Note

Cloning and Expression of β -Fructofuranosidase Gene from *Arthrobacter* sp. K-1

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A gene encoding an extracellular β -fructofuranosidase (β -FFase), designated *bffA*, was cloned from the chromosomal DNA of *Arthrobacter* sp. K-1, and expressed in *Escherichia coli*. The putative *bffA* consisted of 1734 bp encoding β -FFase of 578 amino acids. Comparison of the DNA sequence data with the N-terminal amino acid sequence of the purified enzyme indicated the presence of a precursor protein with a signal sequence of 36 residues. The deduced amino acid sequence of the β -FFase demonstrated a high degree of homology with that of levansucrase from *Acetobacter diazotrophicus*. The N-terminal amino acid sequence and some enzymatic properties of the recombinant enzyme were identical to those of the native enzyme.

β -D-fructofuranosidase (EC3.2.1.26; β -FFase) catalyzes the hydrolysis of sucrose as well as the transfructosylation from sucrose. This transfructosylation activity is useful for the production of fructose-containing oligosaccharides. The transfer ratio, defined as the amount of fructose transferred divided by the amount of aldose moiety liberated, in addition to acceptor specificity and the structures of transfer products are dependent on the origin of the enzymes and on reaction conditions. Fungal β -FFase, one example being *Aspergillus niger*, catalyzes mostly hydrolysis of sucrose at a low concentration, whereas transfructosylation mostly occurs in high sucrose concentration. *A. niger* ATCC 20611 β -FFase selectively transfers the fructosyl residue of one sucrose molecule to the C-1 position of the fructose residue of another sucrose, and produces 1-kestose, nystose and fructosyl-nystose.¹⁾ On the other hand, *Arthrobacter* sp. K-1 β -FFase catalyzes mostly hydrolysis when incubated with sucrose alone even at high concentration. In the presence of suitable acceptors

such as lactose, xylose, isomaltose and stevioside, the enzyme preferentially transfers the fructosyl residue of sucrose to the acceptor molecules, not including sucrose. In so doing, lactosucrose, fructosyl-xyloside, isomaltosylfructoside and fructosyl-stevioside are produced, respectively.²⁾ The enzyme from *Arthrobacter* sp. K-1 has a wider range of acceptor specificities than other β -FFases, and is the most suitable agent for the production of heterooligosaccharides containing the fructose molecule. Information concerning the structure of this enzyme will be useful to help determine how β -FFase exhibits fructosyl transfer activity. In this paper, we describe the molecular cloning and the nucleotide sequences of β -FFase gene (*bffA*) derived from *Arthrobacter* sp. K-1.

We purified β -FFase from the cultured supernatant of *Arthrobacter* sp. K-1 according to a previously described procedure.³⁾ The purified enzyme was digested with V8-protease by the method of Cleveland *et al.*⁴⁾ to analyze the N-terminal and inner amino acid sequences of β -FFase using a Model 473A gas-phase protein sequencer (Perkin-Elmer).

