig. 1. Nucleotide and deduced amino acid sequences of *abnc* from *P. chrysogenum* 31B.

The N-terminal amino acid sequences of the mature *P. chrysogenum* Abnc and a proteolytic fragment, determined by Edman degradation, are underlined.

was found at position 95–105 in the deduced amino acid sequence. So far, two genes encoding fungal endo-arabinanase (*A. niger* ABNA¹³⁾ and *A. aculeatus* ARA1¹²⁾) have been cloned and sequenced. The deduced amino acid sequence of the mature Abnc exhibited high identity to those of mature forms of ABNA (66%) and ARA1 (65%). Cleavage sites of signal peptides of precursors of ABNA and ARA1 were predicted using the signal-peptide-prediction program signalP 3.0 server (<http://www.cbs.dtu.dk/services/SignalP/>).^{17,18)} Both *Aspergillus* endo-arabinanases have been grouped into GH family 43 based on their amino acid sequence [<http://afmb.cnrs-mrs.fr/CAZY/index.html>], indicating that Abnc belongs to the same family. *O*-Glycoside hydrolases hydrolyze glycosidic bonds via general acid catalysis that requires two essential residues: a proton donor and a nucleophile/base. In most cases, Asp and/or Glu residues have been found to be involved in the catalysis.¹⁹⁾ On the basis of X-ray crystallographic studies with the arabinanase 43A of *Cellvibrio japonicus*, the Asp³⁸ and Glu²²¹ residues have been proposed to act as the catalytic base and acid, respectively, in enzymes belonging to the GH family 43.²⁰⁾ These amino acid residues were also conserved at Asp³⁴ and Glu¹⁹⁹ in Abnc. As mentioned earlier, Abnc is a cold-adapted endo-arabinanase and the *Aspergillus* endo-arabinanases are mesophilic. Nevertheless, high identity was observed in amino acid sequences among the enzymes. In order to compare the specific activities of Abnc and *A. niger* ABNA towards debranched arabinan, the latter enzyme was isolated as described previously.¹⁰⁾ Typical assay for arabinanase activity was performed by measuring the release of reducing groups in a reaction mixture containing 200 μ L of 0.1% debranched arabinan (Megazyme International Ireland Ltd., Ireland) in 20 mM acetate buffer (pH 5.0) and the enzyme sample. Reducing sugars were measured by the method of Somogyi.²¹⁾ Specific activities of Abnc and ABNA at 0°C and 10°C were similar. However, specific activities of Abnc at 20°C and 30°C

Fig. 2. Temperature dependence of the recombinant MBP-Abnc.

●, stability; ○, enzyme activity. The temperature stability was evaluated by measuring the residual activity after 1 h pre-incubation of the enzyme at temperatures between 0°C and 50°C in 20 mM acetate buffer (pH 5.0) containing 50 μ g/mL of bovine serum albumin. For determination of optimum temperature of the enzyme, the enzyme reaction was performed at various temperatures for 15 min.

Fig. 3. Analysis of the enzymatic products of arabinooligosaccharides with the recombinant Abnc.

A reaction mixture containing 25 μ L of the recombinant Abnc (1.5 mU) and 25 μ L of 5 mM arabinooligosaccharide in 20 mM acetate buffer (pH 5.0) was incubated at 30°C for the times shown. Aliquots were taken at intervals, diluted 2 times with 10 mM HgCl₂, boiled for 5 min to inactivate the enzyme, and analyzed with HPAEC. The area ratios given are not true product ratios although PAD responses are often approximately equimolar. ×, arabinose; ◇, arabinobiose; ●, arabinotriose; ○, arabinotetraose; ■, arabinopentaose; □, arabinohexaose; ▲, arabinheptaose.

were calculated to be 85% and 44% of those of ABNA, respectively, indicating that Abnc may exhibit the optimum enzyme activity at low temperatures due to losing the thermo-stability under moderate temperatures. Further studies using site-directed mutagenesis or DNA shuffling are needed to determine the amino acids involved in the thermo-stability of the enzymes.

