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Two Types of β-Fructofuranosidases from *Aspergillus oryzae* KB

MASAHIRO KURAKAKE,* KENJI OGAWA, MOTOKI SUGIE, AKIHIRO TAKEMURA, KOUJI SUGIURA, AND TOSHIAKI KOMAKI

Faculty of Life Science and Biotechnology, Department of Applied Biological Science, Fukuyama University, Sanzou, Gakuenchou 1 banchi, Fukuyama, Hiroshima 729-0292, Japan

Aspergillus oryzae KB produces two types of β-fructofuranosidases, F1 and F2. F1 produces 1-kestose, nystose, and fructosyl nystose from sucrose through its transfructosylation action. F2 hydrolyzes sucrose to glucose and fructose. N-Terminal amino acid sequences of the purified enzymes were DYNAAPPNLST for F1 and YSGDLRPQ for F2. Each enzyme encoding gene was identified in the genome of *Aspergillus oryzae*. Although the KB strain showed a higher production of F2 than F1 in a low sucrose liquid medium, F2 production gradually decreased, whereas F1 production increased with increasing sucrose concentration in the medium. Synthesis of F1 and F2 *m*RNAs analyzed on reverse-transcription polymerase chain reaction corresponded to individual enzymatic production. During liquid culture of the KB strain, F1 synthesizes fructooligosaccharides from sucrose through transfructosylation, and F2 gradually hydrolyzes it. In a highly concentrated sucrose medium, intake of sucrose into the KB strain was depressed by F1 through synthesis of transfer products, fructooligosaccharides.

KEYWORDS: β-Fructofuranosidase; fructosyltransferase; transfructosylation; fructooligosaccharides; *Aspergillus*

INTRODUCTION

Fructooligosaccharides such as 1-kestose, nystose, and fructosylnystose (**Figure 1**) are produced by an enzymatic reaction and are used as sweeteners having beneficial health functions in humans (*1*), making the resident bifidobacteria in the intestinal flora more vigorous. Some β-fructofuranosidases can transfer the fructosyl residue to the sucrose molecule at a high concentration of sucrose (*2–9*). *Aspergillus niger* β-fructofuranosidase is used for producing commercial fructooligosaccharides from sucrose through its transfructosylation reaction in which fructosyl residues are transferred to sucrose by β−2,1 glycosidic bonds (*10, 11*). β-Fructofuranosidase-related enzymes have six well-conserved regions in the DNA sequence (*12*), whereas β-fructofuranosidase from *Aspergillus* origin generally does not conserve such regions (*13–15*). Although the structure and mechanism of the active site has been discussed for yeast invertase (*16*), there is almost no information about *Aspergillus*-type β-fructofuranosidases.

β-Fructofuranosidases have fructosyl-transferring activity to various extents. Hidaka et al. (*11*) investigated hydrolyzing (U_h) and fructosyl-transferring (U_t) activities of β-fructofuranosidase from various origins such as *A. niger*, *A. oryzae*, *Penicillium nigricans*, and *Saccharomyces cerevisiae*. The ratios of Ut/Uh were widely distributed for species of different origin (*11*). The two types of β-fructofuranosidase from *Aspergillus niger* have been separated, and the enzymatic properties have been discussed (*17, 18*). It was, however, difficult to clearly discriminate between hydrolases and fructosyltransferases and to explain their physiological functions in the microorganism. In many flowering plants, fructosyltransferase synthesizes fructan from sucrose for accumulation as the reserve polysaccharide (*19*). The synthesized products in plants are hydrolyzed by individual hydrolases as the occasion demands. In fungi, the role of fructooligosaccharides produced by fructosyltransferase is poorly understood.

A. oryzae KB was separated from malted rice, which produced high β-fructofuranosidase activity. Interestingly, the strain produced two types of β-fructofuranosidase having mainly transfructosylating and hydrolyzing activities under the conditions of pH 5 and 40 °C, respectively. In this study, we investigated the properties of the two enzymes, their production, their physiological functions in the KB strain, and the improvement of fructooligosaccharides production by controlling both enzyme secretions.