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Genomic DNA of *Arthrobacter* sp. K-1 was used as a template for PCR with DNA polymerase (Ex-Taq, Takara). The following two oligonucleotides were used as primers for PCR: 5'-GGSCTSCAGGACGGSCCSGA-3' corresponding to the amino acid sequence GLQDGPE in the N-terminal region of β -FFase, and 5'-TCCASACCASACCTGCTC-3' corresponding to EQVWVD in a V8-protease cleaved fragment. PCR was performed using a DNA Thermal Cycler (Model 394, Perkin-Elmer) according to the following thermocycle program: 1 min at 94°C, 2 min at 50°C, and 3 min at 72°C. A major product of 0.2 kbp was purified from agarose gel after electrophoresis, and inserted into pT-Adv vector (CLONTECH) to generate pBFF200. The DNA fragment derived from pBFF200 was labeled using a rapid DNA labeling kit (Pharmacia Biotechnology), and then used as a probe for colony hybridization. Genomic DNA of *Arthrobacter* sp. K-1 was digested with *Bam*HI because the probe hybridized with a *Bam*HI fragment approximately 10 kbp in length according to Southern blot analysis. DNA fragments 9–11 kbp in length were obtained by agarose gel electrophoresis and ligated into the *Bam*HI site of Charomid 9–36 (Toyobo). The ligated mixture was introduced into *E. coli* JM109 using an *in vitro* packaging kit (Gigapack III Gold Packaging Extract, Stratagene) to construct a limited genomic library of *Arthrobacter* sp. K-1. As a result of colony hybridization, 7 positive clones were obtained out of approximately 4000 transformants from the library. All 7 clones contained the same *Bam*HI fragment. The nucleotide sequence of the region that hybridized with the probe was determined by a Model 373A DNA sequencer (Perkin-Elmer) using the Taq DyeDeoxy terminator cycle sequencing kit (Perkin-Elmer).

The nucleotide sequence from the *Bal*I-*Fse*I region (2307 bp) of the cloned fragment is shown in Fig. 1. An open reading frame (ORF) sequence starting at position 258 and ending at position 1995 was located in this region. A Shine-Dalgarno-like sequence (5'-AGGAGA-3') was found 6–11 bp upstream from the initial codon of *bffA*, and the palindromic sequence (5'-CGGATGCCTCGG-ACCGGCGATCATCGCGGTCCGAGGCATCCG-

3') was found 5–46 bp downstream from the stop codon. The ORF of 1734 bp encodes a protein consisting of 578 amino acids with a molecular mass of 62,838 Da. The DNA sequence from position 373–593 in this ORF was completely identical to that of the probe amplified by PCR. From the N-terminal amino acid sequence of the purified protein, the first amino acid residue of a mature protein was identified as Gln37 in the deduced amino acid sequence, the ORF of which would encode a mature protein of 542 amino acids with a signal peptide of 36 amino acids. The amino acid sequences of peptides prepared from purified enzyme were found in the deduced amino acid sequence. The molecular mass of the deduced mature protein was 59,366 Da. This value is in good agreement with the 60,000 Da** of the β -FFase purified from *Arthrobacter* sp. K-1. This ORF is therefore evidently the structural gene of β -FFase for *Arthrobacter* sp. K-1. The nucleotide sequence data reported in this paper are catalogued in the DDBJ, EMBL and GenBank nucleotide sequence databases with the accession number AB062134.

An amino acid sequence homology search was performed using the FASTA program on the GenBank DNA database. The whole amino acid sequence of β -FFase from *Arthrobacter* sp. K-1 demonstrated homology to the sequence of levansucrase from *Acetobacter diazotrophicus*⁵⁾ (51.0% identity), *Rahnella aquatilis*⁶⁾ (31.1%), *Pseudomonas syringae*⁷⁾ (30.3%) and *Zymomonas mobilis*⁸⁾ (29.6%), according to the result of multiple alignment by the software CLUSTAL W (Fig. 2).⁹⁾ Previously reported reaction analysis determined that this enzyme belongs to β -FFase rather than levansucrase.²⁾ However, the sequence of β -FFase from *Arthrobacter* sp. K-1 was found to be similar to sequences of levansucrase produced by Gram-negative bacteria. Recently, enzymes with β -FFase activity are classified under two families, based on their amino acid sequences. One is β -FFases of family 32 of the glycosyl hydrolases, and the other is levansucrase type of enzymes. The deduced amino acid sequences of *Z. mobilis* extracellular

