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Review

Fungal Inulinases: Enzymology, Molecular Biology and Biotechnology

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Kazuyoshi Ohta,* Hidetoshi Akimoto and Satoshi Moriyama

Department of Biochemistry and Applied Biosciences, Faculty of Agriculture, University of Miyazaki
(1–1, Gakuen Kibanadai Nishi, Miyazaki 889–2192, Japan)

Abstract: Inulin is a fructan in which linear chains of β-(2→1)-linked D-fructofuranose molecules are attached to sucrose at the reducing end. Inulinases have been encountered in higher plants and in microorganisms, including filamentous fungi, yeasts and bacteria. Exoinulinases exo- and endoinulinases from filamentous fungi have been purified and characterized as monomeric glycoproteins. The inulinase genes from *Aspergillus* and *Penicillium* spp. have been cloned and the deduced amino acid sequences included conserved sequences in the β-fructofuranosidase superfamily. A phylogenetic analysis showed that fungal exo- and endoinulinases have independently evolved the respective hydrolytic activities toward terminal and internal β-(2→1)-fructofuranosidic linkages in inulin. *Aspergillus niger* inulinases in an immobilized form were applied to the continuous production of fructose syrup or inulo-oligosaccharides from inulin. High concentrations of ethanol were produced from pure inulin or Jerusalem artichoke tubers by a simultaneous saccharification and fermentation process using *A. niger* and *Saccharomyces cerevisiae.*

Key words: ethanol fermentation, filamentous fungi, immobilized enzyme, inulinase, molecular evolution

Inulin occurs as a reserve carbohydrate in the roots or tubers of some plants such as Jerusalem artichoke (*Helianthus tuberosus* L.), chicory (*Cichorium intybus* L.) and dahlia (*Dahlia variabilis* L.).[1] It is a fructan in which linear chains of β-(2→1)-linked D-fructofuranose molecules are attached to the 1-fructosyl position of sucrose (Fig. 1). The degree of polymerization of inulin should be 30 or greater; inulin has a low solubility in cold water but it is readily soluble in warm water. Some studies provide evidence suggesting that fructans are responsible for the cold hardiness of such plants.[2]

Microbial inulinases are of considerable interest both in fundamental studies and in industrial applications. Inulin sources have received attention as a substrate for the enzymatic production of fructose syrup and as a potential feedstock for direct fermentation by inulinase-producing microorganisms to produce ethanol, acetone-butanol or succinate.[1,3] In our laboratory, fungal inulinases have been studied from the viewpoint of enzymology, molecular biology and their applications to biotechnology during the last decade. This review article focuses on biochemical properties, molecular characterization and evolutionary significance of fungal inulinases. It also describes the potential applications of fungal inulinases to the production of fructose syrup, inulo-oligosaccharides and fuel ethanol from inulin or Jerusalem artichoke tubers.

Classification of inulinases and their modes of action.

Inulinases can be divided into exo- and endo-acting enzymes by their modes of action on inulin.[4] Exoinulinases (β-D-fructan fructohydrolase, EC 3.2.1.80) split off terminal fructose units successively from the nonreducing end of the inulin molecule. The reaction catalyzed by exoinulinase proceeds without dissociating from the residual fructan molecule by a single-chain mechanism of action as shown by kinetic studies of Snyder and Phaff.[5] The exo-acting enzymes hydrolyze sucrose and the fructose portion of raffinose in addition to inulin. Microbial exoinulinases consist of two types of enzymes distinguished by their activity toward β-(2→6)-fructofuranosidic linkages in bacterial levan, β-(2→6)-linked fructan.[6] However, fructan exohydrolases (EC 3.2.1.80) purified from the tubers of Jerusalem artichoke are specific for β-(2→1) linkages, and are inactive against sucrose.[7] Meanwhile, endoinulinases (2,1-β-D-fructan fructanohydrolase, EC 3.2.1.7) are specific for inulin and hydrolyze the internal β-(2→1)-fructofuranosidic linkages to yield inulotriose, inulotetraose and inulopentaose as the main products. Exo- and endoinulinases, invertases (EC 3.2.1.26) and levanases (EC 3.2.1.65) are members of the superfamily β-fructofuranosidase, and belong to family 32 in the numerical classification of glycoside hydrolases.[8]

Occurrence and biochemical properties of fungal inulinases.

