

CLONING OF THE *Penicillium minioluteum* GENE ENCODING DEXTRANASE AND ITS EXPRESSION IN *Pichia pastoris*

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Introduction

Dextranase (EC 3.2.1.11, α -1,6 glucan-6 glucanohydrolase) hydrolyzes the α (1,6) glucosidic bond contained in dextran to release smaller isomaltosaccharides, commonly 3 to 5 glucose units long. Dextranases have been found in a wide variety of *Mycomycetes* species, *Actinomycetes* species and bacteria. In sugarcane processing this enzyme is used to degrade the dextran polymer to smaller molecules that do not create excessive viscosity, and there exists in the sugar industry a considerable demand for dextranase. The cloning and expression of a fungal dextranase gene in *P. pastoris* would make it possible to generate large amounts of enzyme with relative ease to be of potential utility in industrial fields.

Previously we have isolated by differential hybridization a cDNA encoding an extracellular dextranase from *P. minioluteum* (García B, *et al.* Unpublished data). In this report we describe the cloning and characterization of the dextranase gene, as well as the expression of this gene in the methylotrophic yeast *P. pastoris*. We also compare the enzymatic properties of the dextranases from *P. minioluteum* and *P. pastoris* cells.

Materials and Methods

The strains used in this study were the *P. minioluteum* HI-4 (MUCL 38929) and *P. pastoris* (*his3* mutant) MP36 (MUCL 38930). The dextranase protein was purified from the culture supernatant according Raices, *et al.* (1). The N-terminal sequencing of purified dextranase was performed by Edman degradation procedure. *Bam*HI genomic library was constructed using the pUC19 vector. DNA sequencing was performed by dideoxynucleotide chain termination method using Sequenase (USB, Amersham). The integrating plasmid pPS7 (2) was used for dextranase expression in *P. pastoris*. MP36 strain was transformed using the electroporation procedure.

Results and Discussion

The *DEX* gene encoding an extracellular dextranase was isolated from a partial genomic DNA library of

P. minioluteum by hybridization using the dextranase cDNA as a probe. In contrast with the majority of the isolated filamentous fungal genes, sequencing data showed that the dextranase gene does not contain introns in its nucleotide sequence. The longest open reading frame (ORF) determined from the gene sequence encodes a protein composed of 608 aa with a predicted molecular weight of 65942 Da. There are four potential initiation codons in the ORF, however only the second initiation codon has a nucleotide sequence, CACAAUG in its vicinity, which matches very well to the consensus sequence around reported initiation codons of filamentous fungi (3). According to the 'leaky scanning mechanism' proposed by Kozac (4), it seems that in the dextranase mRNA the ribosomes initiate translation from the first and second AUG codons. Comparison of the amino acid sequence of the N-terminus of the mature dextranase protein with the deduced protein sequence translated from the first AUG codon indicates the presence of a leader peptide of 34 aa. However the -1,-3 rule and the analysis by the S-factor method (5) predict the cleavage by signal peptidase occurs at least 13 aa preceding the N-terminus of mature dextranase protein. This analysis suggests that dextranase is synthesized as a preproenzyme which means that the protein undergoes another posttranslational modification: the propeptide removal by proteolytic processing. The gene encoding dextranase was expressed in the methylotrophic yeast *P. pastoris*. The fusion of the coding region of mature dextranase protein to the yeast invertase (*SUC2*) signal under the control *AOX1* promoter led to the secretion of high level (over 3.1 g/L) of active dextranase enzyme to the culture medium. The N-terminus of *Pichia* secreted dextranase was determined to be identical to the native product. This result implies the correct recognition and processing of the *SUC2* signal peptide. The native (*P. minioluteum*) and the recombinant (*P. pastoris*) dextranases enzymes are N-glycosylated and the extent of N-glycosylation between the enzymes seems to be different, however having both the same specific activity (1 000 DU/mg protein).

1. Raices M, *et al.* *Biotechnologia Aplicada* 1991;8(2):248-255.

2. Yong V, *et al.* *European Patent Application* 438200 A1.

3. Ballance DJ *Molecular Industrial Mycology*. Marcel Dekker NY 1991,p.1-29.

4. Kozac M. *NAR*1984;12:3873-3893.

5. von Heijne G. *NAR* 1986;14(11):4683-4690.