** The molecular mass, 52,000 Da, reported previously by Fujita *et al.*³⁾ was corrected to 60,000 Da in this report.

<u>TGGCCA</u> CCACTCACCAGGTGTCTCTGCACCGATGTCGATGGGTGTCTCGTAGCCCGCGCCTTGAGGGTCGCGAGCGCATCGGCGAACTCTTCTCTCGTTC	100
<i>Bal I</i>	
CACGGCTCGTCGAGTTAGCAGGGTTGTCAAGTTCCGCGAGCGATGCAGTAGCCCGCGCGACCATGTCCCCGAAATACCTACAAACCTATGCGTTGTCTT	200
ATGAGAGCATGTAGAGGTGCAAATCGATTTCGCATACCTTTCCACATAGGAGAGCACGATGACTCACTCGACGAGAGGGCGCGTTCGTCGCGTCTCGGCC	300
RBS M T H S T R G R V R R V L A	14
GGAGGGCTGGCGACAGTACCTTGCAGCGCCGCTCTGATCGCCGAGCGCCCTGCGACTGCTCAGTCAGGGCTCCAGGACGGCCCGGAGCGACAA	400
G G L A T S T L A A A V L I A G A A P A T A <u>Q S G L Q D G P E P T</u>	47
TTCACTCAGCAGGCGTACGCCCGGAGGATGATTTCACGCCCAAGTGACACGCGCCGACGCCCGCAGTTGCAGCGCATGTCCGACCCACCGGGCC	500
I H T Q Q A Y A P E D D F T A K W T R A D A R Q L Q R M S D P T A P	81
GTCGCGCAGAACTCGATGCGCGCGTCCGTCACTGCCAACTGTGCCGAGGACTTCCCGGACATGTCCAACGAGCAGGTGTGGGTGTGGGACACCTGG	600
S R E N S M P A S V T M P T V P Q D F P D M S N E <u>Q V W V W D T W</u>	114
CCGTGACGGATGAGGATGCCAACAGTACTCCGTCAATGGCTGGGAGATCATCTTCTCGTGGTGGCCGACCCGCAACTCGGCTTGACGACCGGCACG	700
P L T D E D A N Q Y S V N G W E I I F S L V A D R N L G F D D R H	147
TGTTCGCGAAGATCGGCTACTTCTACCGTCCCGCGCGGTACCGCGCGCAGAGCGTCCCGAGAACGGCGGCTGGACGTATGGCGGGCTGGTGTCAAGGA	800
V F A K I G Y F Y R P A G V P A A E R P E N G G W T Y G G L V F K E	181
AGGCGTCACCGCGCAGATCTTCGAGGATCAGTCGTTACGCCACAGACCCAGTGGTGGGGTGGCGCGTGTGTCCAAGAAGCGGAGATCAAGCTGTTC	900
G V T G Q I F E D Q S F S H Q T Q W S G S A R V S K N G E I K L F	214
TTCAACGACGTCGCGTTCTACCGCAACTCTGACGCGACGAACATCAAGCCCTATGACCCCGCATCGCGCTGAGCGTCGGCAAGGTGAAGGCGAACAAAGA	1000
F T D V A F Y R N S D G T N I K P Y D P R I A L S V G K V K A N K	247
AGGCGTCACCCCTCACTGGTTTCAATAAGGTGACCGACCTGTGTCAGGCGGACGGCAGTATTACAGACGGGGCGCAGAACGAGTTCTTCAACTTCCG	1100
K G V T L T G F N K V T D L L Q A D G T Y Y Q T G A Q N E F F N F R	281
