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Molecular Cloning and Expression of an Isomalto-Dextranase Gene from *Arthrobacter globiformis* T6

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The gene encoding an extracellular isomalto-dextranase, designated *imd*, was isolated from the chromosomal DNA of *Arthrobacter globiformis* T6 and cloned and expressed in *Escherichia coli*. A single open reading frame consisting of 1,926 base pairs that encoded a polypeptide composed of a signal peptide of 39 amino acids and a mature protein of 602 amino acids (M_r, 65,900) was found. The primary structure had no significant homology with the structures of any other reported carbohydrases, including two other dextranases. Transformed *E. coli* cells carrying the 2.3-kb fragment overproduced isomalto-dextranase into the periplasmic space under control of the promoter of the *imd* gene itself.

Dextran, which consists predominantly of α-1,6-linked glucose units, is widely used in the pharmaceutical and biochemical fields as an alternative to blood plasma (11) and as a support for column chromatography. Dextran-hydrolyzing enzymes, known generically as dextranases, have been found in a wide variety of *Mycomycetes* species (9, 10, 35), *Actinomycetes* species (9), and bacteria (3, 22, 28, 38). In 1974, Sawai et al. described a bacterial isomalto-dextranase (IMD) (EC 3.2.1.94; 1,6-α-D-glucan isomaltohydrolase) that was a novel *exo*-type enzyme capable of hydrolyzing dextran to release isomaltose units successively from the nonreducing ends of dextran chains (26, 27). This enzyme is produced extracellularly by *Arthrobacter globiformis* T6, a gram-positive soil bacterium identified by Sawai et al. (26), and some properties of the purified enzyme have been reported by Sawai et al. and by our group (18, 32, 34). Isomaltose, the sole product generated from dextran by this enzyme, inhibits the biosynthesis of mutan (7), which is a water-insoluble glucan composed predominantly of α-1,3-linked glucose units. Since mutan is a major component of dental plaque, isomaltose may be of significant importance for the prevention of dental caries. Thus, manufacture of the oligosaccharide by using an IMD seems to be of potential utility in industrial fields. Cloning and expression of the gene for IMD (*imd* gene) in *Escherichia coli* would make it possible to generate large amounts of pure enzyme with relative ease.

With respect to the structure of genes that encode dextranases, only two reports, concerning a dextran glucosidase (23) and an *endo*-dextranase (20), have been published. However, no regions of homologous amino acids can be found in the amino acid sequences of the two enzymes. Comparison of gene structures among various dextranases should help to elucidate the mechanism of the hydrolysis of dextran. In this report, we describe the cloning and sequencing of a gene that encodes an IMD from *A. globiformis* T6 on a 2.3-kb DNA fragment, as well as the analysis of the gene product expressed in *E. coli* cells. We also compare the enzymatic properties of the IMDs from *A. globiformis* T6 and *E. coli* cells.

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Amino acid sequencing and preparation of probes. IMD was produced in the cell-free medium of a culture of *A. globiformis* T6 (NRRL B-4425, IMA 12103). The enzyme preparation was obtained by methods described previously (18, 32) and purified further by fast-performance liquid chromatography on a Mono-S column (Pharmacia Fine Chemical, Uppsala, Sweden). The purified IMD (6 nmol) was digested with 60 pmol of protease I from *Achromobacter lyticus* (Lys-C protease; Wako Pure Chemical Ind., Ltd., Osaka, Japan) at 30°C for 20 h. The resulted peptides were separated by reverse-phase high-performance liquid chromatography on a TRI ROTER-VI system (Japan Spectroscopic Co., Ltd., Tokyo, Japan) equipped with a Finepak C8-P column (Asahi Chemical Industry, Co., Ltd., Tokyo, Japan). The sequence of each peptide and those of N-terminal regions were determined with a gas-phase protein sequencer (model 477A-120A; Applied Biosystems Inc., Foster, Calif.). Partial amino acid sequence data were obtained from the N terminus of the mature protein and from seven peptide fragments (underlined amino acid sequences marked N and LA through LG in Fig. 1). Three DNA probes, consisting of 29, 17, and 30 bases and designated probes c, e, and g, were synthesized on the basis of the LC, LE, and LG sequences, respectively. The sequences of probes c, e, and g were 5′-TACCAGCAGATCGG(A/C/G)AACAACGC(A/C/G)TGGGT-3′, 5′-GA(C/T)(C/T)TIGGGA(A/G)GA(A/G)CC-3′, and 5′-ATCAACCTIAACTGGATCGA(A/G)CTIGACATG-3′, respectively (overlined nucleotide sequences marked c, e, and g in Fig. 1).

