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ORIGINAL RESEARCH PAPER

Cloning, sequencing and expression of a dextransucrase gene (*dex*YG) from *Leuconostoc mesenteroides*

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Abstract The gene *dex*YG encoding the dextransucrase from an industrial strain of *Leuconostoc mesenteroides* 0326 was isolated by PCR. The nucleotide sequence of the *dex*YG gene consists of an open reading frame (ORF) of 4,584 bp, coding for a 1,527 aa protein with a Mr of 170 kDa. The results were analysed by a BLAST similarity search of the GenBank database, which revealed the amino acid sequence was similiar to *dsr*D derived from *L. mesenteroides* Lcc4. The *dex*YG gene was subcloned into the plasmid pET28a(+) and was expressed in *E. coli* BL21 (DE3) by IPTG induction. The pH value was one of the main reasons which caused the degradation of enzyme activity in the later stage of induction. The highest activity was reached 36 U/ml after 5 h induction in medium at pH 6.0. Biotransformation yield of the enzyme reached 65% and the molecular weight of transformed dextran was more than 68 kDa in 2 h.

Keywords Dextran · Dextransucrase · Glucosyltransferase · *Leuconostoc mesenteroides*

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Introduction

Dextransucrase (EC 2.4.1.5) is an extracellular glucosyltransferase that is usually produced by oral *streptococci* and various strains of *Leuconostoc mesenteroides*. Dextransucrase is induced when strain *L. mesenteroides* 0326 is grown on sucrose-rich media. It catalyses the transfer of D-glucosyl units from sucrose to acceptor molecules. Thus, two different products can be synthesized: α-dextran or α-oligosaccharides when efficient acceptors (like maltose) are present (Dols et al. 1997; Monchois et al. 1999). Dextran that is produced by dextransucrase of *L. mesenteroides* has 95% α(1 → 6) linkages in the main chains and 5% α(1 → 3) branch linkage (Brown et al. 1989). Dextran has important medical applications in the production of fine chemicals such as plasma substitutes and Sephadex. However, they can also be used in texture improvement in the food industry, e.g. in milk drinks, yoghurts and ice creams (Neubauer et al. 2003).

A gene coding for dextransucrase was isolated from *L. mesenteroides* NRRL B-512F and sequenced (Wilke-Douglas et al. 1989). The amino acid residues were identified, which are composed of two different functional domains: the *N*-terminal catalytic domain (about 900 aa) and the *C*-terminal domain

(300–400 aa) (Monchois et al. 1999). The dextransucrase of *L. mesenteroides* NRRL B-512F was expressed in the presence of carbon sources other than sucrose but at low activities (Quirasco et al. 1999).

In this paper, we describe the cloning and sequencing of a dextransucrase gene (*dex*YG) of *L. mesenteroides* 0326, and the alignment of the deduced amino acid sequences with those expressed from other dextransucrase genes. Expression of the dextransucrase *dex*YG and its activity are also reported.

Materials and methods

Materials, bacterial strains, plasmid and medium

Restriction endonuclease, LA Taq DNA polymerase, phenol/chloroform and T_4 DNA ligase were purchased from TaKaRa. *E. coli* DH5α was used as a host in standard cloning experiments, and was grown in Luria Bertani (LB) medium. *E. coli* BL21 (DE3) was used for dextransucrase expression and the strain bearing plasmid pET28a(+) were grown in LB supplemented with 50 μg kanamycin/ml.

Molecular techniques

Genomic DNA from *L. mesenteroides* 0326 was extracted according to the established protocols (Sambrook et al. 1989). Restriction endonucleases, LA Taq DNA polymerases were used as described by the suppliers (TaKaRa Inc.). Transformation of plasmids into *E. coli*, DNA purification, digestion and agarose gel electrophoresis were performed according to standard procedures (Sambrook et al. 1989). pUCm-T vector (Shanghai Sangon) was used for cloning of PCR products. DNA sequencing was carried out by Invitrogen biotechnology company (China).