CGACCCGTTCACTTCGAGGACCCCGCGCACCCCGCGGAGACTTTCATGGTTTTGAGGGCAACTCCGCCATGCAGCGCAGACCGCGACCTGCAATGAG	1200
D P F T F E D P A H P G E T F M V F E G N S A M R E T A T C N E	314
GCCGACCTCGGCTACCGCCAGGGCGGATCCGTACCGCGAGACCGTCGACGATGTCAACGCTTCCGGCGCGACCTACAGATCGGGAACGTGGGTCTCGCGA	1300
<u>A D L G Y R Q G D</u> P Y A E T V D D V N A S G A T Y Q I G N V G L A	347
AGGCGAAGAACAAGCAACTGACGGAGTGGGAGTTCCTCCCGCGATCTGTCCGCGAACTGCGTTACAGACAGACCGAGCGCGCAGATCTACTTCAA	1400
K A K N K Q L T E <u>W E F L P P I L</u> S A N C V T D Q T E R P Q I Y F K	381
GGATGGTAAGTCGTACCTCTTACGATCAGCCACCGCGGACGTTTCGCGCGGAGTCTGATGGCCCTGAGGGCGTATACGATTCTCGCGCAGCGGTATC	1500
D G K S Y L F T I S H R G T F A A G L D G P E G V Y G F V G D G I	414
CGCAGCGACTACCGCCCTCAACGGCGGATCGGGCCTTGCCCTCGGCAACCCGACGAACCTGAACCTTCTGGGCGGGCAGCGGTCGCCCCTGACTTCA	1600
R S D Y Q P L N G G S G L A L G N P T N L N F L G G Q P F A P D F	447
ATCAGCACCGGGGCACTTCCAGGCGTACTCCCACTACGTCATGCCGGCGGCTTGTCCAGTCGTTTCATGCACACCATCGGAACGCACGATGACTTCGT	1700
N Q H P G H F Q A Y S H Y V M P G G L V Q S F I D T I G T H D D F V	481
TCGCGGCGGCGACGCTCGCACCGACGGTGAATAAGGACATCGGCGTGGGGGCGACCCGACCAAGACGGCCGTCGACTACTCGTACGGCAGCGAGGGGCTG	1800
R G G T L A P T V K M D I G V G G D P T K T A V D Y S Y G S E G L	514
GGCGGCTGGGCGGATATCCCGGCGAACAAGCACTGTTCACAAACGGCAAGTTCGGCGTGGCGGTCTCCGACGAGCGCGCAGAAGATCCGCAAGATCC	1900
G G W A D I P A N K H L F T N G K F G V A V S D E A A Q K I R K I	547
TCGGCTCGAAGTTCGACGACTACCTCGACGCGAGCCGCTCTCAGCCAGGTGCGAGCCCTCATCGAGAAGCTGTGSCACAATCGGCGGCTGACATGA	2000
L G S K F D D Y L D G K P V S A T V R A L I E K L L A Q Y G G *	578
CGGATGCCTCGGACCGGCGATCATCGCCGTCGAGGCATCCGACAGCACCATCACACACATCGAGGAGTCGAGCATGACCGAGGACACACCCGACAG	2100
CCACAGCCGTCTCCGCGCAAGCAGCACGATCGCGCTGGTTCGCGGCGCGCGCAGTCGACCGCTTCTCGTGATCGTGGCGCGGTACTCGTCTGGCCG	2200
CGACCCAGCGTCGCGCCGCTCCCCCGGATGCCGCAACCCGAGCGCCGACGCGAGCGAGGGGGTGTACCGTCTCCCGCTTCGGAAGGCGCGCTTGC	2300
<u>GGCCGGCC</u>	2307
<i>Fse I</i>	