Isolation of the *imd* gene. Chromosomal DNA of *A. globiformis* T6 was prepared as described by Ausubel et al. (2). A *Sau*3AI partial digest of chromosomal DNA was fractionated by centrifugation on a gradient of 5 to 25% NaCl at 37,000 rpm (SW 40Ti rotor; Beckman, Fullerton, Calif.), for 4.5 h, and DNA of 9 to 20 kb was recovered. The fragments were inserted into the *Bam*HI site of λEMBL3 phage vector (8) (Stratagene Cloning Systems, La Jolla, Calif.). The recombinant DNAs were packaged into bacteriophage particles by using packaging extract (Amersham Japan, Tokyo, Japan) and propagated in *E. coli* NM539 (8).

A genomic library consisting of approximately 4×10^5 recombinant phages was screened first with ^{32}P-labeled probe e, and five phage plaques were detected as positive clones. Two

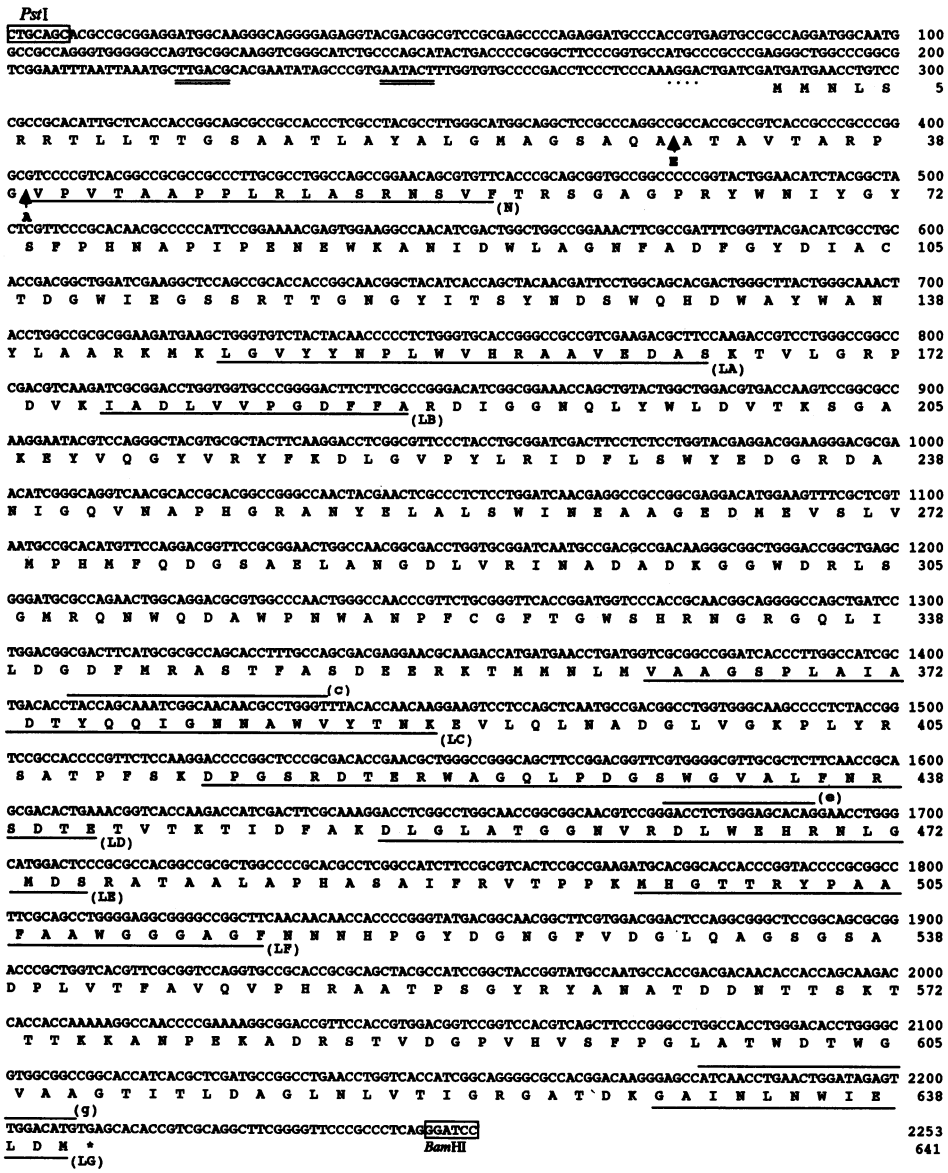

FIG. 1. Nucleotide sequence of the *Pst*I-*Bam*HI fragment. The putative ribosome-binding site is indicated by dots. The −10 and −35 regions of a possible promoter sequence are double underlined. The deduced amino acid sequence is given below the nucleotide sequence. The overlining marked c, e, and g indicates the synthesized oligonucleotide probes. The underlining marked N and LA through LG indicates the amino acid sequences that were determined with a protein sequencer. The arrows marked A and E indicate the site of cleavage of the signal peptide in *A. globiformis* T6 and *E. coli* IMDA, respectively.

of them were rehybridized with ^{32}P-labeled probe c as a second screening, and phage DNAs were isolated from confluent plate lysates by the standard method (24). Hybridizations were carried out at 37°C for the first screening and at 55°C for the second screening in standard buffer (24). Two phage DNAs contained inserts of about 12 kb with an identical restriction pattern (data not shown). To determine the location of the *imd* gene in the fragment, we prepared various subclones that carried