To obtain the mature Abnc, PCR was performed using two primers (cold-N/Eco and cold-stop/Xba) and the cloned *abnc* cDNA as the template. Primer cold-N/Eco (5'-GGAATTCTACGCGAATCCCGGAGC) has an *Eco*R I site at its 5' end. The amplified fragment was digested with *Eco*R I and *Xba* I, and ligated to the restriction enzyme sites of the pMAL-c2X vector (New England BioLabs), forming a new plasmid termed pMAL-AC1. The recombinant Abnc was produced by *E. coli* DH5 α transformed with the plasmid pMAL-AC1 as previously reported.¹⁶⁾ The recombinant maltose binding protein (MBP)-fusion Abnc was purified by an Amylose Resin column (New England BioLabs) as previously reported.¹⁶⁾ The yield of the MBP-fusion Abnc from 1 L of the transformant culture medium was approximately 40 mg. Figure 2 shows the influence of temperature on enzyme activity and stability of the recombinant MBP-Abnc. Pre-incubation of the recombinant MBP-Abnc at 40°C for 1 h significantly inactivated the activity of the enzyme. Optimum activity occurred at 30°C. The enzyme activity was approximately 40% of maximum even at 0°C. These results were similar to those of the *P. chrysogenum* Abnc. Degradation of arabinooligosaccharides with different DPs (DP3 to DP7; Megazyme) by the recombinant Abnc was studied with high-performance anion-exchange chromatography (HPAEC) using a Dionex DXc-500 system with a CarboPac PA-1 column (4×250 mm; Dionex) (Fig. 3). Reaction products were eluted at a flow rate of 1 mL/min with 0.1 M NaOH for 5 min followed by a linear gradient from 0 M to 0.45 M sodium acetate in 0.1 M NaOH for 30 min. The effluent was monitored with pulsed amperometric detection (PAD). Arabinotriose was not hydrolyzed by the enzyme under experimental conditions. Arabinotetraose and arabinopentaose were slightly degraded to arabinose and arabinotriose for arabinotetraose, and arabinobiose and arabinotriose for arabinopentaose, respectively. The activity of the recombinant Abnc was great with

arabinohexaose and arabinheptaose among oligosaccharides tested. Progression of products released by the recombinant enzyme on debranched arabinan was also analyzed with HPAEC. The enzyme produced several arabinooligosaccharides at the initial stage of hydrolysis and accumulated arabinotriose at longer reaction times (data not shown). The data suggest that the enzyme hydrolyzes the substrate in an endo manner and that the major final product is arabinotriose, which is similar to the catalytic properties of native Abnc.¹⁰⁾ The recombinant Abnc expressed from the plasmid pMAL-AC1 is a MBP fusion protein. Taking into account the binding ability of MBP to carbohydrate, enzyme activity of the recombinant Abnc towards various polymeric substrates including wheat arabinoxylan, lupin galactan, polygalacturonic acid, soluble starch, and carboxymethyl cellulose, was assayed. The enzyme showed no activity towards all the substrates tested, indicating that the existence of MBP in the recombinant enzyme did not affect the substrate specificity of native Abnc.

Cold-adapted enzymes have recently received much attention due to their many applications in the textile and food industries, and in bioremediation and in basic studies of the mechanism of their adaptation to low temperatures.²²⁾ So far, cold-adapted polysaccharidases involving cellulase,^{23,24)} amylase,²⁵⁾ xylanase,²⁶⁻²⁸⁾ and polygalacturonase²⁹⁾ have been characterized. In respect of the fruit juice industry, pectinases are often used to improve juice extraction and to clarify the final products. On the other hand, there is a haze problem in apple juice concentrates due to the formation of α -1,5-L-arabinan,³⁰⁾ which can be degraded by endo-arabinanases. These enzymatic treatments of fruit juice should be carried out at low temperatures to prevent deterioration of quality. Cold-adapted endo-arabinanase would be useful in the field.

This research was financially supported in part by the Japan Science and Technology Agency.

REFERENCES

- 1) K. Tagawa and A. Kaji: Preparation of L-arabinan and 1,5-L-arabinan. *Methods Enzymol.*, **160**, 542-545 (1988).