Fig. 1. Nucleotide and deduced amino acid sequences of the *bffA*.

The deduced amino acid sequence of the *bffA* is described below the nucleotide sequence in one-letter notation. The determined amino acid sequences from purified enzyme are underlined below the deduced sequence. The putative ribosome-binding site is underlined and labeled RBS. Convergent arrows indicate the palindrome sequence of the putative terminator.

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AR 1  --MTHSTRGRVRRVLGGTATSTLAAAVLIAG-----AAEPATA
AC 1  MAHVRRKRVATLNMAAGSLMLVLGAQSALAQCNFSRQEAARMAHRPGVMPPRGGLFPGRS
RA 1  -----
PS 1  -----
ZY 1  -----MSTS

AR 37  QSGLDQGEPTIHTQOAYAEEDFTAKWTRADARQLQRMSDPTAPSRENSVPASVTMPTV
AC 61  LAGVPGFELPSIHTQOAYDEQSDFTARWTRADALQKAHSDATVAAGQNSLPAQLTMPNI
RA 1  -----MTNLNYTE-----TIWTRADALKVNEN-----DPTTTOPIV
PS 5  SSASVQLKNSPLAGNINYE-----TVWSRADALKVNEN-----DPTTTOPIV
ZY 1  -----MLNKAGIAEE-----SLWTRADAMKVHTD-----DPTTAMPPTI
      * * * * *

AR 97  EODFPDMSNEQVWVWDTPWLTDEEDANCYSVNGWEIIFSLVADR--NLG-----
AC 121  PADFPVLN-PDVVWVWDTPWLTIDKHADQESYNGWEVIFCLTADPNAGYC-----
RA 32  DADFPVMS-DEWFIWDTMPLRSIDGTVVSVGWSVIFLTAQRNNNSEYLDAGNYDIT
PS 48  SADFPVMS-DTWFIWDTMPLRELDGTVVSVNGWSVILTLTADRHPNDPOYLDANGRYDIK
ZY 34  DYDFPIWT-DKYVWVWDTPWLRDINGQVVSFCGWSVIFALVADRTRYG-----
      *** * * * *

AR 143  --FDDRHVHARIGIFYRPAQVPAERPEPNCGWYGGIVFEQVTCQIFEDQSFHQWQS
AC 168  --FDDRHVHARIGIFYRRACIPASRRPNCGWYGGIHPDGCASAOVYAGQTYTNOAQS
RA 91  SDWNNRHGRATICYWYSRTCK-----DWIFGGRVMAEGVSPTSR-----EWA
PS 107  RDWDRHGRARMSYWYSRTCK-----DWIFGGRVMAEGVSPTTR-----EWA
ZY 80  --WHNRDGRIGIFYRSRGS-----NWIFGCHLLKCANPRSW-----EWS
      * * * * *

AR 201  GSARVSKNG--ETKLFETDVAFYRNSDGTNIKEYPRIALSVGKVKANKKGVTLTGFKVK
AC 226  GSSRLMQIHGNTVSVEYTDVAENRDANANNITEPOAITITQLGRIHADFNHVFMTGTAH
RA 133  CTPILLN-EDGDIDLYTCTVTPG-----ATIAKVRGKVLTSSEGVTLAGEV
PS 149  CTPILLN-DKGDIDLYTCTVTPG-----AAIAKVRGRIVTSDQGVELKDETQV
ZY 120  CCTIMAPGTANSVEVFETSNNDTPS-----ESVPAQCKGYIYADDKSVWFDGDKV
      * * * * *

AR 259  TDLLQADGTYTQGAQNEFFNFRDPFTFEDPAHPGETFMVFEGNSAMORETATCNEADLG
AC 286  TELLQADGTYTQGAQNEFFNFRDPFTFEDPKHPEGVNYMVFEGNATAGQGVANCTEADLG
RA 180  KSLFSADGTYTQTESQNPYNFRDESPFIDP-EDGKLYMVFEGNVAGERGSHVIGKQEMG
PS 196  KKLFEADGTYTQTEAQNSSWNFRDESPFIDP-NDGKLYMVFEGNVAGERGSHVIGKQELG
ZY 171  TDLEQADGTYTQTEAENNEWDERDPHEVINE-EDGKTYALFEGNVAMORGAVAVGEEIG
      * * * * *

AR 319  YRQGDFAETVDDVNASGATYQIGNVGLAKAKNKLTWEFELPPIISANCVTQOTERPOI
AC 346  RRPNDENAEITLQEVLDGAYYOKANIGLATATDSTLSKWKFLSPLISANCVNDQTERPOV
RA 239  TLEPG-----HRDVGNAFYQACIGMAVAKDLSGDEWEILPPLVTAAGVNDQTERPHF
PS 255  PVPPG-----HEDVGAREQVGCIGLAVAKDLSGDEWEILPPLVTAAGVNDQTEREHY
ZY 230  PVPFK-----TETPDGARVYAAATGIAQADNEARTEWKLPLPLVTAAGVNDQTEREHV
      * * * * *