MATERIALS AND METHODS

Materials. 1-Kestose, nystose, and fructosyl nystose were purchased from Wako Pure Chemical Industries Ltd. All other chemicals were reagent grades. The *Aspergillus oryzae* KB strain was separated from malted rice for production of Sake (Japanese traditional liquor).

* Corresponding author. Telephone: 81-084-936-2111, fax: 81-084-936-2023, e-mail: kurakake@fubac.fukuyama-u.ac.jp.

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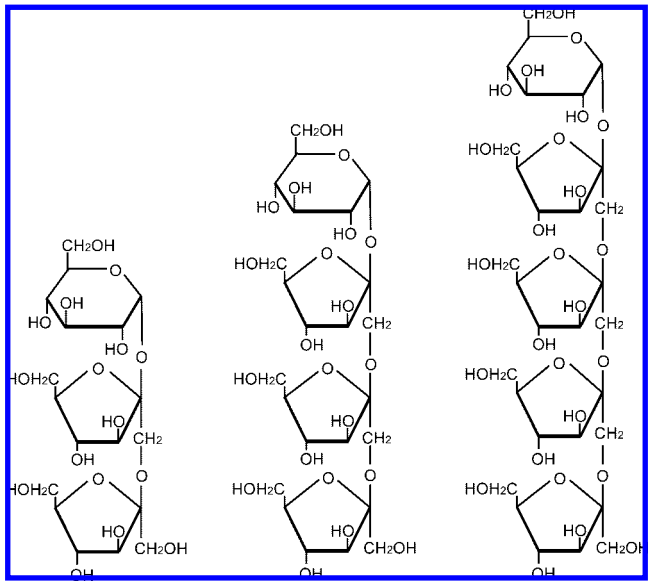

Figure 1. Structures of 1-kestose (left), nystose (center), and fructosyl nystose (right).

Productions of β-Fructofuranosidases in Liquid Culture. The KB strain was cultured in liquid medium (30 mL) containing 0.5–10% sucrose, 0.5% yeast extract, and 0.2% $Na_2HPO_4 \cdot 12H_2O$ in 100 mL flasks at 30 °C, and the culture was mixed at 140 rpm for 1–3 days. The supernatant and suspension following homogenization were used for determination of β-fructofuranosidase activity. The grown mycelial pellets were washed, dried, and weighed.

For the purification of each β-fructofuranosidase, the KB strain was cultured in liquid medium containing 1% (for F2) or 10% (for F1) sucrose, 1.0% yeast extract, and 0.2% $Na_2HPO_4 \cdot 12H_2O$ (400 mL) in 2 L flasks at 30 °C, and the cultures were mixed at 140 rpm for 4 days. Enzymes were released from mycelial pellets by homogenization (Biomixer BM-2, Nihonseiki Kaisha Ltd., Tokyo, Japan), and proteins in the culture supernatant were desalted using saturated 90% ammonium sulfate. Following centrifugation at 1500*g* and 4 °C for 10 min, the precipitate was dissolved in 100 mM sodium acetate buffer (pH 5) and was purified according to methods described in a following section.

Determination of Enzymatic Activities. Hydrolyzing (U_h) and fructosyl-transferring (U_t) activities were measured under high sucrose concentration (20%), although measurements at a low sucrose concentration (1%) were performed for determination of β-fructofuranosidase activity. Twenty percent (w/v) sucrose was incubated with the enzymes at pH 5 and 40 °C for 1 h. The reaction was stopped by incubating for 10 min in boiling water, and the reaction mixture was subjected to HPLC analysis after dilution and filtration through a 0.22 μm membrane filter. The two enzymatic activities were calculated from amounts of glucose (*G*) and fructose (*F*) produced. Total activity ($U_{tot} = U_t + U_h$) corresponding to degradation of sucrose was defined as the amount of enzyme that could produce 1 μmol of glucose from sucrose. Values of U_t and U_h were obtained by multiplying U_{tot} by the transfer ratio $(G - F)/G$ and the hydrolysis ratio F/G, respectively; and they were defined as the amount of enzyme that could hydrolyze 1 μmol of sucrose and transfer 1 μmol of fructosyl residues, respectively.