- 2) G. Beldman, H.A. Schols, S.M. Pitson, M.J.F. Searle-van Leeuwen and A.G.J. Voragen: Arabinans and arabinan degrading enzymes. *Adv. Macromol. Carbohydr. Res.*, **1**, 1-64 (1997).
- 3) K. Komae, A. Kaji and M. Sato: An α -L-arabinofuranosidase from *Streptomyces purpurascens* IFO 3389. *Agric. Biol. Chem.*, **46**, 1899-1905 (1982).
- 4) L. Weinstein and P. Albersheim: Structure of plant cell walls. IX. Purification and partial characterization of a wall-degrading endo-arabinase and an arabinosidase from *Bacillus subtilis*. *Plant Physiol.*, **63**, 425-432 (1979).
- 5) A. Kaji and K. Tagawa: Purification, crystallization and amino acid composition of α -L-arabinofuranosidase from *Aspergillus niger*. *Biochim. Biophys. Acta*, **207**, 456-464 (1970).
- 6) F.M. Rombouts, A.G.J. Voragen, M.F. Searle-van Leeuwen, C. C.J.M. Geraeds, H.A. Schols and W. Pilnik: The arabinanases of *Aspergillus niger*-purification and characterisation of two α -L-arabinofuranosidases and an endo-1,5- α -L-arabinanase. *Carbohydr. Polym.*, **9**, 25-47 (1988).
- 7) T. Sakai and T. Sakamoto: Purification and some properties of a protopectin-solubilizing enzyme that has potent activity on sugar beet protopectin. *Agric. Biol. Chem.*, **54**, 879-889 (1990).
- 8) T. Sakamoto and J.-F. Thibault: An exo-arabinanase of *Penicillium chrysogenum* able to release arabinobiose from α -1,5-L-arabinan. *Appl. Environ. Microbiol.*, **67**, 3319-3321 (2001).
- 9) T. Sakamoto and H. Kawasaki: Purification and properties of two type-B α -L-arabinofuranosidases produced by *Penicillium chrysogenum*. *Biochim. Biophys. Acta (General subjects)*, **1621**, 204-210 (2003).
- 10) T. Sakamoto, H. Ihara, S. Kozaki and H. Kawasaki: A cold-adapted endo-arabinanase from *Penicillium chrysogenum*. *Biochim. Biophys. Acta (General subjects)*, **1624**, 70-75 (2003).
- 11) G. Beldman, M.J.F. Searle-van Leeuwen, G.A. De Ruiter, H. A. Siliha and A.G.J. Voragen: Degradation of arabinans by arabinanases from *Aspergillus aculeatus* and *Aspergillus niger*. *Carbohydr. Polym.*, **20**, 159-168 (1993).
- 12) M. Skjt, S. Kauppinen, L.V. Kofod, C. Fuglsang, M. Pauly, H. Dalbge and L.N. Andersen: Functional cloning of an endo-arabinanase from *Aspergillus aculeatus* and its heterologous expression in *A. oryzae* and tobacco. *Mol. Genet. Genomics*, **265**, 913-921 (2001).
- 13) M.J.A. Flippin, H. Panneman, P. van der Veen, J. Visser and L.H. de Graaff: Molecular cloning, expression and structure of the endo-1,5- α -L-arabinase gene of *Aspergillus niger*. *Appl. Microbiol. Biotechnol.*, **40**, 318-326 (1993).
- 14) H. Ihara, T. Uemura, M. Masuhara, S. Ikawa, K. Sugimoto, A. Wadano and M. Himeno: Purification and partial amino acid sequences of the binding protein from *Bombyx mori* for CryIAa delta-endotoxin of *Bacillus thuringiensis*. *Comp. Biochem. Physiol. B*, **120**, 197-204 (1998).
- 15) T. Sakamoto, M. Yamada, H. Kawasaki and T. Sakai: Molecular cloning and nucleotide sequence of an endo-1,5- α -L-arabinase gene from *Bacillus subtilis*. *Eur. J. Biochem.*, **245**, 708-714 (1997).
- 16) T. Sakamoto, H. Ihara, A. Shibano, N. Kasai, H. Inui and H. Kawasaki: Molecular characterization of a *Penicillium chrysogenum* exo-1,5- α -L-arabinanase that is structurally distinct from other arabinan-degrading enzymes. *FEBS Lett.*, **560**, 199-204 (2004).
- 17) J.D. Bendtsen, H. Nielsen, G. von Heijne and S. Brunak: Improved prediction of signal peptides: SignalP 3.0. *J. Mol. Biol.*, **340**, 783-795 (2004).
- 18) H. Nielsen, J. Engelbrecht, S. Brunak and G. von Heijne: Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. *Protein Eng.*, **10**, 1-6 (1997).
- 19) G. Davies and B. Henrissat: Structures and mechanisms of glycosyl hydrolases. *Structure*, **3**, 853-859 (1995).
- 20) D. Nurizzo, J.P. Turkenburg, S.J. Charnock, S.M. Roberts, E.J. Dodson, V.A. McKie, E.J. Taylor, H.J. Gilbert and G.J. Davies: *Cellvibrio japonicus* α -L-arabinanase 43A has a novel five-blade beta-propeller fold. *Nat. Struct. Biol.*, **9**, 665-668 (2002).
- 21) M. Somogyi: Notes on sugar determination. *J. Biol. Chem.*, **195**, 19-23 (1952).
- 22) C. Gerday, M. Aittaleb, M. Bentahir, J.-P. Chessa, P. Claverie, T. Collins, S. D'Amico, J. Dumont, G. Garsoux, D. Georgette, A. Hoyoux, T. Lonhienne, M.-A. Meuwis and G. Feller: Cold-adapted enzymes: from fundamentals to biotechnology. *Trends Biotechnol.*, **18**, 103-107 (2000).
- 23) S. Violot, R. Haser, G. Sonan, D. Georgette, G. Feller and N. Aghajari: Expression, purification, crystallization and preliminary X-ray crystallographic studies of a psychrophilic cellulase from *Pseudoalteromonas haloplanktis*. *Acta Crystallogr. D Biol. Crystallogr.*, **59**, 1256-1258 (2003).
- 24) A.H. Iyo and C.W. Forsberg: A cold-active glucanase from the ruminal bacterium *Fibrobacter succinogenes* S85. *Appl. Environ. Microbiol.*, **65**, 995-998 (1999).
- 25) G. Feller, T. Lonhienne, C. Deroanne, C. Libioulle, J. Van Beeumen and C. Gerday: Purification, characterization, and nucleotide sequence of the thermolabile α -amylase from the antarctic psychrotroph *Alteromonas haloplanktis* A23. *J. Biol. Chem.*, **267**, 5217-5221 (1992).
- 26) I. Petrescu, J. Lamotte-Brasseur, J.P. Chessa, P. Ntarima, M. Claeysens, B. Devreese, G. Marino and C. Gerday: Xylanase from the psychrophilic yeast *Cryptococcus adeliae*. *Extremophiles*, **4**, 137-144 (2000).
- 27) J. Gomes, I. Gomes and W. Steiner: Thermolabile xylanase of the Antarctic yeast *Cryptococcus adeliae*: production and properties. *Extremophiles*, **4**, 227-235 (2000).
- 28) T. Collins, M.A. Meuwis, I. Stals, M. Claeysens, G. Feller and C. Gerday: A novel family 8 xylanase, functional and physicochemical characterization. *J. Biol. Chem.*, **277**, 35133-35139 (2002).
- 29) H. Birgisson, O. Delgado, L.G. Arroyo, R. Hatti-Kaul and B. Mattiasson: Cold-adapted yeasts as producers of cold-active polygalacturonases. *Extremophiles*, **7**, 185-193 (2003).
- 30) J.R. Whitaker: Pectic substances, pectic enzymes and haze formation in fruit juices. *Enzyme Microbiol. Technol.*, **6**, 341-349 (1984).

