Cloning and expression of *Lipomyces starkeyi* dextranase-encoding gene in yeasts

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ABSTRACT

Lipomyces starkeyi produces a novel glucanhydrolase containing endo-dextranase and α -amylase activities. A cDNA from *L. starkeyi* encoding a dextranase was isolated and characterized. The 2,052 kb cDNA fragment (*lsd1*) carrying dextranase gene showed one open reading frame (ORF) composed of 1,824 bp flanked by a 41 bp 5'-UTR and a 184 bp 3'-UTR including a poly(A) tail of 27 bp. The ORF encodes for a 608 amino acid with a predicted molecular mass of 67.6 kDa. There was 77% deduced amino acid sequence identity between the LSD1 dextranase and the dextranase from *Penicillium minioluteum*. The primary structure of the dextranase from *L. starkeyi* has distant similarity with enzymes belonging to glycosyl hydrolase family 49. The *lsd1* protein was expressed in the *Saccharmyces cerevisiae* under control of *GAL1* promoter and active dextranase was produced.

INTRODUCTION

L. starkeyi produced an endo-dextranase and/or an α-amylase [1, 2]. This dextranase has been demonstrated to be an effective agent for removing dextran during sugar processing [3]. Except for a few bacterial dextranase, microbial dextranase generally are inducible [2]. Kim and Day reported the isolation of a derepressed and partial

constitutive mutant for dextranase and amylase [4]. They characterized its dextranase and amylase, and reported its use for the production of small-size dextran using sucrose and/or starch.

There have been a number of recent reports on cloning and expression of bacterial genes encoding dextranase [5, 6]. However, the cloning of a yeast dextranase gene has not been reported. We believe that the cloning and expression to high levels of a yeast gene would make it possible to produced large quantities of enzyme for industrial applification. The aims of this study were the isolation and characterization of the dextranase encoding DNA from *L. starkeyi*.

MATERIALS AND METHODS

RT-PCR Amplification of LSD1. A fresh *L. starkeyi* culture was used to inoculate LW medium containing 1% (w/v) starch and incubated at 28℃ for 36 h. Total RNA extraction was prepared with hot acid phenol method using glass-beads. First strand cDNA synthesis was performed by reverse transcribing 0.5 μg of total RNA of *L. starkeyi* using a modified oligo-dT primer. For PCR, 10 μl of the first strand cDNA synthesis was used to amplify part of the coding sequence for dextranase. A degenerate primer, DC-F and DC-R, were designed according to seven conserved regions of dextranases reported by Aoki and Sakano [7]. The peptide sequences TWWH(D/N)(N/S/T) (conserved region I) and YKQVG(S/A) (conserved region V) was chosen. To complete the sequence locating upstream and downstream from the 1.1 kb fragment of dextranase gene and to complete the full length of cDNA, 5'RACE (rapid amplification of cDNA ends) and 3'RACE were carried out using 5' and 3' full RACE Core Set (TaKaRa, Japen). Under this condition, a major PCR product of 180 bp corresponding to the 5' region and 900 bp corresponding to the 3' region were obtained after the PCR. A fragment of 2 kb was finally obtained.

Construction of the *S. cerevisiae* vector pYLSD1 (pYES2-LSD1) and transformation of *S. cerevisiae*. The DNA fragment corresponding to the dextranase gene (*lsd1*) was amplified from the genomic DNA by PCR and the synthetic oligonucleotide primers DX-F and DX-R. PCR fragment was cloned into vector pYES2 (Invitogen, USA) resulted in the plasmid pYLSD1. *S. cerevisiae* was transformed using method described by Gietz

et al [8]. The isolation of plasmid DNA from yeast transformants was performed according to Adam and Polaina [9].

S. cerevisiae transformants were selected on induction medium [induction medium refers to synthetic complete (SC) containing 2% galactose, 0.3% blue dextran and lacking uracil]. Plate were incubated at 30℃ for 2-6 days and digestion of dextran was indicated by appearance of halos surrounding the colonies. For isolation of intracellular protein SC broth was inoculated to 0.1 OD_{600} from overnight culture and grown at 30℃ for 72 h. Cell were harvested and resuspened in 5 ml breaking buffer (50 mM sodium phosphate, pH 7.4, 1 mM EDTA, 5% glycerol, 1 mM PMSF). 0.45 mm glass beads were added and the cells were vigorously vortexed. After centrifugation, the supernatant was carefully removed and used for the enzyme assay.

RESULTS AND DISCUSSIONS

Isolation of LDS1 dextranase gene from L. starkeyi. In order to isolate the dextranase-coding gene, RT-PCR amplification was employed to clone the gene. Sequence analysis showed that the cDNA was 2,052 bp long and contained a 1,824 bp ORF flanked by a 41 bp 5'-UTR and a 184 bp 3'-UTR including a poly(A) tail of 27 bp. The open reading frame encodes for a 608 amino acid polypeptide with a predicted molecular mass of 67.6 kDa.

The primary structure of dextranase protein from L. starkeyi (LSD1) was compared with sequences in CLUSTALW program [10]. This search revals a significant homology (77% identity) with a dextranase from P. funiculosum [11]. No significant homology with the primary structure of any other reported glycosyl-hydrolase, including other dextranases [5, 6].

Construction of expression vector and expression of dextranase in *S. cerevisiae*. The DNA fragment encoding LSD1 protein was amplified by PCR from the chromosomal DNA from *L. starkeyi*. LSD1 PCR product cloned into vector pYES2. The *S. cerevisiae* expression vector pYLSD1 (pYES2-LSD1) contains the coding sequence of the dextranase gene of *L. starkeyi*. Its expression vector also contain the galactose-regulated *GAL1* promoter of *S. cerevisiae*, the terminator sequence of the *CYC1* gene from the *S. cerevisiae*, and the *S. cerevisiae URA3* gene. Transformation by LiAc method of the

INVSc1 (*ura3*) strain with this DNA yielded Ura+ transformants. A screening step to find the clones producing active dextranase was performed on minimal agar plates containing blue dextran, and the expression was induced with galactose. All the clones tested presented a halo of dextran hydrolysis.

Properties of dextranase expressed by *S. cerevisiae* transformants. We tested the ability of non-dextranlytic yeast *S. cerevisiae* INVSc1 to secrete active dextranase when transformed with pYLSD1. INVSc1 transformants were able to form blue halos on BDSC-Ura plates within 96 h, indicating the stable expression of the *L. starkeyi* KSM22M LSD1 gene under control of the galactose-regulated GAL1 promotor, and effective secretion of the encoded gene product by *S. cerevisiae* INVSc1. The dextranase activity of the transformed *S. cerevisiae* strain was primary cell wall associated.

Intracellular extracts of INVSc1-pYLSD1 were used in the determination of the LSD1 activity at differenct temperatures (10℃ and 60℃). The optimum temperature for LSD1 was 37℃. At temperatures below 28℃, LSD1 activity decreased gradually, whereas incubation at temperatures over 40℃, the activity drastically decreased LSD1 activity. The optimum pH of dextranase is 5.5. At pH 7.7, the activity was reduced by almost 35%, while the activity at pH 6.5 was reduced to 10% of maximum.

The enzyme showed high reactivity towards dextran (100%), starch (54%), but only small amounts of reducing sugars were liberated from mutan (8%), inulin (7%), levan (3%).

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