1. Distribution of inulinase-producing microorganisms.

Inulinases are found mainly in higher plants[6] and in microorganisms, including filamentous fungi,[9–18] yeasts[5,19–21] and bacteria.[22–26] These inulinase-producing microorganisms are often isolated from rhizosphere soil samples of inulin-containing plants.[11,16,18] Among the inulinolytic filamentous fungi, black aspergilli (*e.g., Aspergillus niger*,[10,11] *Aspergillus ficuum*[13] and *Aspergillus awamori*[14]) and penicillia (*e.g., Penicillium* sp. strain 1,[9] *Penicillium purpurogenum*,[15] *Penicillium* sp. strain TN-88[16] and *Penicil-*

* Corresponding author (Tel. & Fax. +81–985–58–7217, E-mail: k.ohta@cc.miyazaki-u.ac.jp).

Abbreviations: F, D-fructofuranose; G, D-glucopyranose; I/S, inulinase activity/invertase activity; SSF, simultaneous saccharification and fermentation.

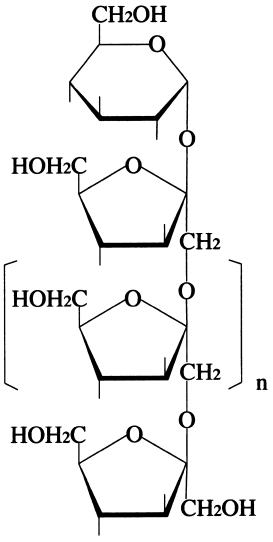

Fig. 1. Structural formula of inulin.

lium trzebinskii[17]) are predominant. Nakamura *et al*.[10] isolated *A. niger* strain 12, which produces extremely high levels of inulinases regardless of the carbon source. Recently, *A. niger* mutant strain 817 was generated from *A. niger* strain 12, and showed 4-fold higher inulinase activity than the wild-type strain in submerged calture.[27] Except for *A. niger* strain 12 and its mutant strain 817, these filamentous fungi were shown to produce inulinase in response to the presence of inulin. *A. niger* strain 12 was first shown to produce an extracellular endoinulinase as well as two forms of exoinulinase.[28–30] Later, endoinulinases were found in filamentous fungi, including *A. ficuum*,[31] *P. purpurogenum*,[15] *Chrysosporium pannorum*[32] and *Penicillium* sp. strain TN-88,[16] and also in bacteria, including *Arthrobacter* sp. strain S37[23] and *Paenibacillus* sp. strain CDB 003.[24] Inulinolytic yeasts include *Kluyveromyces marxianus* (formerly *Kluyveromyces fragilis* or *Saccharomyces fragilis*)[5,20,21] and *Candida kefyr*,[19] and they produce only exoinulinases. The occurrence of exoinulinases in bacteria was described for *Streptomyces rochei*,[22] *Bacillus polymyxa*[25] and *Geobacillus stearothermophilus*.[26]

2. *Fungal inulinase production and standard enzyme assay conditions.*

Nakamura *et al*.[10] optimized conditions for inulinase production by submerged culture of *A. niger* strain 12. They developed basal medium B (initial pH 4.5) that included the following (per liter): 30 g of inulin; 20 g of corn steep liquor; 12 g of $NH_4H_2PO_4$; 0.7 g of KCl; 0.5 g of $MgSO_4 \cdot 7H_2O$; and 10 mg of $FeSO_4 \cdot 7H_2O$. For *A. niger* mutant strain 817, they modified the basal medium by replacing the carbon source with inexpensive sucrose and adding sucrose fatty acid ester as a surfactant to release readily intracellular inulinases.[27] Liquid cultures (120 mL) in 500-mL Erlenmeyer flasks were grown on a rotary shaker (140 rpm) at 30°C for 5 days. Similar media and culture conditions were described for *Penicillium* species.[9,16]

To characterize inulinolytic enzymes, inulinase activity [I] is commonly compared with the invertase activity [S] of the same enzyme preparation; I/S ratio is a useful criterion that may reflect the differences in affinity of each enzyme for inulin and sucrose.[1] Nakamura and Hoashi[9] described the standard assay conditions for inulinase and invertase activities. A reaction mixture consisting of 0.5 mL of 0.5% (w/v) inulin or sucrose dissolved in deionized water and 0.5 mL of suitably diluted enzyme solution in 0.1 M acetate buffer (pH 5.0) was incubated at 40°C for 30 min. The extracellular inulinase and invertase activities in culture filtrates were assayed by measuring reducing sugars released from inulin and sucrose, respectively. One unit of inulinase activity was defined as the amount of enzyme that liberated 1 μmol of fructose equivalents from inulin per min. One unit of invertase activity was defined as the amount of enzyme that hydrolyzed 1 μmol of sucrose per min.