Penicillium chrysogenum の生産する 低温適応性エンドアラビナーゼの遺伝子解析

阪本龍司¹, 居原 秀², 芝野麻子²,
永広久雄¹, 川崎東彦¹

¹ 大阪府立大学大学院生命環境科学研究科応用生命科学専攻
(599-8531 堺市学園町 1-1)

² 大阪府立大学大学院理学系研究科生物科学専攻
(599-8531 堺市学園町 1-1)

Penicillium chrysogenum 31B 株は培養上澄中に少なくとも 4 種のアラビナン分解酵素を分泌生産する。その中の一つであるエンドアラビナーゼ (Abnc) は反応最適温度が 30-40℃ であり, 40℃ でも不安定な低温適応性酵素である。本研究では Abnc をコードする遺伝子 (*abnc*) をクローン化し, その塩基配列を決定した。また, *abnc* を大腸菌で発現させ, その産物の酵素学的性質を検討した。本遺伝子の塩基配列を決定した結果, Abnc は 320 アミノ酸残基から構成されるタンパク質であることが推定された。現在までにカビ由来のエンドアラビナーゼとしては, *Aspergillus niger* および *A. aculeatus* 由来の 2 種の中温性酵素の遺伝子の塩基配列が決定されているが, Abnc はこれらのタンパク質とそれぞれ 66% および 65% と高い同一性を示した。*Aspergillus* 由来の両酵素ともに糖加水分解酵素ファミリー 43 に分類されていることから, Abnc も同ファミリーに属することが判明した。また, このファミリーで推定されている触媒残基は本酵素にも保存されており, Asp³⁴ は塩基触媒, Glu¹⁹⁹ は酸触媒として働くことが予想された。大腸菌で発現させた組換え型 Abnc の温度特性は野生型酵素と同様の性質を示したことから, 目的とする遺伝子を単離できたものと考えられる。組換え型 Abnc を用いて, 重合度 3 から 7 までのアラビノオリゴ糖に対する分解活性を測定した結果, 本酵素は重合度 6 以上のオリゴ糖に対して高活性を示した。また, アラビノトリオースに対しては本実験条件下では分解活性を有しなかった。