plasmids in which *Pst*I, *Hin*dIII, and *Bam*HI digests of the fragment had been ligated at the appropriate sites in pUC119 (36). Three positive clones for the *Pst*I digest only were obtained from the various clones by colony hybridization (24) at 55°C using ^{32}P-labeled probes c and g. The plasmids from the three clones contained the same insert (3.5-kb *Pst*I fragment) with an identical restriction map in each case (data not shown). The plasmid pIMD1, one of the three, was selected for analysis of its nucleotide sequence.

Analysis of nucleotide sequence. The 2,253-bp *Pst*I-*Bam*HI fragment containing the *imd* gene was sequenced by the dideoxy chain-termination method (25) (Fig. 1). In this sequence, a single open reading frame (ORF), starting at position 286 and ending at position 2211, was found. A Shine-Dalgarno-like sequence, AGGA, was found upstream of the initiation codon of the ORF (positions 275 to 278; indicated by dots in Fig. 1). Putative promoter regions of the *imd* gene were detected upstream of the Shine-Dalgarno-like sequence: at positions 243 to 248, the sequence 5′-AATACT-3′ was found as a putative −10 region, and at positions 220 to 225, the

sequence 5′-TTGACG-3′ was found as a putative −35 region (double-underlined sequences in Fig. 1). The ORF could encode a mature protein of 602 amino acids with a putative signal peptide of 39 amino acids. From the results of N-terminal sequencing, the signal peptide appeared to be removed by cleavage between residues 39 and 40 (arrow marked A in Fig. 1). All amino acid sequences from the purified IMD were discovered in the deduced amino acid sequence. Furthermore, the predicted amino acid composition of the mature protein was consistent with that of the purified IMD. The relative molecular weight (M_r, 65,900) of the deduced mature protein agreed very closely with that (M_r, 66,000) of the purified enzyme (32). These data suggest that the ORF encodes an IMD.