3. *Purification and properties of fungal endo- and exoinulinases.*

In 1978, Nakamura *et al*.[28] first purified extracellular endoinulinase P-III from the culture filtrate of *A. niger* strain 12 by ethanol precipitation, anion-exchange chromatography and gel-permeation chromatography, and obtained the crystalline form by ammonium sulfate precipitation. The enzyme contains 6.7% carbohydrate, of which the major components are mannose and galactose.[4] Later, some other endoinulinases were purified from a commercial inulinase preparation of *A. ficuum* ATCC 16882 (Novozyme 230; Novo A/S, Bagsvaerd, Denmark)[31] and the culture filtrates of *P. purpurogenum*,[15] *C. pannorum*,[32] *A. niger* mutant 817 (P-IA and P-IB)[27] and *Penicillium* sp. strain TN-88.[16] Table 1 summarizes some properties of fungal endoinulinases. They are monomeric glycoproteins with M_rs ranging from 58 to 70 kDa. Apparent K_m values for inulin hydrolysis are in the range of 0.20 to 8.1 mM. Their optimum pH values and temperatures are in the range of 4.8 to 7.0 and 45 to 55°C, respectively.

Fungal extracellular exoinulinases have been purified from *A. niger* strain 12 (P-I and P-II),[29,30] *Penicillium* sp. strain 1 (P-I, P-II and P-III),[33] *P. trzebinskii*,[17] *C. pannorum* (F2 and F3),[34] *A. ficuum* (ExoI and ExoII),[35] *A. awamori*[14] and *Penicillium* sp. strain TN-88.[36] Table 2 compares some properties of fungal exoinulinases. I/S ratios of most exoinulinases vary from about 2.0 to 0.02, and the ratios of the fungal enzymes are generally higher than those of the yeast enzymes.[7] *Penicillium* sp. strain TN-88 enzyme had an extremely high specific activity, 743 U/mg, toward inulin with an I/S ratio of 7.9.[36] Some fungal exoinulinases from *Penicillium* sp. strain 1,[33] *C. pannorum* (F3)[34] and *A. awamori*[14] hydrolyze both β-(2$\rightarrow$1)- and β-(2$\rightarrow$6)-fructofuranosidic linkages as described for yeast exoinulinases.[5,19–21] These fungal enzymes are also monomeric glycoproteins with M_rs ranging from 59 to 87 kDa and exhibit apparent K_m values of 0.003 to 15 mM. Their optimum pH values and temperatures are in the range of 4.0 to 5.2 and 45 to 60°C, respectively.

Molecular characterization and phylogenetic analysis of fungal inulinases.

1. *Molecular cloning and sequence analysis of fungal inulinase genes.*

Endo- and exoinulinase genes from *Aspergillus* and

Table 1. Comparison of the properties of fungal endoinulinases.

Fungal strain	M_r* (kDa)	pH optimum (stability)	Temperature optimum (stability)	K_m (mM)	Specific activity (U/mg) toward: inulin	Specific activity (U/mg) toward: sucrose	Inulin hydrolysis (%)	Hydrolysis products	Ref.
Aspergillus niger strain 12	66	5.3 (4.0–7.5)	45°C (<40°C)	1.25	101	0.00	45	F_3, F_4, F_5	28
Aspergillus niger strain 817									
P-IA	70	5.3 (5.0–7.0)	50°C (<50°C)	0.48	352	0.00	53	F_3, F_4	27
P-IB	68	5.3 (3.5–9.0)	50–55°C (<50°C)	0.50	338	0.00	51	F_3, F_4	27
Aspergillus ficuum	64	4.8–5.2 (NA**)	NA** (<60°C)	8.1	348	0.00	NA**	F_3	31
Crysosporium pannorum	58	6.0–7.0 (4.5–8.5)	50°C (<45°C)	NA**	106	0.00	NA**	F_3, F_4, F_5	32
Penicillium purpurogenum	64	5.1 (5.0–7.5)	NA** (<55°C)	0.21	82.8	0.02	32	F_3, F_4, F_5	15
Penicillium sp. strain TN-88	68	5.2 (5.0–7.0)	50°C (<40°C)	0.20	105	0.00	70	F_3	16

*M_r, determined by SDS-PAGE. **NA, not available.