Activities of F1 and F2 were expressed by eqs 3 and 4, which were derived from eqs 1 and 2. I_t and I_h represented U_t/U_{tot} and U_h/U_{tot} for F1, respectively; and II_t and II_h represented U_t/U_{tot} and U_h/U_{tot} for F2, respectively.

$$U_t = F1 \bullet I_t + F2 \bullet II_t \quad (1)$$

$$U_h = F1 \bullet I_h + F2 \bullet II_h \quad (2)$$

$$F1 = (II_h \bullet U_t - II_t \bullet U_h)/(II_h \bullet I_t - II_t \bullet I_h) \quad (3)$$

$$F2 = (I_t \bullet U_h - I_h \bullet U_t)/(II_h \bullet I_t - II_t \bullet I_h) \quad (4)$$

Purification of β-Fructofuranosidases. Crude KB β-fructofuranosidases were purified using the following chromatography steps at 4 °C. The crude enzyme solution (0.1 g/mL in water) was subjected to gel filtration on a 50 × 2.5 cm i.d. Sephadex G-25 column pre-equilibrated with 10 mM sodium acetate buffer (pH 5). Proteins were eluted with the same buffer at a flow rate of 1.3 mL/min. Fractions were collected every 3 min. Fractions with β-fructofuranosidase activity were pooled and subjected to anion-exchange chromatography on a 50 × 1.0 cm i.d. Super Q Toyopearl column (Toso Ltd., Japan) pre-equilibrated with 10 mM sodium acetate buffer (pH 5). Proteins were eluted with a linear gradient of 0–0.4 M NaCl at a flow rate of 0.16 mL/min.

Fractions with β-fructofuranosidase activity were pooled, and aliquots were subjected to gel filtration on a 100 × 1.0 cm i.d. Sephacryl S-200 column pre-equilibrated with 10 mM sodium acetate buffer (pH 5). Proteins were eluted at a flow rate of 0.1 mL/min, and fractions were collected every 15 min.

Analysis of Sugars. Fructooligosaccharides were analyzed by HPLC with the following conditions: column, 250 mm x 4.6 mm i.d. NH2P-50 (Asahi Chemical Industry Co., Ltd., Kawasaki, Kanagawa, Japan); mobile phase, acetonitrile−water (70:30 v/v); flow rate, 1.0 mL/min; detector, Hitachi model L-3300 differential refractive index monitor.

N-Terminal Amino Acid Sequencing of β-Fructofuranosidases. Purified β-fructofuranosidases were adsorbed on a PVDF (polyvinylidene difluoride) membrane and subjected to N-terminal amino acid sequencing by automatic Edman degradation using a Procise 491 protein sequencing system (Applied Biosystems Ltd., MA, USA).

In-Gel Digestion and Tryptic Peptide Mass Fingerprinting. F1 enzyme separated in SDS-PAGE was excised from the gel and washed in 25 mM ammonium hydrogencarbonate−acetonitrile (1:1) and applied to reduction and tryptic digestion treatments (*20*). L-1-Tosylamido-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin (0.05 mg/mL) (Nacalai Tesque Ltd., Kyoto, Japan) was incubated at 37 °C for 20 h. Peptide extract (5 μL) was mixed with 20 mg/mL 3,5-dihydroxybenzoic acid (5 μL), and then an aliquot (1 μL) was spotted on a sample plate. After drying in a desiccator, this was subjected to matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) with a Voyager DE STR mass spectrometer (Applied Biosystem Ltd., MA, USA). Detection was performed in the reflector mode with an accelerating voltage of 20 kV.

Total RNA Separation and Reverse-Transcription Polymerase Chain Reaction. *Aspergillus oryzae* KB was cultured in medium containing 0.5 or 10% sucrose, 0.5% yeast extract, and 0.2% $Na_2HPO_4 \cdot 12H_2O$ at 30 °C and mixed at 140 rpm for 27–72 h. Total RNA was extracted from the mycelial pellet using a cationic surfactant followed by a lithium chloride-based method with a Catrimox-14 RNA Isolation Kit (Takara Bio Ltd., Otsu, Shiga, Japan). Reverse-transcription polymerase chain reaction (RT-PCR) was performed using total RNA (1.5 μg/mL) and two combination patterns of four designed primers (0.5 μM): (1) F1 forward: 5′-TTACACTGATCCTGACAC-3′ and reverse:, 5′-ACCGAAGACGTTACCGGT-3′, (2) F2 forward: 5′-CTTGCGGCAGTTGCACAAGC-3′ and reverse: 5′-TACCACTGAGCCGCATAG-3′. Reverse transcription (RT) was performed for 30 min at 50 °C, and PCR was performed with the following conditions: denaturation for 5 min at 99 °C, followed by 50 cycles of 30 s at 94 °C, 30 s at 60 °C, 2.5 min at 72 °C, and a final extension step for 5 min at 72 °C.