The deduced amino acid sequence was compared with other published sequences in various databases of the National Center for Biotechnology Information by the BLAST Network Service. However, no remarkable homologous sequence was found. Although a part of the IMD sequence had weakly homology with the C-terminal region of α-galactosidase from *Cyamopsis tetragonoloba* (21), we obtained insufficient data to discuss at present. We also examined sequence comparisons between IMD and other reported dextranases from *Arthrobacter* sp. strain CB-8 (20) and *Streptococcus mutans* (23) with a DNASIS computer system (Hitachi Soft Engineering, Yokohama, Japan), but we could not detect any homologous region. Although the dextranase from *S. mutans*, DEXB, is an *exo*-type enzyme, as is IMD, DEXB includes three out of four conserved regions that are commonly found in members of the α-amylase family of enzymes (16, 31, 33). It is believed that these regions are involved in the binding of substrate and in the catalytic sites of these carbohydrases. However, such conserved regions were absent in the deduced sequence of IMD. IMD may attack its substrate in a different manner from such carbohydrases.

Expression of the *imd* gene in *E. coli* cells. For expression of the *imd* gene in *E. coli* cells, four plasmids were constructed as follows. (i) For pIMD-A, a *Pst*I-*Bam*HI fragment from the pIMD1 plasmid was ligated into the *Pst*I-*Bam*HI sites of pUC119. (ii) For pIMD-B, pIMD1 was digested with *Bam*HI, blunt ended with a Blunting kit (Takara Shuzo Co., Ltd., Kyoto, Japan), and inserted into a *Hin*dIII linker, 5′-CCAAGCTT-3′. The resultant plasmid, pIMD-A2, was introduced into *E. coli* JM110 cells (24) in order to use the *Cla*I site methylated by Dam methylase (4). A *Cla*I (blunt)-*Hin*dIII fragment from pIMD-A2 was inserted into the *Eco*RI (blunt)-*Hin*dIII sites of pKK223-3 (6). (iii) For pIMD-C, two fragments, namely, *Sma*I-*Aat*II and *Aat*II-*Bam*HI fragments, were prepared from pIMD1 and ligated into the *Eco*RI (blunt)-*Bam*HI sites of pTrc99A (1). (iv) For pIMD-R, the same fragment as described in section i was inserted into pUC118 (36). Transformed *E. coli* cells carrying plasmids pIMD-A, -B, -C, and -R were designated *E. coli* IMDA, IMDB, IMDC, and IMDR, respectively. Each strain of transformed *E. coli* was cultured at 30°C in 20 ml of LB broth (24) until the A_{600} reached about 1.0, then isopropyl-β-D-thiogalactopyranoside (IPTG; final concentration, 1 mM) was added to the culture medium and incubation was continued for 48 h. One milliliter of sample was removed at appropriate intervals, and the cells were harvested and suspended in a buffer (20 mM Tris-HCl [pH 7.5]) that contained 10 mM 2-mercaptoethanol, 5% glycerol, and 0.15% Triton X-100) at 100 μl/4 × 10^8 cells. We estimated that in *E. coli* and *A. globiformis* T6 culture the concentration of the cells was 4 × 10^8 cells per ml at an A_{600} of 1. The suspended cells were sonicated for 15 min and centrifuged at 100,000 × *g* for 20 min. The supernatant was assayed for IMD activity under standard conditions (18) by the Somogyi and Nelson methods (17, 29). As shown in Table 1, the activity was at maximum for *E. coli* IMDA cells after 48 h of culture and was 0.57 to 0.58 U/ml of culture regardless of the addition of IPTG. The numbers of *E. coli* IMDA and *A. globiformis* T6 cells were 1.7 to 1.8 and 3.3 × 10^9 cells per ml, respectively, after 48 h of culture. Therefore, the IMD activity per *E. coli* IMDA cell was about threefold greater than that obtained with *A. globiformis* T6. Although production of IMD by *E. coli* IMDB and IMDC was controlled by *tac* and *trc* promoters, respectively, and was positively induced by IPTG, the associated activities were lower than that of *E. coli* IMDA. Our data on the expression of the *imd* gene showed that *E. coli* cells recognized a promoter on the genomic fragment of *A. globiformis* T6. Shine-Dalgarno-like and promoter-like sequences, similar to the consensus sequences of *E. coli*, were present in the 5′-upstream region of the cloned *imd* gene. Deletion analysis of the region indicated that these sequences were active in *E. coli* cells as a promoter. *E. coli* IMDA and IMDR cells constitutively expressed the IMD protein in the absence and in the presence of IPTG. However, the maximum activity in *E. coli* IMDA was about twice that in *E. coli* IMDR. We constructed a plasmid in which the *imd* promoter and the gene were inserted in pBR322 without a promoter (data not shown). *E. coli* cells carrying this plasmid constitutively produced an IMD protein, and the maximum activity was 0.29 U/ml of culture for 24 h. Since the pIMD-A plasmid includes a *lac* promoter and the *imd* promoter in the same orientation and the pIMD-R plasmid has the two promoters in the inverse orientation, synthesis of IMD in *E. coli* IMDA might be expected as a result of synergism of the two promoters, but no expression was induced by IPTG.