AR 379  YEKDGKSYLFTISHRGTFAAGLDGPPGVYGFVGDG-IRSDYQPLNGSGSLALGNFTNLNF
AC 406  YELNGKYYLFTISHRTTFAAGVDGPDGVYGFVGDG-IRSDYQPLNGSGSLTMGNPTDLNT
RA 292  VEQDGKYYLFTISHKFTYADGLTGPDGVYGFVGLSDN-LTGPYSPMN-ASGIVLGNPPSQ--
PS 308  VEQDGKYYLFTISHKFTYAEGLTGPDGVYGFVGEH-LFGPYSPMN-ASGIVLGNPPSQ--
ZY 283  VEQNGTYLFTISHHSTYADGLSGPDGVYGFVSENGTFGPYSPMN-ASGIVLGNPPSQ--
      * * * * *

AR 438  LGGQPTAPDFNOHFGHFQAYSHYVMPGGLVQSFIDTICTHD--DFVRGGTLAPTVMKMDTG
AC 465  AAGTDEDPSPDQNERAFQSYSHYVMPGGLVESFIDTVENR-----RGGLTAPTVRVRIA
RA 348  -----PFQYSHCVMPNGLVTSFIDNVETSD-GNYRIGGTETAPTVRKIVLK
PS 364  -----PFQYSHCVMPNGLVTSFIDSVETEG-EDYRIGGTETAPTVRILLK
ZY 340  -----PYQAYSHYVMTNGLVTSFIDTIPSSDPNVYRIGGTAPTVMKLELV
      * * * * *

AR 496  VGGDPKTKTAVDYSGSEGLGGWADI PANKHLFTNGKEGVAVSDEAAQKIRKILGSKFDDY
AC 519  QN----ASAVDLRYGNGGLGGYCDIPANRADVNIAGEIQDLFGQPTSGLAAQASTNN---
RA 392  GN----RSFVERVDYGYIPPMKNITLN-----
PS 408  GD----RSFQEEYDYGYPAMKDVTL-----
ZY 385  GH----RSFTEVKGYGYPQIEWLAEDESSNSAAALSLLNK-----
      *

AR 556  LDGKPVSEATVRELIEKLTAQYGG
AC 572  -----AQLAQRVROFLNQ---
RA -----
PS -----
ZY -----

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Fig. 2. Comparison of deduced amino acid sequences of *Arthrobacter* sp. K-1 β -FFase with those of levansucrase.

Arthrobacter sp. K-1 β -FFase (AR), *Acetobacter diazotrophicus* levansucrase (AC), *Rahnella aquatilis* levansucrase (RA), *Pseudomonas syringae* levansucrase (PS) and *Zymomonas mobilis* levansucrase (ZY) are aligned by the CLUSTAL W program. Asterisks indicates identical amino acids.

invertase⁸) and *Bacillus* sp. V230 β -FFase¹⁰) have also been reported to be similar to those of levansucrase.