Cloning of the dextransucrase gene

Based on the sequence of gene *dsr*D (AY017384, GenBank), two primers [sense (5′-ATTTATGCCA TTTACAAAAAAGCT-3′) and antisense (5′-CTTA TGCTGACACAGCATTTCC-3′] were designed to amplify the gene by PCR. The 50 μl reaction mixture contained 5 μg genomic DNA, 0.5 μM of each primers, 250 μM of each dNTPs, 5 U LA Taq DNA polymerase, 2.5 mM $MgCl_2$ and 1 × LA Buffer (Ryu et al. 2000). The PCR amplification protocol consisted of a denaturation at 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 47°C, for 30 s, extension at 72°C for 5 min, and a final hold for an extra 10 min at 72°C. The resulting PCR-amplified fragments were ligated into plasmid pUCm-T to generate plasmid pYGdex, and the plasmid pYGdex was transformed into the *E. coli* DH5α strain.

The nucleotide sequence of the *dex*YG gene has been submitted to the GenBank databases under accession no. DQ345760.

Construction of expression plasmid pETdex

Using the plasmid pYGdex as template, a pair of specific primers were designed as follows: Sense 5′-CCGTAGATCTTCCATGCCATTTACAGAAAAAGT-3′ with a *Bgl*II site (underlined) and Antisense 5′-CCGCTCGAGTTATGCTGACACAGCATTT-3′ with a *Xho*I site (underlined). The PCR product obtained was cleaved with *Bgl*II and *Xho*I and ligated into the vector pET28a(+) previously digested with *BamH*I and *Xho*I to construct an expression plasmid *pETdex*. Plasmid *pETdex* was then transformed into *E. coli* BL21 (DE3) to obtain recombinant *E. coli* BL21 (DE3)/*pETdex*.

Expression and SDS-PAGE of dextransucrase

Cells of BL21 (DE3)/*pETdex* were grown at 37°C in LB medium containing 50 μg kanamycin/ml, and the expression of dextransucrase was induced by adding IPTG to 1 mM when the OD_{600} reached 0.6. The cultivation was continued for 5 h and the cells were harvested by centrifugation at 12,000 × *g* for 5 min (Malten et al. 2005). Bacteria pellet was disrupted by pulse sonication in lysis buffer as crude enzyme (Funane et al. 2001).

Proteins from the lysed cells were separated by 8% (w/v) SDS-PAGE (Sambrook et al. 1989) and were stained with Coomassie Brilliant Blue. After SDS-PAGE, the gel was washed three times with 20 mM sodium acetate buffer, pH 5.4, containing 0.05 g $CaCl_2$/l to eliminate the SDS.

Activity assay and enzymatic biotransformation

Dextransucrase activity was determined spectrophotometrically by measuring the initial rate of fructose production using the dinitrosalicylic acid method (Monchois et al. 1997). The enzymatic reaction was carried out at 30°C with magnetic stirring in 20 mM sodium acetate buffer (pH 5.4) containing 100 g sucrose/l and 0.05 g $CaCl_2$/l. Samples were centrifuged for 8 min at 9,000 × *g* before measuring the absorbance. A calibration curve was obtained with a 1 g fructose/l solution. One unit of dextransucrase activity was defined as that catalyzing the formation of 0.1 mg fructose/h under the above-mentioned conditions.

Results and discussion

Sequence analysis of the cloned *dex*YG and comparison of *dex*YG with others

The nucleotide sequence of the *dex*YG gene was cloned and sequenced. The nucleotide sequence of the *dex*YG gene was 4,584 bp in length (see Supplementary Table 1) and encodes a protein of 1,527 aa (Fig. 1) with a molecular mass of 170 kDa. The highly conserved residues identified in the *N*-terminal domain (Neubauer et al. 2003) were also conserved in *dex*YG. Analysis of the *dex*YG protein sequence revealed that it consists of a hydrophilic core domain

Fig. 1 Deduced amino acid sequence of the *L. mesenteroides* 0326 dextransucrase. The *N*-terminal amino acids of the secreted dextransucrase are shown in bold and marked by an arrow

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1    MPFTEKVMRKKLYKVGKSWVVGGVCAFALTASFALATPSVLGDSSVPDVSANNVQSASDN
                                 N-terminus of secreted dexYG