RESULTS AND DISCUSSION

Identification of Two Types of β-Fructofuranosidases. *Aspergillus oryzae* KB produced two types of β-fructofuranosidases: F1 (high transferring activity) and F2 (high hydrolyzing activity). We found that the two enzymes were selectively produced by changing sucrose concentration in the medium. F1 and F2 were preferentially produced in the presence of high sucrose concentration and in the presence of low sucrose concentration, respectively. Two enzymes were produced in each optimal medium and purified by column chromatographies as shown in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Their molecular weights were estimated to be 96 000 Da for F1 and 79 000 Da for F2, and N-terminal

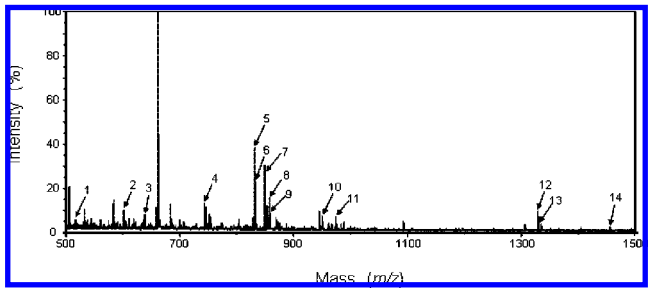

Figure 2. TOF-MS spectrum of peptides from F1 protein digested by trypsin.

Table 1. Molecular Sizes of Peptides Detected by TOF-MS

no.	MH^+	amino acid sequence
1	518	GDQK
2	602	SGAPDR
3	639	ELHIK
4	744	ITEPER
5	831	GQSGIELK
6	832	EALMSGPK
7	850	EEGSSWR
8	858	TLGIDIAR
9	859	YVFCDGR
10	951	FALSTWAR
11	974	AHILPPNGR
12	1330	TISNVVDNELAR
13	1335	TTNGIVSTNESGR
14	1457	NDPVAVFDGSVIPK

amino acid sequences of the two enzymes were DYNAAPPNLST for F1 and YSGDLRPQ for F2. Both enzymes were found to contain glycoprotein using PAS (periodic acid Schiff) staining (*21*) (data not shown). Sequencing of the *A. oryzae* genome (37 Mb) was accomplished in 2005 (*22*). The gene encoding β-fructofuranosidases (amino acid sequence 524, molecular weight 57 459; accession no. AP007164–1378) corresponded to the F2 enzyme according to information of N-terminal amino acid sequencing (YSGDLRPQ). In comparison, the F1 β-fructofuranosidase gene was identified with the exon in 1 626 689–1 628 470 (accession no. AP007167) using N-terminal amino acid sequencing (DYNAAPPNLST) and peptide mass fingerprinting. **Figure 2** shows the TOF-MS spectrum in peptide mass fingerprinting for F1 enzyme. Fourteen signals were identified with the calculated tryptic peptide mass in the amino acid sequence translated from the exon of *A. oryzae* (**Table 1**). The F1 encoding gene was registered to accession no. BR000414 (amino acid sequence 593, molecular weight 64 140). The translated ORF showed high identities to *A. niger* β-fructofuranosidase (65%, accession no. O13388) (*23*), *A. sydowii* IAM2544 fructosyltransferase (62%, accession no. Q9P853) (*14*), and *A. niger* ATCC20611 β-fructofuranosidase (60%, accession no. Q96VC5) (*15*). Although molecular weights of both enzymes on SDS-PAGE were estimated more accurately because of the addition of carbohydrate chains, the ratio between their enzymes was similar.