A cell extract of *E. coli* IMDA was analyzed for production of IMD by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (14) and Western blotting (immunoblotting) with rabbit immunoglobulin G against native IMD (5). A polypeptide with a relative molecular weight of 66,000 was overproduced (Fig. 2, lane 4). The polypeptide was transferred to a polyvinylidene difluoride membrane (15) from a large preparative SDS-PAGE gel and subjected to amino acid sequencing. The N-terminal sequence was determined as

TABLE 1. Production of IMD proteins by recombinant *E. coli* cells and *A. globiformis* T6

Strain[a]	Culture time (h)	IPTG[b]	A_{600}	Concn of 10^9 cells/ml[c]	IMD activity (U/ml of culture)	10^9 U/cell
E. coli IMDA	48	−	4.2	1.7	0.58	0.34
		+	4.4	1.8	0.57	0.32
E. coli IMDB	24	−	7.3	2.9	0.12	0.04
		+	7.2	2.9	0.29	0.10
E. coli IMDC	24	−	4.2	1.7	0.02	0.01
		+	4.1	1.6	0.34	0.21
E. coli IMDR	48	−	5.1	2.0	0.23	0.12
		+	5.0	2.0	0.28	0.14
A. globiformis T6	48		8.3	3.3	0.33	0.10

[a] Recombinant *E. coli* cells were cultured in LB broth and *A. globiformis* T6 was cultured in broth that contained 2% dextran, as previously described (18).
[b] −, no IPTG added; +, IPTG added.
[c] These cell concentrations were calculated at 4 × 10^8 cells per ml when A_{600} equaled 1.

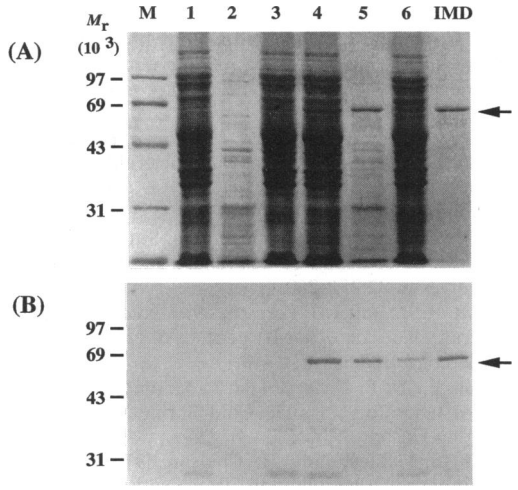

FIG. 2. Expression of the cloned IMD protein from *E. coli* IMDA cells. Lane M, marker proteins, namely, phosphorylase *b* (M_r, 97,000), bovine serum albumin (M_r, 69,000), ovalbumin (M_r, 43,000), and carbonic anhydrase (M_r, 31,000); lanes 1, 2, and 3, total cell extract, periplasmic fraction, and cytoplasmic fraction, respectively, from *E. coli* cells that carried pUC119 as controls; lanes 4, 5, and 6, the same fractions from E. coli IMDA; lane IMD, purified IMD from *A. globiformis* T6. The arrows indicate the positions that correspond to the relative mass of IMD. (A) SDS-PAGE; (B) Western blotting analysis.