Table 2. Comparison of the properties of fungal exoinulinases.

Fungal strain	M_r (kDa)	pH optimum (stability)	Temperature optimum (stability)	K_m (mM)	Specific activity (U/mg) toward: inulin	Specific activity (U/mg) toward: sucrose	Activity toward levan	I/S	Ref.
Aspergillus awamori	72	4.5 (3.0–5.0)	60°C (<45°C)	0.003	75	NA*	+	NA*	14
Aspergillus niger strain 12									
P-I	70	4.0 (4.0–7.0)	55°C (<50°C)	0.4	1.68	7.30	−	0.23	29
P-II	59	5.0 (4.0–7.0)	55°C (<50°C)	1.87	7.60	9.50	−	0.80	30
Aspergillus ficuum									
Exo I	76	4.7	60°C	15	NA*	NA*	NA*	0.16	35
Exo II	74	4.7	60°C	11	NA*	NA*	NA*	0.36	35
Crysosporium pannorum									
F2	84	5.0 (5.0–7.5)	55°C (<50°C)	NA*	41.5	62.3	−	0.67	34
F3	70	6.0 (5.0–8.5)	55°C (<45°C)	NA*	10.50	36.2	+	0.29	34
Penicillium sp. strain 1									
P-I	86	4.5 (4.0–6.0)	45°C (<40°C)	0.17	44.3	22.7	+	2.0	33
P-II	64	5.0 (4.0–6.0)	50°C (<40°C)	0.23	16.3	25.0	+	0.65	33
P-III	66	4.0 (4.0–6.0)	45°C (<40°C)	0.16	1.39	1.22	+	1.1	33
Penicillium trzebinskii	87	5.2 (4.0–9.0)	NA* (<50°C)	0.04	103	61.7	−	1.7	17
Penicillium sp. strain TN-88	81	4.0 (5.0–7.0)	55°C (<50°C)	0.09	743	93.8	−	7.9	36

*NA, not available.

Penicillium spp. have been cloned and sequenced recently (Fig. 2).[14,37–42] All the fungal endoinulinase genes lacked introns, whereas the exoinulinase genes contained single introns at the conserved position. *A. niger* strain 12 possessed two copies of the endoinulinase gene, *inuA* and *inuB* (*inu* being derived from inulin), in the genome.[37] The *inuA* and *inuB* genes encoded a 23-amino acid signal peptide and a 493-amino acid mature protein with a calculated M_r of 53 kDa. The *inuB* gene was transcribed constitutively, but the *inuA* gene was silenced irrespective of the carbon source in submerged culture.[38] However, both the endoinulinase genes, *inuA* and *inuB*, were functionally expressed in *Escherichia coli*[37] and in *Saccharomyces cerevisiae* (our unpublished data). Interestingly, the nucleotide sequence of the independently cloned *A. ficuum* endoinulinase gene, *inu2*,[41] was virtually identical to that of the *A. niger inuA* gene. However, it remains unclear if the *A. ficuum* strain has the second copy of the endoinulinase gene corresponding to the *A. niger inuB* gene. The equivalent gene, *inuC*, from *Penicillium* sp. strain TN88 exists as a single copy in the genome.[39] *Penicillium* endoinulinase genes encoded a 25-amino acid signal peptide and a 490-amino acid mature protein with a calculated M_r of 53 kDa.[39,40]

Sequence information about fungal exoinulinase genes has been limited to those from *A. awamori*,[14] *Penicillium* sp. strain TN-88[36] and *A. niger* strain 12.[42] *Aspergillus* exoinulinase genes encoded a 19-amino acid signal pep-