Reactivity to sucrose was very different between the two enzymes, with the value of U_t/U_h for F1 (22.0) being 110 times larger than that for F2 (0.20). **Table 2** shows the sugar composition of reaction mixtures with 20% sucrose at pH 5 and 40 °C for 24 h. With F1, fructooligosaccharides such as 1-kestose and nystose were efficiently produced by transfructosylation, but with F2, glucose and fructose were mainly released from sucrose by hydrolysis, and the degree of transfructosylation was small. Essential enzyme activities of F1 and

Table 2. Sugar Composition of Reaction Mixture Produced by F1 and F2

enzyme	fructose	glucose	sucrose	1-kestose	nystose	fructosyl nystose
F1	2.8	29.3	9.2	33.4	23.8	1.5
F2	34.0	38.2	19.2	7.8	0.8	0.0

F2 were calculated from the values of U_t/U_h of their purified enzymes using eqs 1−4.

Enzymatic activity was measured at various pH values under appropriate conditions, using phosphate−citrate (McIlvain, pH 2.2–8.0) and glycine−NaCl−NaOH (Söresen, pH 9–10) buffers. Residual activity was measured after incubation at each pH and at 40 °C for 60 min. **Figure 3** shows the effects of pH on purified F1 and F2 β-fructofuranosidases. Optimum pH values of F1 and F2 were found to be around 6 and 5, respectively. F1 showed a stable activity between pH 5 and 7. F2 was more stable at alkaline pH (pH 4–9), and its residual activity at pH 10.8 was about 50%. Optimum temperatures for F1 and F2 activities were almost the same around 50 °C, and stable enzymatic activities at pH 5 for 20 min were found to be below 40 and 50 °C for F1 and F2, respectively.

Induction of F1 and F2 Productions in Liquid Culture. Next, selective production of the two enzymes F1 and F2 were investigated in liquid culture of the KB strain. Sucrose is the optimal carbon source for the production of β-fructofuranosidases from this strain. Relationships between sucrose concentrations in the medium and enzymatic productions were investigated in liquid culture at 30 °C and at 140 rpm for 3 days. As

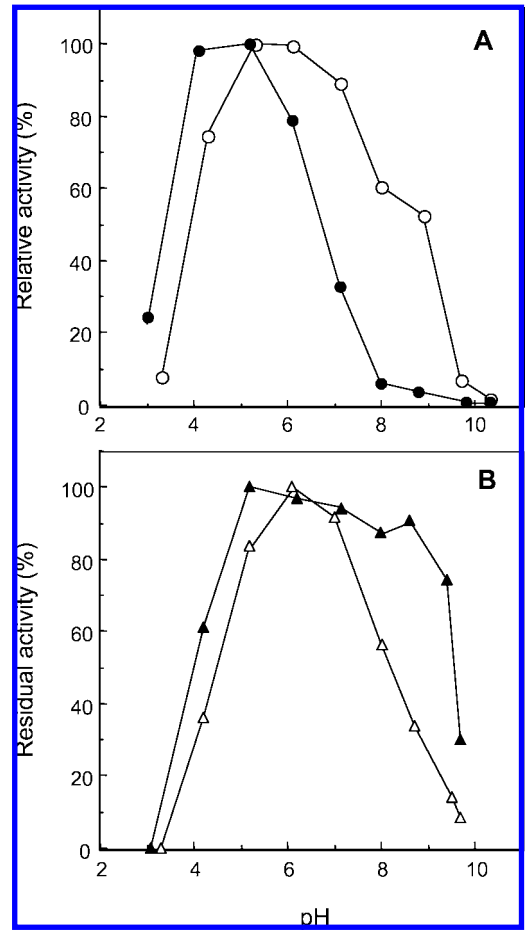

Figure 3. Effects of pH on β-fructofuranosidase activities of F1 and F2. Symbols: (○) and (△) are relative activities and (●) and (▲) are residual activities for F1 (**A**) and F2 (**B**), respectively.