Ala-Thr-Ala-Val-Thr-Ala-Arg-Pro, showing that the signal peptide of the IMD produced in *E. coli* IMDA cells had been removed by cleavage between residues 30 and 31 (arrow marked E in Fig. 1). These results indicate that *E. coli* cells were able to utilize the promoter of the *imd* gene rather than a *tac* or *trc* promoter and that they recognized the signal sequence of the IMD that was produced, even though the cleavage site was different from that in the original bacterium. Therefore, the IMD produced in *E. coli* IMDA is clearly secreted from the cytoplasm.

Secretion of the cloned IMD in *E. coli* cells. The transformed *E. coli* cells were cultured as described above. Two milliliters of sample was removed at appropriate intervals, and the cells were harvested. Fractionation of periplasmic and cytoplasmic proteins was performed by osmotic shock as described by Koshland and Botstein (13). Each fraction was assayed for IMD activity and checked for production of the IMD protein by SDS-PAGE and subsequent Western blotting. As we expected, 72% of the IMD activity in the total cell extract was detected in the periplasmic fractions of *E. coli* IMDA and IMDB cells (Table 2). With *E. coli* IMDC, most of the activity remained in the cytoplasmic fraction (Table 2). The activities of β-lactamase (37) and malate dehydrogenase (12) were measured as those of marker enzymes of periplasmic and cytoplasmic fractions, respectively. About 90% of the former activity and 80% of the latter activity were found in the periplasmic fraction and the cytoplasmic fraction, respectively.

TABLE 2. IMD activity of each fraction from transformed *E. coli* cells

Strain[a]	IMD activity (U/ml of culture) from the following fraction[b]		
	Total cells	Periplasmic	Cytoplasmic
E. coli IMDA	0.57 (100)	0.41 (72)	0.13 (23)
E. coli IMDB	0.29 (100)	0.21 (72)	0.06 (21)
E. coli IMDC	0.34 (100)	0.05 (15)	0.30 (88)

[a] *E. coli* IMDA cells were cultured for 48 h without IPTG. *E. coli* IMDB and IMDC cells were cultured for 24 h after IPTG had been added to the cultures.
[b] Numbers in parentheses are percentages indicating recovery in each fraction when the activity in total cells is taken as 100%.

The proteins produced by *E. coli* IMDA were examined by SDS-PAGE and Western blotting (Fig. 2, lanes 4 to 6). The cloned IMD produced *E. coli* IMDA cells was secreted into the periplasmic space and found as a polypeptide with an M_r of 66,000, which reacted with the antibodies raised against native IMD. The cloned IMD from *E. coli* IMDA cells was not secreted into the growth medium but accumulated in the periplasmic fraction. E. coli IMDC cells also produced IMD protein, but most of the IMD activity was located in the cytoplasmic fraction. These results imply that, in *E. coli*, the homologous promoter of the *imd* gene is active and the signal peptide is functional. The production of the original IMD in *A. globiformis* T6 was triggered by dextran, while that of the cloned IMD in *E. coli* was observed to be unaffected by dextran (data not shown).

Some properties of the cloned IMD. After *E. coli* IMDA has been cultured in 500 ml of LB broth at 30°C for 48 h, the cells were harvested and subjected to osmotic shock. Pellets resulting from centrifugation at 10,000 × *g* were resuspended in 200 ml of an ice-cold solution of 20% sucrose and 10 mM Tris-HCl (pH 7.5). Then 6.5 ml of ice-cold 0.5 M EDTA (pH 8.0) was added and incubation on ice was continued for 10 min. The mixture was centrifuged at 10,000 × *g*, and the pellet was resuspended in 200 ml of ice-cold distilled water. The mixture was incubated for 10 min on ice and then centrifuged again. The supernatant was saved as the periplasmic fraction. The cloned IMD was highly purified from this supernatant by the procedure of Okada et al. (18).