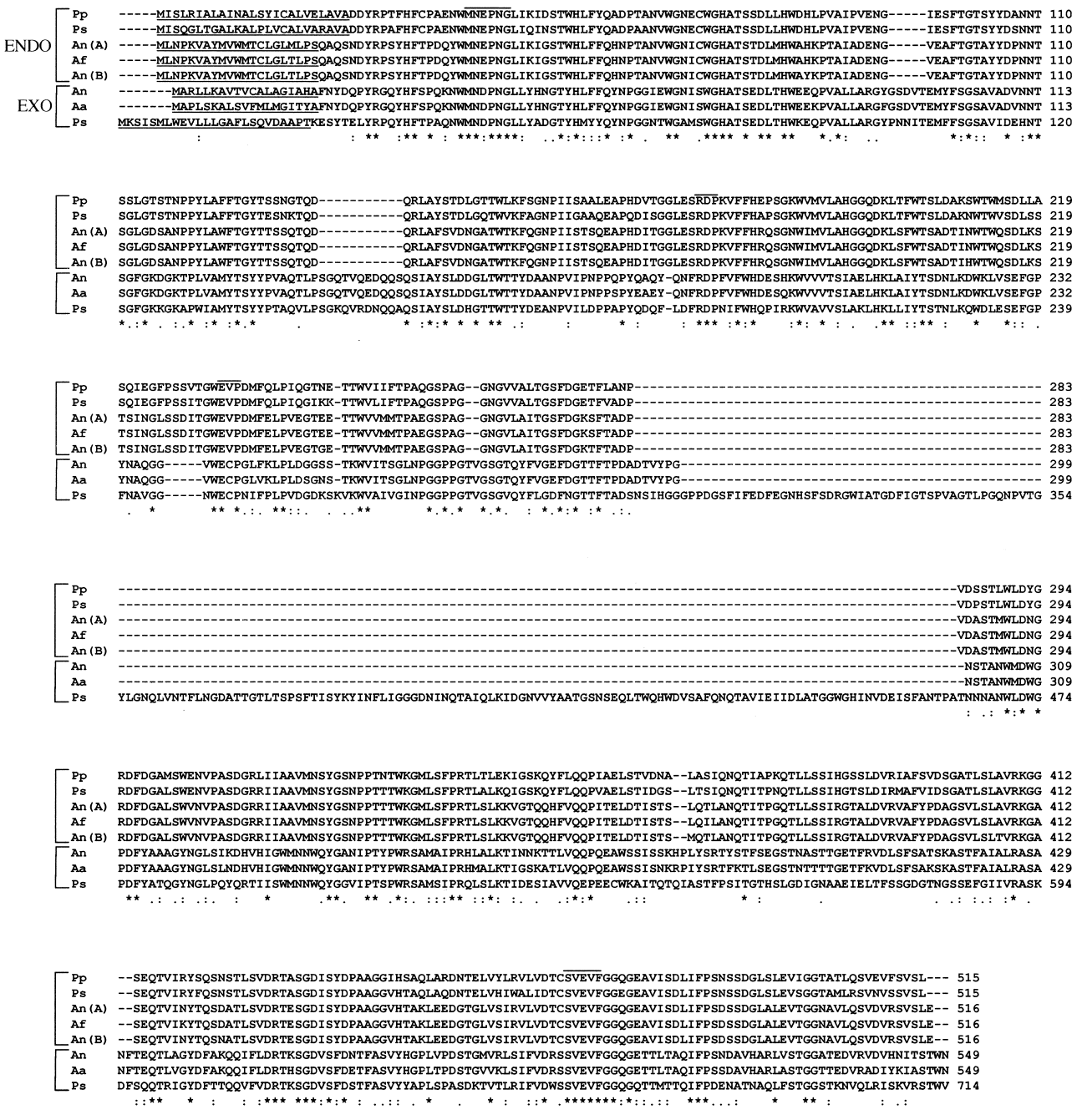

Fig. 2. Multiple sequence alignment of endo- and exoinulinases from *Aspergillus* and *Penicillium* species.

The alignment was done by using the CLUSTAL W program. Dashes (−) indicate gaps introduced during alignment. Asterisks indicate identity, and single and double dots (. and:) indicate lower and higher degrees of amino acid similarity, respectively. Numbering of the amino acids starts at the N-termini of the proteins. The signal peptide sequences are underlined. The overlined amino acid sequences are discussed in the text. The abbreviations used are: ENDO, endoinulinases; EXO, exoinulinases; Pp, *P. purpurogenum*; Ps, *Penicillium* sp. strain TN-88; An(A), *A. niger* (*inuA*); Af, *A. ficuum*; An(B), *A. niger* (*inuB*); An, *A. niger*; Aa, *A. awamori.*

tide and a 518-amino acid mature protein with a calculated M_r of 57 kDa.[14,42] An exoinulinase gene *inuD* from *Penicillium* sp. strain TN-88 encoded a 25-amino acid signal peptide and a 677-amino acid mature protein with a calculated M_r of 75 kDa. The TN-88 exoinulinase included a large insertion of 157 amino acid residues as compared to the *Aspergillus* enzymes. The *inuD* gene was located 860-bp upstream of the endoinulinase gene *inuC* in the opposite direction of transcription.[36] The clustering of *inuC* and *inuD* genes in *Penicillium* sp. strain TN-88 may be an interesting system for further study of the functional significance of their proximity. The methylotrophic yeast *Pichia pastoris* is a suitable host system for heterologous expression of exoinulinases because of the lack of invertase activity and its high secretion efficiency.[42] The *P. pastoris* transformant carrying the *A. niger* exoinulinase gene *inuE* secreted the active recombinant enzyme in high yields.