61   TTDTQQNTTVTEENDKVQSAATNDNVTTAASDTTQSADNNVTEKQSDDHALDNEKVDNKQ
121  DAVAQTNVTSKNEESAVASTDTDPAETTTDETQQVSGKYVEKDGSWYYYFDDGKNAKGLS
181  TIDNNIQYFDESGKQVKGQYVTIDNQTYYFDKDSGDELTGLQSIDGNIVAFNDEGQQIFN
241  QYYQSENGTTYYFDDKGHAATGIKNIEGKNYYFDNLGQLKKGFSGVIDGQIMTFDQETGQ
301  EVSNTTSEIKEGLTTQNTDYSEHNAAHGTDAEDFENIDGYLTASSWYRPTDILRNGTDWE
361  PSTDTDFRPILSVWWPDKNTQVNYLNYMADLGFISNADSFETGDSQSLLNEASNYVQKSI
421  EMKICAQQSTEWLKDAMAAFIVTQPQWNETSEDMSNDHLQNGALTYVNSPLTPDANSNFR
481  LLNRTPTNQTGEQAYNLDNSKGGFELLLANDVDNSNPVVQAEQLNWLYYLMNFGTITAND
541  ADANFDGIRVDAVDNVDADLLQIAADYFKLAYGVDQNDATANQHLSILEDWSHNDPLYVT
601  DQGSNQLTMDDYVHTQLIWSLTKSSDIRGTMQRFVDYYMVDRSNDSTENEAIPNYSFVRA
661  HDSEVQTVIAQIVSDLYPDVENSLAPTTEQLAAAFKVYNEDEKLADKKYTQYNMASAYAM
721  LLTNKDTVPRVYYGDLYTDDGQYMATKSPYYDAINTLLKARVQYVAGGQSMSVGSNDVLT
781  SVRYGKDAMTASDTGTSETRTEGIGVIVSNNAELQLEDGHSVTLHMGAAHKNQAYRALLS
841  TTADGLAYYDTDENAPVAYTDANGDLIFTNESIYGVQNAQVSGYLAVWVPIGAQQDQDAR
901  TASDTTTNTSDKVFHSNAALDSQVIYEGFSNFQAFATDSSEYTNVVIAQNADQFKQWGVT
961  SFQLAPQYRSSTDTSFLDSIIQNGYAFTDRYDLGYGTPTKYGTADQLRDAIKALHASGIQ
1021 AIADWVPDQIYNLPEQELATVTRTNSFGEDDTDSDIDNALYVVQSRGGGQYQEMYGGAFL
1081 EELQELYPSLFKVNQISTGVPIDGSVKITEWAAKYFNGSNIQGKGAGYVLKDMGSNKYFK
1141 VVSNTEDGDYLPKQLTNDLSETGFTHDDKGIIYYTLSGYRAQNAFIQDDDNNYYYFDKTG
1201 HLVTGLQKINNHTYFFLPNDIELVKSFLQNEDGTIVYFDKKGHQVFDQYITDQNGNAYYF
1261 DDAGVMLKSGLATIDGHQQYFDQNGVQVKDKFVIGTDGYKYYFEPGSGNLAILRYVQNSK
1321 NQWFYFDGNGHAVTGFQTINGKKQYFYNDGHQSKGEFIDADGDTFYTSATDGRLVTGVQK
1381 INGITYAFDNTGNLITNQYYQLADGKYMLLDDSGRAKTGFVLQDGVLRYFDQNGEQVKDA
1441 IIVDPDTNLSYYFNATQGVAVKNDYFEYQDNWYLTDANYQLIKGFKAVDDSLQHFDEVTG
1501 VQTKDSALISAQGKVYQFDNNGNAVSA
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with a hydrophobic *N*-terminal region. This *N*-terminal region displayed the sequence characteristics of a typical signal peptide sequence for secretion. The signal peptidase cleavage site at residues between position 42 and 43 (MPFTEKVMRKKLYKVGK SWV VGGVCA FALTASFALATPSVLG-DSSVPD) was predicted using SignalP 3.0 server (www.cbs.dtu.dk/services/SignalP). Dextransucrase can be secreted into the culture medium of *L. mesenteroides* strain. However, the recombinant protein (dextransucrase) cannot be secreted expressed into the culture medium of recombinant *E. coli* (DE3)/pETdex since it does not have a signal sequence of the correspondent *E. coli* host.