The cloned IMD was purified from the periplasmic fraction of *E. coli* IMDA cells to a final specific activity of 40.5 U/mg of protein. In our previous report (18), the specific activity of authentic IMD was given as 30.3 U/mg of protein. In the present study we purified the enzyme still further and determined its properties. Both enzymes migrated as a single band during SDS-PAGE, and the relative molecular weight of each was estimated to be 66,000. The theoretical pI values of the native and cloned IMD were calculated to be 5.44 and 5.54, respectively, from the deduced amino acid sequences. The optimum pH of both enzymes was 5.3. The K_m for dextran of the cloned enzyme, 0.28 mg/ml, was similar to that (0.30 mg/ml) of native IMD from *A. globiformis* T6. Furthermore, antibodies to the native IMD reacted with the cloned IMD (Fig. 2), indicating that the proteins were immunologically identical. These results suggest that, although the cloned IMD had nine more amino acid residues than the native IMD, its properties as an enzyme and a protein were not influenced by the extra amino acids.

We are now trying to isolate the genes for a glucodextranase (19) from *A. globiformis* I42 and an isomaltotrio-dextranase (30) from *Brevibacterium fuscum* subsp. *dextranlyticum*. Resembling IMD, both enzymes are *exo*-type dextranases that are found in the cell-free culture medium, so the structures of their genes may reveal common important regions within the primary sequences of dextranases.

Nucleotide sequence accession number. The nucleotide sequence data of *imd* gene have been submitted to the DDJB, EMBL, and GenBank databases under the accession no. D30761.