Reddy and Maley[43] identified functional residues in the β-fructofuranosidase superfamily by site-directed mutagenesis using *S. cerevisiae* invertase as a model; the Asp residue within the motif MNDPNG and the Glu residue in the conserved sequence ECP are involved in the catalytic reaction as a nucleophile and a proton donor, respectively.

The Glu residue instead of the Asp within the motif in the endoinulinases seems to be essential for the enzyme activity because substitution of Glu-43 with an Asp residue in *A. niger* endoinulinase caused the reduction of the activity to 3.5% of its original level (our unpublished data). The Cys residue within the ECP is replaced by Val in the endoinulinases. The sequence SVEVF is conserved among fungal exo- and endoinulinases and bacterial levanases, but not in yeast invertases or yeast exoinulinase. Its existence in the C-terminal parts of the proteins may play an important role in the binding of the high-M_r fructans.

2. *Regulation of inulinase gene expression by carbon sources.*

Many microbial enzymes exhibiting hydrolytic activity are under the dual control of induction and carbon catabolite repression by growth substrates. *Aspergillus* and *Penicillium* inulinase genes included the sequence 5′-GGAAATT-3′ in their promoter regions,[38,39,42] which is reported to be responsible for the starch induction of α-amylase and glucoamylase genes in *Aspergillus oryzae*.[44] The *cis*-acting element is also expected to participate in inulin induction in the fungal inulinase genes. In contrast to the constitutive expression of the endoinulinase gene *inuB* in *A. niger* strain 12, an expression of the exoinulinase gene *inuE* in the same strain was inducible by its substrate inulin or sucrose and repressed in the presence of glucose or fructose.[42] The CREA repressor mediates carbon catabolite repression in *Aspergillus nidulans*.[45] The differential regulation of exo- and endoinulinase genes in *A. niger* strain 12 coincided with the fact that potential CREA-binding sites (5′-SYGGRG-3′) are present in the *inuE* 5′-noncoding region[42] but not in the *inuB* 5′-noncoding region.[38]

3. *Molecular evolution of fungal inulinases.*

Laloux *et al*.[46] suggested a common evolutionary origin for the yeast *K. marxianus* exoinulinase and the *S. cerevisiae* invertase on the basis of the high similarity (67%) of their amino acid sequences. In fact, the *S. cerevisiae* invertase also hydrolyzes inulin at lower efficiency than the yeast exoinulinase.[1] Phylogenetic analysis of β-fructofuranosidases showed that the yeast exoinulinase formed a cluster with invertases of *S. cerevisiae* and *Pichia anomala* (Fig. 3). The cluster of yeast invertases and exoinulinase is linked to that of fungal exoinulinases. *Aspergillus* and *Penicillium* endoinulinases are clustered at a distant position from their exoinulinases and share a branch of the tree with *Actinomyces naeslundii* lavanase. It was suggested that the intron-less endoinulinase genes have arisen from a common ancestor of bacterial origin, which could have been horizontally transferred to the filamentous fungi.[42] The *inuA* and *inuB* genes could have arisen from a recent gene duplication in *A. niger* after the branching of the ancestral gene between the *Aspergillus* and *Penicillium* species. *Bacillus* levanases were located at intermediate positions between the clusters of fungal exo- and endoinulinases. Thus exo- and endoinulinases found in *Aspergillus* and *Penicillium* spp. have independently evolved the respective hydrolytic activities toward terminal and internal β-(2→1)-fructofuranosidic linkages in inulin.