The obtained results were analysed by a BLAST similarity search of the GenBank database (http://www.ncbi.nlm.nih.gov.cn/BLAST). Comparison of the deduced amino acid sequence of *dex*YG with the sequences of other proteins revealed similarities corresponding to some conserved regions of dextransucrases. The highest level of homology was found with DsrD (GenBank AY017384) from *L. mesenteroides* Lcc4 (98.8% identity). The amino acid sequence is also highly similar to DEX (GenBank LMU81374; 97.4% identity). *dex*YG also shows significant similarities to other dextransucrases such as DsrB (66.7% identity) (Monchois et al. 1998) and DsrC (GenBank LME250172, 65.5% identity).

Expression of *dex*YG in *E. coli*

Dextransucrase can be efficiently expressed in *E. coli* BL21 (DE3) by IPTG induction at 25°C. SDS-PAGE analysis clearly showed a band after 3 h induction, corresponding to a Mr 170 kDa. This value is consistent with the theoretical molecular mass (Fig. 2).

Effect of pH on the enzymatic activity

The dextransucrase activity of *dex*YG was detected by measuring the release of reducing sugars in the presence of sucrose (Fig. 3). The dextransucrase activity was reached 5.4 U/ml after 4 h induction at 25°C. The degradation of activity was observed in a 5 h culture after induction. The pH value was increasing from 7.4 to 8.1 during the period of induction. Data showed that the loss of activity at 5 h post induction might be due to the increase in culture pH.

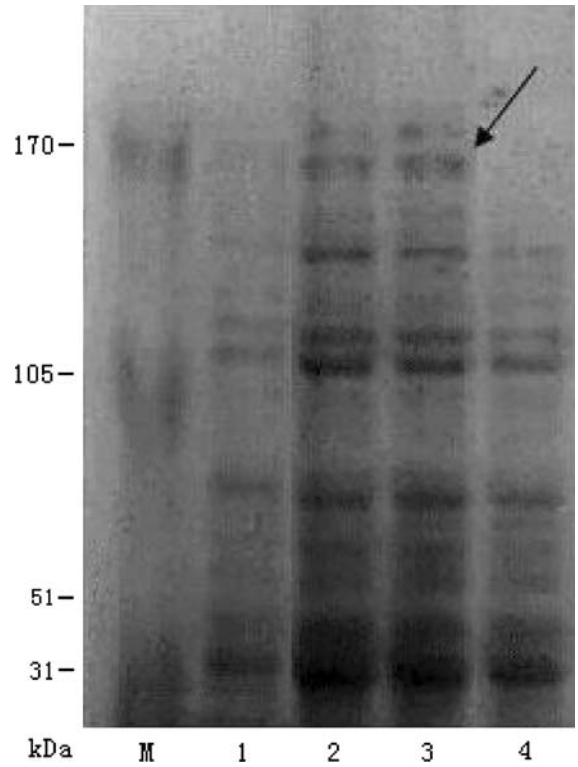

Fig. 2 SDS-PAGE analysis of the *dex*YG gene product, *E. coli* BL21 (DE3) cells bearing pET28a (+) plasmids were cultured at 25°C in LB medium containing 0.1 M Tris/HCl buffer, pH 5.4, and 50 μg kanamycin/ml. They were induced with 1 mM IPTG. 1 ml cultures were collected at different times of incubation (2, 3 and 4 h). Proteins were extracted by boiling bacterial pellets in a denaturation buffer and analyzed by 8% (w/v) SDS-PAGE and stained with Coomassie Brilliant Blue. Lane M: molecular weight markers; Lane1: 2 h after induction; Lane2: 3 h after induction; Lane3: 4 h after induction; Lane4: 0 h after induction. *Arrow* indicates an induced protein band with an Mr = 170 kDa

The effect of pH value on dextransucrase activity produced by engineered strain were studied (Fig. 4). The highest activity was reached 35.6 U/ml under the optimal culture conditions after 5 h induction in medium with pH 6.0 buffer, which was 3.5 times as that of in LB medium without pH-adjustment. The data demonstrated that the pH value was one of the main reasons which caused the degradation of enzyme activity in the later stage of induction. These results showed that dextransucrase could be efficiently heterologous expressed in *E. coli* and a strong dextransucrase activity had been detected and activity was shown to be pH dependent.