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REFERENCES

1. **Amann, E., B. Ochs, and K.-J. Abel.** 1988. Tightly regulated *tac* promoter vectors useful for the expression of unfused and fused proteins in *Escherichia coli*. Gene **69:**301–315.
2. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.).** 1987. Current protocols in molecular biology. John Wiley, New York.
3. **Bailey, R. W., and R. T. J. Clarke.** 1959. A bacterial dextranase. Biochem. J. **72:**49–54.
4. **Brooks, J. E., R. M. Blumenthal, and T. R. Gingeras.** 1983. The isolation and characterization of the *Escherichia coli* DNA adenine methylase (*dam*) gene. Nucleic Acids Res. **11:**837–851.
5. **Coligan, J. E., A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, and W. Strober (ed.).** 1991. Current protocols in immunology. John Wiley, New York.
6. **deBoer, H. A., L. J. Comstock, and M. Vasser.** 1983. The *tac* promoter: a functional hybrid derived from the *trp* and *lac* promoters. Proc. Natl. Acad. Sci. USA **80:**21–25.
7. **Ebisu, S., A. Misaki, K. Kato, and S. Kotani.** 1974. The structure of water-insoluble glucans of cariogenic *Streptococcus mutans*, formed in the absence and presence of dextranase. Carbohydr. Res. **38:**374–381.
8. **Frischauf, A. M., H. Lehrach, A. Poustka, and N. Murray.** 1983. Lambda replacement vectors carrying polylinker sequences. J. Mol. Biol. **170:**827–842.
9. **Hattori, A., and K. Ishibashi.** 1981. Screening of dextranase-producing microorganisms. Agric. Biol. Chem. **45:**2347–2349.
10. **Hiraoka, N., J. Fukumoto, and D. Tsuru.** 1972. Studies on mold dextranases. III. Purification and some enzymatic properties. J. Biochem. **71:**57–64.
11. **Ingelman, B.** 1947. Dextran and its use as a plasma substitute. Acta Chem. Scand. **1:**731–738.
12. **Kitto, G. B.** 1969. Intra- and extramitochondrial malate dehydrogenase from chicken and tuna heart. Methods Enzymol. **13:**106–116.
13. **Koshland, D., and D. Botstein.** 1980. Secretion of beta-lactamase requires the carboxy end of the protein. Cell **20:**749–760.
14. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:** 680–685.
15. **Matsudaira, P.** 1987. Sequence from picomole quantities of proteins electroblotted onto polyvinylidene difluoride membranes. J. Biol. Chem. **262:**10035–10038.
16. **Nakajima, R., T. Imanaka, and S. Aiba.** 1986. Comparison of amino acid sequences of eleven different α-amylases. Appl. Microbiol. Biotechnol. **23:**355–360.
17. **Nelson, N.** 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. **153:**375–380.
18. **Okada, G., T. Takayanagi, and T. Sawai.** 1988. Improved purification and further characterization of an isomalto-dextranase from *Arthrobacter globiformis* T6. Agric. Biol. Chem. **52:**495–501.
19. **Okada, G., T. Unno, and T. Sawai.** 1988. A simple purification procedure for an further characterization of a glucodextranase from *Arthrobacter globiformis* I42. Agric. Biol. Chem. **52:**2169–2176.
20. **Okushima, M., D. Sugino, Y. Kouno, S. Nakano, J. Miyahara, H. Toda, S. Kubo, and A. Matsushiro.** 1991. Molecular cloning and nucleotide sequencing of the *Arthrobacter* dextranase gene and its expression in *Escherichia coli* and *Streptococcus sanguis*. Jpn. J. Genet. **66:**173–187.
21. **Overbeeke, N., A. J. Fellinger, M. Y. Toonen, D. van Wassenaar, and C. T. Verrips.** 1989. Cloning and nucleotide sequence of the alpha-galactosidase cDNA from *Cyamopsis tetregonoloba* (guar). Plant Mol. Biol. **13:**541–550.
22. **Richards, G. N., and M. Streamer.** 1974. Studies on dextranases. Part IV. Mode of action of dextranase D_1 on oligosaccharides. Carbohydr. Res. **32:**251–260.
23. **Russell, R. R. B., and J. J. Ferretti.** 1990. Nucleotide sequence of the dextran glucosidase (*dex*B) gene of *Streptococcus mutans*. J. Gen. Microbiol. **136:**803–810.
24. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:** 5463–5467.
26. **Sawai, T., K. Toriyama, and K. Yano.** 1974. A bacterial dextranase releasing only isomaltose from dextrans. J. Biochem. **75:**105–112.
27. **Sawai, T., Y. Ukigai, and A. Nawa.** 1976. Identification of an isomalto-dextranase producing bacterium, *Arthrobacter globiformis*. Agric. Biol. Chem. **40:**1249–1250.
28. **Sery, T. W., and E. J. Hehre.** 1956. Degradation of dextrans by enzymes of intestinal bacteria. J. Bacteriol. **71:**373–380.
29. **Somogyi, M.** 1952. Notes on sugar determination. J. Biol. Chem. **195:**19–23.
30. **Sugiura, M., A. Ito, and T. Yamaguchi.** 1974. Studies on dextranase. II. New *exo*-dextranase from *Brevibacterium fuscum* var. *dextranlyticum*. Biochem. Biophys. Acta **350:**61–70.
31. **Svensson, B.** 1988. Regional distant sequence homology between amylases, α-glucosidases and transglucanosylases. FEBS Lett. **230:** 72–76.
32. **Takayanagi, T., G. Okada, and S. Chiba.** 1987. Quantitative study of the anomeric forms of isomaltose and glucose produced by isomalto-dextranase and glucodextranase. Agric. Biol. Chem. **51:** 2337–2341.
33. **Tonozuka, T., M. Ohtsuka, S. Mogi, H. Sakai, T. Ohta, and Y. Sakano.** 1993. A neopullulanase-type α-amylase gene from *Thermoactinomyces vulgaris* R-47. Biosci. Biotech. Biochem. **57:**395–401.
34. **Torii, M., K. Sakakibara, A. Misaki, and T. Sawai.** 1976. Degradation of alpha-linked D-gluco-oligosaccharides and dextrans by an isomalto-dextranase preparation from *Arthrobacter globiformis* T6. Biochem. Biophys. Res. Commun. **70:**459–464.
35. **Tsuchiya, H. M., A. Jeanes, H. M. Bricker, and C. A. Wilham.** 1952. Dextran-degrading enzymes from molds. J. Bacteriol. **64:** 513–519.
36. **Vieira, J., and J. Messing.** 1987. Production of single-stranded plasmid DNA. Methods Enzymol. **153:**3–11.
37. **Waley, S. G.** 1974. A spectrophotometric assay of β-lactamase action on penicillins. Biochem. J. **139:**789–790.
38. **Yamaguchi, T., and S. Gocho.** 1973. Production and properties of alkaline dextranase from a newly isolated *Brevibacterium*. Agric. Biol. Chem. **37:**2527–2533.