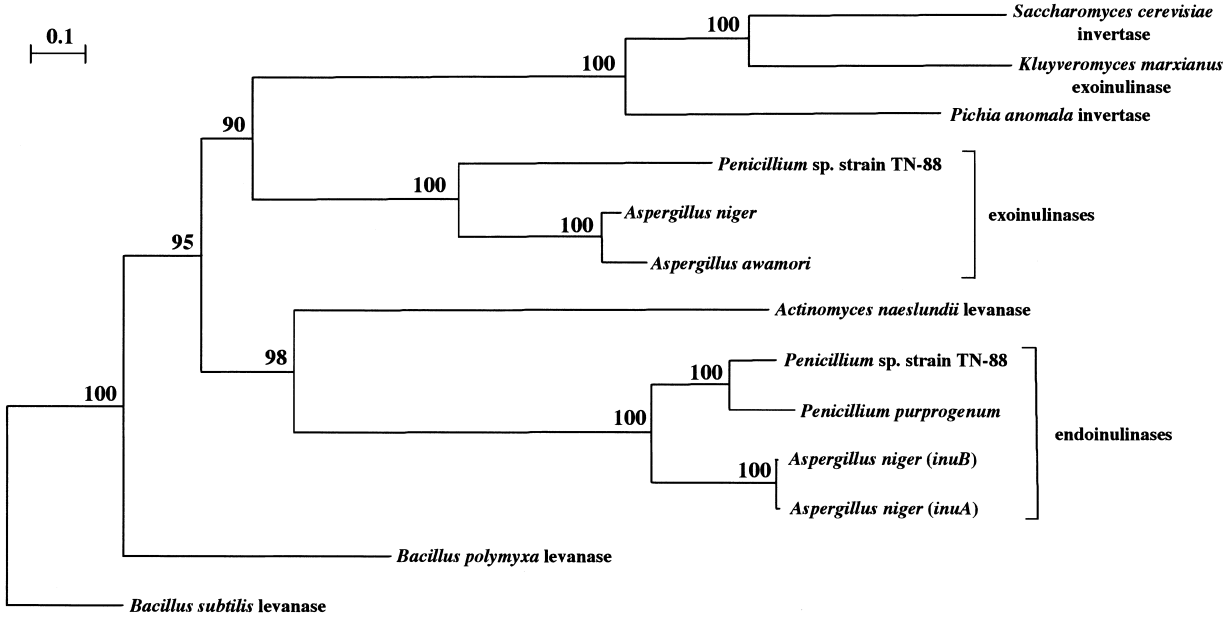

Fig. 3. Phylogenetic tree showing evolutionary distances among fungal inulinases and other homologous β-fructofuranosidases.

The phylogenetic tree was inferred by the neighbor-joining method on the basis of the deduced amino acid sequences. The numbers at internal nodes represent bootstrap confidence values (%) based on 1000 replications. The scale bar denotes 0.1 amino acid substitutions per site. Sources of sequence data (accession numbers are given in parentheses): exoinulinases of *A. niger* (*inuE*, AB100243); *Penicillium* sp. strain TN-88 (*inuD*, AB041337) and *A. awamori* (*inul*, AJ315793); levanases of *A. noeslundii* (*levJ*, U12274), *B. subtilis* (*sacC*, X05649) and *B. polymyxa* (*lelA*, Z26651); endoinulinases of *Penicillium* sp. strain TN-88 (*inuC*, AB041337), *P. purpurogenum* (*INU A*, D84360) and *A. niger* (*inuA*, AB012771; *inuB*, AB012772); invertases of *S. cerevisiae* (*SUC2*, V01311) and *P. anomala* (*INV1*, X80640); *K. marxianus* exoinulinase (*INU1*, X57202).

Applications of fungal inulinases to biotechnology.

1. Continuous production of high-fructose syrup or inulo-oligosaccharides.

High-fructose syrup attracted considerable attention as a low caloric sweetener in the food industry, because D-fructose is two-fold sweeter and more soluble than sucrose.[1] Among the inulinase-producing microorganisms, *A. niger* and *K. marxianus* hold the GRAS (Generally Recognized as Safe) status[47] and are preferred for the production of inulinases to be used in the food industry. Enzyme immobilization may increase processing efficiency and decrease costs. Nakamura *et al*.[48] developed a process for the continuous production of fructose syrups from inulin by using immobilized inulinase from *A. niger* mutant 817. The packed-bed column reactor showed the high volumetric productivity of 410 g of reducing sugars/L/h over 45 days of continuous operation. The reaction product was a mixture of 95% D-fructose and 3% D-glucose.