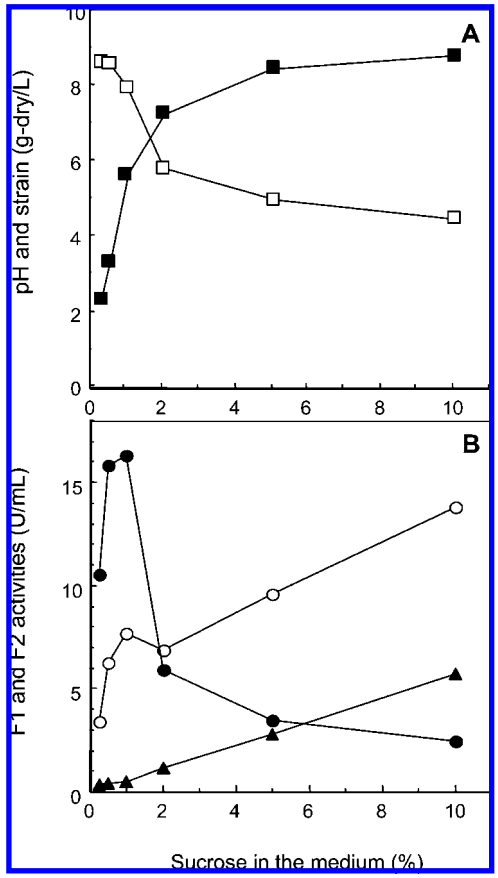

Figure 4. Effects of sucrose in the liquid medium on enzymatic production from the KB strain. KB symbols: (**A**) pH (□), strain dried-weight (■); (**B**) enzymatic activities of F1 (○) and F2 (●), and ratios of F1/F2 activities (▲). Variables are plotted as a function of sucrose concentration present in the medium.

Table 3. F1 and F2 Activities in the Supernatant of the KB Culture Broth and in Homogenized Mycelial Pellets

sucrose (%)	sample	F1 (U/mL)	F2 (U/mL)	F1/F2
1	supernatant	0.83	7.58	0.11
	mycelial pellets	6.55	11.19	0.59
5	supernatant	0.34	0.68	0.50
	mycelial pellets	9.37	0.98	9.60

shown in **Figure 4A**, weight of the grown pellet consisting of mycelia increased, and pH decreased from 8.7 to 4.5 as sucrose concentration in the medium increased. **Figure 4B** shows changes in F1 and F2 activities as a function of sucrose concentration in the medium. While at low sucrose concentrations, production of F2 (with a high hydrolyzing activity) was higher than that of F1 (with a high fructosyl-transferring activity), F2 production gradually decreased whereas F1 greatly increased with increasing sucrose concentration, and F1 production was higher than that of F2 in a medium containing more than 2% sucrose. The F1/F2 ratio increased almost linearly with respect to sucrose concentration in the medium.

Table 3 shows F1 and F2 in extra- and intracellular locations in liquid cultures of KB strain. When the KB strain was cultured in 1 and 5% sucrose media for 3 days, the supernatant of the culture broth and the suspension after homogenizing the grown pellet were used for measurements of each enzymatic activity. In both media, F1 was almost completely localized in mycelial pellets, whereas about 40% of F2 was localized extracellularly.

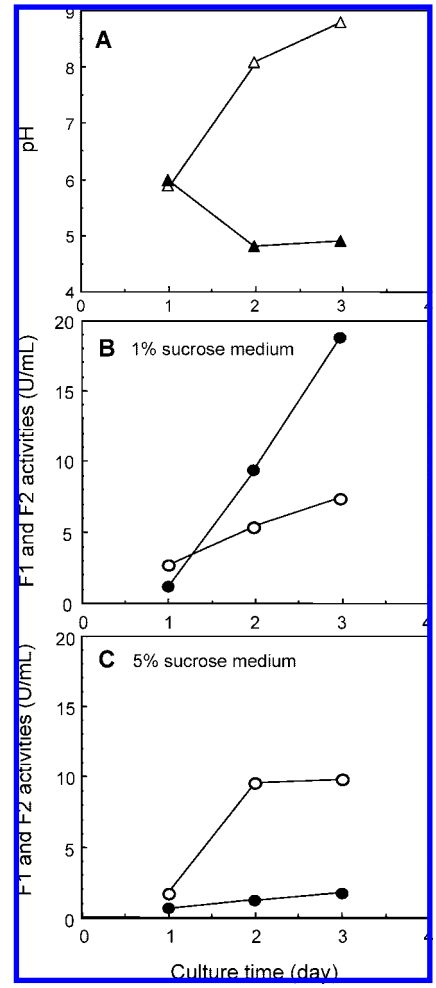

Figure 5. Changes in F1 and F2 activities as a function of growth in liquid culture of the KB strain. Symbols: (**A**) pH (△, ▲) at 1 and 5% sucrose in the medium, respectively; (**B**) activities of F1 and F2 in 1% sucrose-containing medium (○, ●); (**C**) activities of F1 and F2 in 5% sucrose-containing medium (○, ●).