The pK1 plasmid was constructed to express the recombinant *bffA* gene in *E. coli*. The pK1 plasmid that contains the *bffA* gene for the mature protein region was prepared as follows. To introduce *Nde*I site at the translation initiation codon and *Bam*HI site downstream of the stop codon of the *bffA* gene, primer 1 (5'-CATATGCAGTCA-GGGCTCCAG-3') and primer 2 (5'-GAAGGGA-TCCTCATGTCTAGCCG) were synthesized. PCR amplification was performed using 25 cycles each at 94°C for 1 min, 50°C for 2 min and 72°C for 3 min. After confirmation of the sequence between the *Nde*I and *Bam*HI of the amplified fragment, the fragment was cloned to the *Nde*I-*Bam*HI sites of the pET21a plasmid to construct pK1 plasmid. The transformant of *E. coli* BL21 (DE3) harboring the pK1 plasmid was grown at 30°C for 16h on Luria-Bertani broth containing 0.5 mM IPTG. Cells were washed using 20 mM potassium phosphate buffer (pH 6.0) and disrupted by sonication. Cell debris was removed by centrifugation, and the supernatant was used for purification of the recombinant β -FFase. SDS-PAGE and Western blot analysis of the recombinant enzyme revealed a single band corresponding exactly to those of the native β -FFase produced by *Arthrobacter* sp. K-1 (Fig. 3). In addition, the general properties of the recombinant enzyme corresponded well to the native enzyme (Table 1). The recombinant enzyme hydrolyzed β -2,1-fructofuranosidic linkage between fructose and glucose such as sucrose, raffinose, neokestose, and stachyose, but barely hydrolyzed the β -2,1-fructofuranosidic linkage between fructose and fructose such as 1-kestose and nystose. In the presence of acceptors such as lactose and xylose, the recombinant enzyme catalyzed transfructosylation from sucrose to acceptors, and produced lactosucrose and fructosyl-xyloside, respectively. These results indicate that the enzymatic properties of the recombinant enzyme were identical to those of the native enzyme.

In conclusion, the deduced amino acid sequence of the *bffA* gene product is similar to the sequence of levansucrase. Levansucrase transfers a fructosyl

Fig. 3. SDS-PAGE (A) and Western blot (B) analysis of recombinant β -FFase.

Lane 1: β -FFase from *Arthrobacter* sp. K-1. Lane 2: recombinant β -FFase from *E. coli* BL21(DE3)/pK1. The numbers on the left indicate the position of molecular weight markers (lane M).

Table 1. Some properties of recombinant β -FFase.

Properties	Recombinant	Native ^a
Molecular mass	60 kDa	60 kDa
N-Terminal sequence	QSGQLQDGP	QSGQLQDGP
Optimum pH (40°C, 10min)	6.5	6.5
pH stability (40°C, 2h)	5.5–10.0	5.5–10.0
Optimum temp. (pH6.5, 10min)	55°C	55°C
Temp. stability (pH6.5, 30min)	100%(45°C) 68%(60°C)	100%(45°C) 72%(60°C)
K_m (Sucrose)	10.4 mM	9.1 mM

^a β -FFase purified from *Arthrobacter* sp. K-1

residue of sucrose toward 6-OH of fructose of sucrose, to synthesize a β -2,6-linked fructan (levan). However, the *bffA* gene product barely transfers a fructosyl residue of sucrose toward 1-OH of fructose of sucrose, and the resultant product is not levan but oligosaccharide as 1-kestose. Of interest is

why the *bffA* gene product exhibits a different specificity to levansucrase. The *bffA* gene in this study will provide a clue as to the mechanisms by which the *bffA* gene product expresses fructosyl transfer activity different from levansucrase.

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Arthrobacter sp. K-1 株由来の
 β -フルクトフラノシダーゼ遺伝子の
クローニングと大腸菌における発現

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Arthrobacter sp. K-1 株のゲノム DNA から β -フルクトフラノシダーゼ遺伝子 (*bffA*) をクローニングし、その塩基配列を決定した。 *bffA* は 1734 bp からなり、578 残基のアミノ酸をコードしており、この中には 36 残基のシグナル配列が確認された。推定される一次構造の相同性検索を行ったところ、本酵素は β -フルクトシダーゼよりもレバンスクララーゼと相同性を示し、*Acetobacter diazotrophicus* のレバンスクララーゼと最も高い相同性を示した。 *bffA* を組み込んだ大腸菌から発現酵素を精製しその性質を調べたところ、N 末端のアミノ酸配列、一般的な酵素化学的性質は *Arthrobacter* sp. K-1 株から単離した β -フルクトフラノシダーゼと一致した。