

## CLONING AND SEQUENCING OF DEXTRANASE cDNA FROM *Penicillium minioluteum* AND ITS EXPRESSION IN *Escherichia coli*

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### Introduction

Dextranase (EC 3.2.1.11,  $\alpha$ -1,6 glucan-6 glucanohydrolase) is an enzyme that hydrolyzes the  $\alpha$  (1,6) glucosidic bond contained in dextran to smaller molecules. Dextran is not a natural constituent of sugar juice but it is formed by bacteria of *Leuconost* species. This polymer is present in cane juice and it decreases the yield in sugar production. Thus dextranase is used in the sugar industry to degrade the dextran into smaller molecules that do not create excessive viscosity.

Some reports about the cloning of genes encoding dextranase from different bacteria have been published, however, the cloning of a fungal dextranase gene has not been reported. We describe the isolation and nucleotide sequence of dextranase cDNA from *P. minioluteum* as well as its expression in *E. coli*.

### Experimental Procedures

The induction of dextranase in *P. minioluteum* fungus was studied in 5 L fermenters in two mediums containing dextran or glucose as sole carbon sources. The *in vitro* translation was performed using the rabbit reticulocyte lysate kit (Promega). *In vitro* translation products were immunoprecipitated with anti-dextranase polyclonal antibodies (1). The cDNA library was constructed using the Stratagene cDNA synthesis kit (2) and the EcoRI-XhoI digested pBluescript II SK (3). The ligation product was transformed into *E. coli* strain MC 1061 (4). The isolated cDNA was subcloned into pET-3a, pET-3b, pET-3c vectors (5) for the expression in *E. coli* using the T7 RNA polymerase system (6). The DNA sequencing was performed by dideoxynucleotide chain termination method using Sequenase (USB, Amersham).

### Results and Discussion

Dextranase activity was detected in the supernatant of the culture grown in dextran at 40 h of the fermentation process, following an increase in this activity and reaching 380 U/mL of culture at 120 h. Meanwhile the culture grown in glucose did not show dextranase activity

The *in vitro* translation of the poly (A) RNA from the culture grown in dextran showed a major product do not observed in the translation of poly (A) RNA from the glucose culture. *In vitro* translation products were immunoprecipitated and the results corresponding to the dextran cultures revealed two major polypeptides showing similar size to that of the immunoprecipitated dextranase enzyme. The mRNA isolated from the culture grown in glucose did not lead to the synthesis of polypeptides that were immunoprecipitated by the anti-dextranase antibody. The poly (A) RNA isolated from the culture grown in dextran at 56 h of fermentation was used for cDNA synthesis. The cDNA clones corresponding to genes differentially regulated in the induced culture (dextran) with respect to the non-induced (glucose) were isolated by differential hybridization. Crossed hybridization enabled the classification of cDNA clones into four different groups characterized in that each cDNA only hybridize to a cDNA from the same group of clones. Among the selected clones one cDNA fragment from each of the four group was subcloned into pET-3a, pET-3b and pET-3c vectors. The cDNAs were expressed as fusion protein in *E. coli* BL 21 (DE 3)[pLys] strain. No enzyme activity was detected in the whole cell extracts or in the culture supernatant of the transformants, however the Western blot analysis of one transformant evidenced a 67 kDa polypeptide that specifically reacted with the anti-dextranase antibody. No signals were obtained in the uninduced culture of this transformant or with the transformants containing other cDNA fragments. We concluded that the cDNA originating the polypeptide that specifically reacted with anti-dextranase antibody encodes a dextranase enzyme from *P. minioluteum*.

The nucleotide sequence of dextranase cDNA revealed that the longest ORF contains 1824 bp encoding a polypeptide of 604 aa. This ORF also contains four ATG near the 5' end of the cDNA sequence. Translation of the sequence encoding the mature dextranase yields a protein composed of 574 aa. The sequence AATAAG, 17 bp upstream to the polyA tail is similar the canonical eucaryotic polyadenylation signal AATAAA.

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