Figure 5 shows time courses of F1 and F2 activities in liquid cultures of the KB strain. Values of pH were dependent on the concentration of the carbon source, changing to pH 8.8 and 4.9 in 1 and 5% sucrose medium, respectively (**Figure 5A**). Both enzymatic activities in 1% sucrose-containing medium were significantly enhanced, and in particular, F2 activity was greater than that of F1 (**Figure 5B**). In 5% sucrose-containing medium, production of F1 was enhanced at an early period of the culture and was maintained, while that of F2 was very low during culture (**Figure 5C**).

Figure 6 shows each sugar concentration in culture broth during liquid culture with 5% sucrose medium, in which production of F1 was higher than that of F2. In a 20–30 h culture, 1-kestose and nystose were synthesized by transfructosylation of F1 with a decrease in sucrose concentration, and they were gradually hydrolyzed to fructose by F2 during the liquid culture. Synthesis of higher molecular oligosaccharides than nystose did not proceed due to the hydrolysis action of F2.

Expressions of *β*-Fructofuranosidase Encoding Genes. Each set of forward and reverse primers for F1 (forward: 5′-TTACACTGATCCTGACAC-3′ and reverse: 5′-ACCGAAGACGTTACCGGT-3′) and F2 (forward: 5′-CTTGCGGCAGTTGCACAAGC-3′ and reverse: 5′-TACCACTGAGCCGCATAG-3′) was designed from sequence data of *A. oryzae*, and

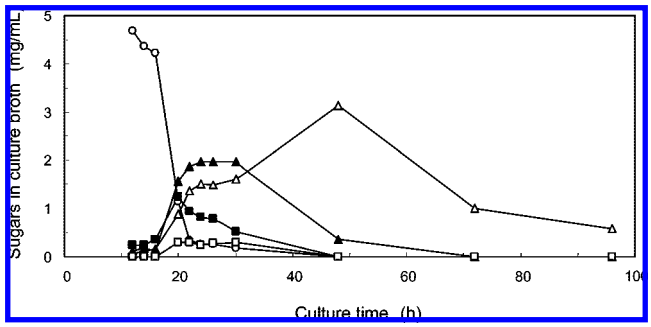

Figure 6. Change in each sugar concentration in the culture broth during the liquid culture with high sucrose medium of the KB strain. Symbols: sucrose (○), glucose (▲), fructose (△), 1-kestose (■), and nystose (□).

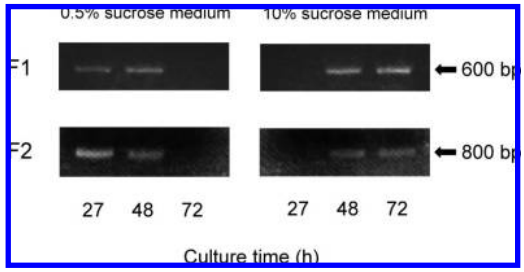

Figure 7. Expressions of F1 and F2 β-fructofuranosidase genes in liquid culture of the KB strain. RT-PCR for RNA expressed in liquid culture with 0.5 and 10% sucrose-containing media.

RT-PCR was carried out to investigate expression of each gene using these primers. **Figure 7** shows expression of each enzyme gene during liquid culture of the KB strain using 0.5 and 10% sucrose-containing media. In 0.5% sucrose-containing medium, both *m*RNAs for F1 and F2 were mainly synthesized in 27 and 48 h cultures and in 48 and 72 h cultures in 10% sucrose-containing medium. Analysis of dye intensities in agarose gels showed that expression of the F2 gene was smaller at high sucrose and that of the F1 gene was higher than that of F2 in 0.5% sucrose-containing medium.