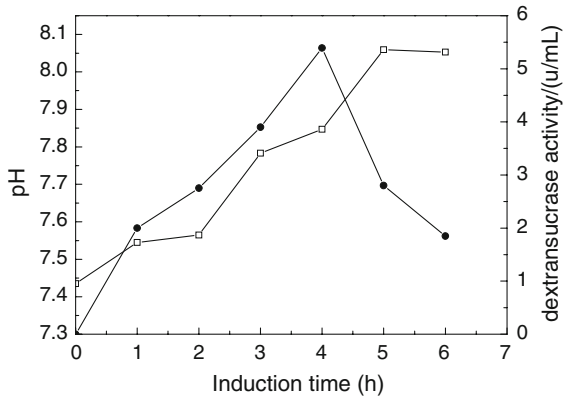

Fig. 3 Time course of pH and dextransucrase activity after induction. The dextransucrase activity was measured by the dinitrosalicylic acid method. They were induced with 1 mM IPTG, and samples were taken at various time points after induction (1, 2, 3, 4, 5 and 6 h). The enzyme reaction is described in 'Materials and methods'. (□) pH; (●) dextransucrase activity

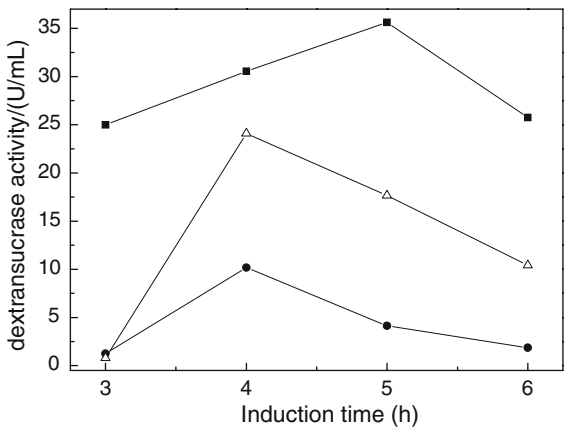

Fig. 4 The effect of different pH value on dextransucrase activity produced by engineered strain. They were induced with 1 mM IPTG, and samples were taken at various time points after induction (3 ,4, 5 and 6 h). The enzyme reaction are described in 'Materials and methods'. Different pH value in experiment: (■) pH 6.0 sodium phosphate buffer, (●) initial pH (not buffered), (Δ) pH 8.0 sodium phosphate buffer

Enzymatic biotransformation

In the biotransformation test of dextransucrase, the substrate, sucrose, was transformed to dextran and fructose within 2 h by crude enzyme and the molecular weight of dextran was greater than 68 kDa (Fig. 5). Biotransformation yield of the enzyme reached 65% by measuring production of fructose using DNS method. These data demonstrated

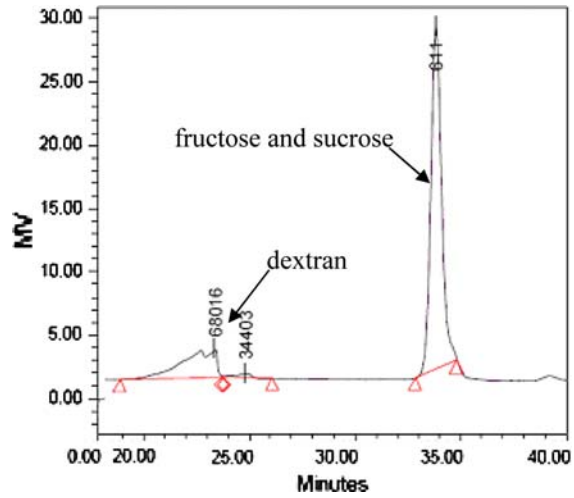

Fig. 5 HPLC analysis of the product of dextransucrase biotransformation. In the biotransformation of dextransucrase, the substrate sucrose was transformed to dextran and fructose within 2 h by crude enzyme and the molecular weight of dextran was bigger than 68 kDa. Biotransformation yield of the enzyme reached 65% by measuring production of fructose using DNS method. The enzyme reaction is described in 'Materials and methods'. HPLC conditions: GPC column, mobile phase: ultrapure water, flow rate: 0.6 ml/min, injection volume: 10 µl, detector temperature: 40°C, column temperature 60°C

that the dextransucrase gene *dexYG* of *L. mesenteroides* 0326 was heterologously expressed in *E. coli* BL21 (DE3) and was able to drive dextran synthesis. Our results on the expression of this enzyme in *E. coli* BL21 (DE3) fermentations may help to optimize the industrial process of dextransucrase production. These may bring new opportunities for industrial applications of dextransucrases.

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