FINAL REPORT

GRDC
GRAINS RESEARCH & DEVELOPMENT CORPORATION

CS104

Genetic manipulation of cellulase genes into nitrogen-fixing bacteria

PROJECT DETAILS

PROJECT CODE:	CS104
PROJECT TITLE:	GENETIC MANIPULATION OF CELLULASE GENES INTO NITROGEN-FIXING BACTERIA
START DATE:	01.01.1989
END DATE:	31.12.1991
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Summary

Genetic Engineering of Cellulase Genes into Nitrogen-fixing Bacteria

The original aims of this project were: 1) to clone a number of different cellulase genes from cellulose-degrading soil bacteria; 2) to refine the size of the DNA fragments carrying the cellulase genes and join them together into so-called cellulase gene cassettes, and 3) to introduce the various cellulase gene cassettes into a number of different nitrogen-fixing (diazotrophic) soil bacteria. The ultimate objective of this research program is to determine whether the introduction of cellulose-degrading properties into diazotrophic bacteria will allow them to utilize the cellulosic components of crop residues (such as wheat stubble) as a source of energy for improved nitrogen fixation. Details of the background and experimenta plan are outlined in the original research proposal.

In summary, we have cloned eight different cellulase-encoding genes from three different species of soil bacteria. Three of these genes have been manipulated in vitro to generate a series of six different cellulase gene cassettes. Each of the cellulase gene cassettes determines the production of the three types of cellulase enzyme when introduced into nitrogen-fixing species of *Azospirillum*, *Azotobacter* and *Pseudomonas* bacteria. Derivatives of *Azospirillum*, containing two of the cellulase gene cassettes, show increased levels of nitrogen fixation when grown in the presence of the hemicellulose xylan. We are continuing to pursue this objective at the present time. Several of the cellulase gene-containing *Azospirillum* and *Azotobacter* derivatives were also shown to utilize cellobiose as an energy source for nitrogen fixation. While these preliminary results are encouraging, further work will be required in order to achieve the ultimate objective of improving biological nitrogen fixation by introducing cellulose-degrading properties into diazotrophic soil bacteria. We are continuing to pursue this objective at

the present time.

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