A. oryzae KB produces two types of β-fructofuranosidases. F1 mainly produces 1-kestose and nystose as well as β-fructofuranosidase from *A. oryzae* and *A. niger*, and F2 mainly hydrolyzes sucrose to glucose and fructose as well as β-fructofuranosidase from *Saccharomyces cerevisiae* and *Chalara paradoxa* (*4, 11*).

In liquid culture of the KB strain, the amount of sucrose (carbon source) in the medium influenced production of the two types of β-fructofuranosidases. The production of F1 with a high fructosyl transferring activity corresponded to the growth of the KB strain. In a low sucrose medium, production of F2 and the expression of its coding gene were more enhanced than those of F1. It seemed that the KB strain produced F2 to obtain monosaccharides in the starvation state in a low sucrose medium because F2 supplies monosaccharides of glucose and fructose immediately from sucrose to the strain through its high hydrolyzing activity. On the other hand, specific times for F1 and F2 gene expressions shifted from 27 to 48 h in a high sucrose medium, but production of F1 was more enhanced than that of F2, and expression of the F1 encoding gene was more enhanced than that in a low sucrose medium as compared to expression of the F2 encoding gene in low and high sucrose-containing media. This suggested that F1 affected accumulation of sucrose by transforming sucrose to fructooligosaccharides.

In general, when the carbon source is lacking or is in excess in liquid cultures, the pH value of the culture broth becomes more alkaline and acidic, respectively. In a low sucrose medium, increase in F2 production may be also be due to a higher stability in alkaline pH as shown in **Figure 3B**. It seems that F2 has an important role in the starvation state and that the pH value is related to production of both enzymes. Roles of F1 and F2 enzymes in the KB strain are very interesting. Although fructosyltransferases from *Penicillium chrysogenum* and *A. sydowii* IAM 2544 synthesize the fructan, inulin with a β−2,1 glycosidic bond (*24, 25*), the physiological function of the fructan is unclear. Many flowering plant species synthesize fructan using two types of fructosyltransferases (*26, 27*) and use it as their main carbohydrate reserve, which is hydrolyzed to monosaccharides by hydrolases.

In a high sucrose medium in which F1 is produced more efficiently than F2, fructooligosaccharides were synthesized from sucrose by F1, but finally hydrolyzed by F2. This role between the two enzymes seems to be regulation of intaking sucrose. In high sucrose concentration, the sucrose intake by the KB strain was depressed and occurred gradually over a longer time through the synthesis step of oligosaccharides by F1. This reaction should be taken into consideration because of a cooperation between intracellular F1 (96%) and F2 (59%), as shown in **Table 3**. This was also reflected in the shifts of expressions of F1 and F2 coding genes from 27 to 48 h after delaying sucrose intake in a high sucrose-containing medium.

In an expression study of the fructosyltransferase gene from *A. sydowii* LAM 2544, protein extracts from transgenic bacteria and yeast were shown to synthesize fructooligosaccharides in vitro, but transgenic potato plants were shown to synthesize inulin molecules of up to 40 hexose units in vivo (*14*). In the KB strain, however, accumulation of fructooligosaccharides or polysaccharides such as inulin was not done within the strain in vivo because synthesized fructooligosaccharides were hydrolyzed successively (**Figure 6**).

As a result, *A. oryzae* KB produces two types of β-fructofuranosidases: F1 with a high transferring activity and F2 with a high hydrolyzing activity. Culture conditions for selective productions were obtained by changing sucrose amounts (carbon source). With respect to physiological functions of these enzymes, in low sucrose conditions, F2 was more efficiently produced and hydrolyzed sucrose to glucose and fructose for rapid intake. In high sucrose conditions, F1 was more efficiently produced and synthesized fructooligosaccharides from sucrose through transfructosylation to delay the sucrose intake. The two enzymes from *A. oryzae* KB are thought to be involved in regulation of ingested sucrose necessary for the growth of the strain.

In industrial production of fructooligosaccharides, a few types of β-fructofuranosidases were used. It was, however, found that F1 β-fructofuranosidase with high fructosyl-transferring activity was preferentially produced from *A. oryzae* KB by increasing sucrose concentration in the liquid medium. It is also effective to depress the expression of F2 (with high hydrolyzing activity) encoding gene. This finding may be confirmed for other strains that produce β-fructofuranosidases with various U_t/U_h ratios.

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