Biochemistry and Molecular Biology of Bacterial Xylanases

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Introduction

Xylanases degrade the hemicelluloses of plant matter and plays a major role in bioconversion in nature. Microorganisms produce these enzymes, and in this project, the xylanase produced by rumen bacteria *Fibrobacter succinogenes* S85 and a thermophilic bacteria *Bacillus coagulans* was studied, including cloning of the respective genes and the determination of their nucleotide sequence. The xylanase enzyme was also purified and characterized.

Materials and Methods

Both bacteria were already available in our laboratory from previous work. The xylanase enzyme from both sources was purified using protein chromatography technique and IEF, and was shown to be pure by SDS-page. The xylanase gene of both bacteria was cloned using standard cloning methods and analysed by Southern Blotting, restriction enzyme mapping and nucleotide sequencing. The nucleotide sequence data was further analysed using computer software makes a comparison of the properties of the xylanase genes from this study with published data. The xylanase gene cloned from *B. coagulans* in a recombinant plasmid could be amplified by PCR, cloned and expressed in *E. coli*, thus establishing the veracity of the gene.

Results and Discussion

The xylanase gene cloned *F. succinogenes* expressed xylanase activity in *E. coli*. The purified enzyme has a MW of 60 kDa, temperature optimum of 37 °C and pH optimum of 7. The xylanase gene of *F. succinogenes* was sequenced. Two ORF with a high homology to xylanase gene sequence in the data bank were obtained, each 1235 bp and 1451 bp in size.

The gene for a thermostable xylanase enzyme was cloned from *B. succinogenes* and expressed in *E. coli*. The purified enzyme has a MW of 20 kDa, temperature optimum of 55 °C and pH optimum of 7.2. A single ORF of 630 bp was obtained after analyzing the nucleotide sequence of the gene. This ORF can be amplified by PCR, recloned into another vector plasmid and transformed into *E. coli*. Enzyme activity was expressed, thus confirming the veracity of the cloned gene.

Both the xylanase genes appear to have strong promoters, enabling good expression in *E. coli*. Thus there is potential use of these promoters to be adjuncted to another separate gene to improve its expression. The recombinant plasmid containing the xylanase gene can be used as marker gene in conjunction with a chromogenic substrate (eg. RBB-xylan), hence replacing antibiotic resistance marker commonly used in molecular cloning.

Conclusions

The xylanase from the rumen bacteria *Fibrobacter succinogenes* S85 was purified and characterized. The gene for this enzyme was further cloned and analysed. Similar work was accomplished on the thermostable xylanase from the thermophilic bacteria *Bacillus coagulans*, in which the enzyme was purified and characterized, and its gene cloned and analysed.

Benefits from the study

Xylanase enzymes can now be produced on a larger scale in a fermenter using the cloned xylanase genes from mesophilic bacteria and from thermophilic bacteria. In case of the latter the thermostable xylanase has the property of being stable a higher operating temperatures.

The xylanase can be used to remove xylans from paper pulp and to decolorize this material, thus eliminating the use of harmful chemicals.

Other uses for the xylanases are in bread making and in pre-treatment of animal fodder to improve digestibility and quality for ruminants.

The recombinant xylanolytic plasmid can be used in teaching kits for teaching recombinant DNA Technology, and for use as a genetic marker for selection, obviating the need to used "unfriendly" antibiotic marker genes.

Many undergraduates and postgraduates have benefited from working on this project, and a reasonably well-equipped laboratory dedicated to biochemistry and molecular biology work has been established.

The study also helped in establishing academic ties and research collaboration with local and overseas universities.

Literature cited in the text

None.

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