Non-digestible fructo-oligosaccharides (GF_n) and inulo-oligosaccharides (F_m) act as a type of soluble dietary fiber with a reduced caloric value (the subscript n or m stands for the number of fructosyl moieties). Hidaka *et al*.[49] selected an *A. niger* ATCC 20611 that produces a desirable β-fructofuranosidase with a high transfructosylating activity. They synthesized fructo-oligosaccharides such as 1-kestose (GF_2), nystose (GF_3), and 1^F- β-fructofuranosyl-nystose (GF_4) on an industrial scale by enzymatic transfer of fructosyl moieties from and to sucrose molecules. In our study, inulo-oligosaccharides were continuously produced from inulin in a packed-bed column reactor using immobilized endoinulinase.[50] In common with the GF_n, the resulting F_3 and F_4 were preferentially utilized by *Bifidobacterium* spp. but not by *E. coli* or *Clostridium perfringens*, which produces toxic substances and causes enteritis. Ingestion of these oligosaccharides is expected to exert a bifidogenic effect; selective proliferation of bifidobacteria in the large intestine eliminates other unfavorable bacteria such as *E. coli* and *C. perfringens.* The growth of bifidobacteria on GF_n- and F_m-type oligomers is probably due to their production of intracellular β-fructofuranosidases capable of hydrolyzing these oligomers.[51]

2. Production of high concentrations of ethanol from inulin by the SSF process.

In 1937, Underkofler *et al*.[52] reported the direct fermentation of a crude inulin extract from Jerusalem artichoke to ethanol using inulinase-producing yeasts. The kinetics of ethanol fermentation from Jerusalem artichoke by *K. marxianus* has been extensively studied.[1] However, *K. marxianus* appears to be less ethanol-tolerant than the usual strains of *S. cerevisiae*. In addition, the concerted action of fungal exo- and endoinulinases on inulin allows more effective hydrolysis to fructose than the sole action of yeast exoinulinases. Recently, Ohta *et al*.[53] described a simultaneous saccharification and fermentation (SSF) process using *A. niger* strain 12 and an ethanol-tolerant *S. cerevisiae* strain 1200 and produced high concentrations (20–21%, v/v) of ethanol from pure inulin in 72 h. Subsequently, Nakamura *et al*.[54] obtained ethanol concentrations of 10.4, 15.0 and 20.1% (v/v) from ground tubers, juice concentrate and flour of Jerusalem artichoke, after 15, 72 and 120 h, respectively, using the SSF process.

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糸状菌イヌリナーゼ：酵素化学，分子生物学およびバイオテクノロジー

太田一良，秋元秀俊，森山　聡
宮崎大学農学部応用生物科学科
（889–2192 宮崎市学園木花台西 1–1）

イヌリンは，キクイモの塊茎などに貯蔵多糖として存在し，フルクトースのβ-(2→1)結合による平均重合度30–40の直鎖状構造をもつ（Fig. 1）．イヌリナーゼは，主として高等植物，糸状菌，酵母，細菌に見出される．数種の糸状菌は，イヌリンの非還元末端からフルクトース単位で順次切断するエキソ型イヌリナーゼとイヌリン内部のβ-(2→1)結合に作用し重合度3–5のオリゴ糖を生成するエンド型イヌリナーゼを分泌する．両酵素はともに単量体の糖タンパク質である．各種糸状菌起源のエンド型とエキソ型酵素の諸性質をそれぞれTable 1とTable 2に示す．*Aspergillus*属と*Penicillium*属糸状菌の各酵素をコードする遺伝子はクローニングされ，その塩基配列から推定されるアミノ酸配列中に分泌のためのシグナル配列とβ-フルクトフラノシダーゼ・ファミリーの保存領域MNDPNG（エンド型酵素ではDの代りにE）とECPが見出された（Fig. 2）．推定アミノ酸配列に基づく分子系統樹では，糸状菌エキソ型イヌリナーゼは酵母インベルターゼと類縁関係を示した．一方，イントロンをもたない糸状菌エンド型イヌリナーゼ遺伝子は細菌から糸状菌へ水平伝播した祖先遺伝子から進化したことが示唆された（Fig. 3）．黒麹菌*Aspergillus niger* No. 12株からイヌリナーゼの生産性が4倍高い変異株No. 817を取得した．その高活性イヌリナーゼを固定化し，充填カラムによるバイオリアクターを構築した．本リアクターの使用により，イヌリンから低カロリー甘味料としてのフルクトース・シロップと機能性イヌロオリゴ糖が連続的に安定して生産された．また，上述の*A. niger*変異株とエタノール耐性酵母*Saccharomyces cerevisiae*からなる並行複発酵系により，イヌリンやキクイモ粉末から短時間に高濃度エタノールが生産された．