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International Centre for Genetic Engineering and Biotechnology
United Nations Industrial Development Organization

# Collaborative Research Programme

# TERMINAL EVALUATION REPORT

**UNIDO contract #** 91/065

**ICGEB ref. #:** CRP/ CHI 90-02

**Project initiation:** June 1991

**Project termination:** December 1994

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## TERMINAL EVALUATION REPORT

Part 1

**Title of Project**

"Saccharification of Straw: use of enzymes from native fungi".

**Keywords:** Penicillium purpurogenum, straw, cellulases, xylanases.

**UNIDO contract #** 91/065 **ICGEB ref. #:** CRP/ CHI 90-02
**Project initiation:** June 1991 **Project termination:** December 1994

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**Abstract:**

A strain of the fungus P. purpurogenum isolated locally from a soil sample has been found to grow well on wheat straw and is therefore an interesting model for the study of lignocellulose saccharification. In this project, the cellulolytic and particularly the xylanolytic enzymes secreted by the fungus have been studied. Initially, culture conditions for the optimal production of these enzymes were determined (World J. Microb. Biotech. 10, 280-284, 1994). The cellulolytic system produced by the fungus was analyzed, and one of its components (ß-glucosidase) was purified and characterized (Biotech. Appl. Biochem. 15, 185-191, 1992). The xylanolytic system has been found to be complex, with the participation of several enzyme forms, two of which have been purified and its properties studied (Xylan and Xylanases, pages 505-510, 1992; Bioch. Bioph. Acta, submitted). Work on the xylan debranching enzymes has also been performed: at least 3 acetyl xylan esterases are produced by the fungus, one of which has been prepared in pure form and characterized. Mutants of P. purpurogenum with increased xylanase production have been obtained. A gene bank of the fungus has been prepared in Saccharomyces cerevisiae, and a clone producing xylanases free of cellulases, with yields comparable to the original fungus has been isolated (Bioch. Bioph. Res. Comm., submitted). This work opens the door for a more detailed study of the application of these enzymes in saccharification.

**OBJECTIVES/METHODOLOGY**
**(proposed at the time of the submission of the research proposal)**

-Determination of optimal culture conditions for the production of cellulases and xylanases by P. purpurogenum using straw as carbon source. Effect of nitrogen source (e.g. corn steep liquor), pH, temperature, will be analyzed. Filter paper hydrolyzing activity, cellulases, ß-glucosidase and xylanases will be followed.

-Identification, purification and characterization of the enzymes. Cellulases, ß-glucosidase, and xylanases will be studied.

-Search of enzyme mixtures that can degrade straw efficiently and identification of the products obtained. Supernatants of cultures and mixtures of purified enzymes will be studied, following the formation of cellulo- and xylooligosaccharides and monosaccharides.

-Preparation of a gene bank of P. purpurogenum for the future cloning of the most promising enzymes.

## RESULTS
(compare against the set objectives)

The following results have been obtained in this project:

a) Culture conditions for the optimal production of cellulases and xylanases by P. purpurogenum.

Details of this work were given in the first year report. The findings have been published in the World Journal of Microbiology and Biotechnology. Photocopy of this paper is enclosed.

b) Purification and characterization of cellulases and xylanases from P. purpurogenum.

The work performed on cellulases and ß-glucosidase has been described in the first-year report. The studies on ß-glucosidase have been published in Biotechnology and Applied Biochemistry 15, 185-191, 1992.

The second-year report describes the details of the work performed on the purification and characterization of xylanases. This work has been published in preliminary form in a chapter of the book "Xylans and Xylanases" pages 505-510, 1992. A detailed account of the work is given in the enclosed manuscript (submitted for publication to Biochimica et Biophysica Acta).

A study has been initiated on the xylan debranching enzymes. The purification and characterization of an acetyl xylan esterase from P. purpurogenum has been carried out. Details of this work are enclosed (see "Purification and characterization of an acetyl xylan esterase").

c) Search of enzyme mixtures that can degrade straw efficiently and identification of the products obtained.

Work on this objective has been recently initiated. Techniques are being set up for the separation of oligosaccharides. A brief description is given separately. See "Analysis of hydrolysis products of lignocellulosics".

d) Preparation of a gene bank and isolation of transformants. Preparation of mutants of P. purpurogenum with increased production of xylanases

In the second-year report, details were given on the isolation of yeast transformants producing xylanases. One of these transformants has been further characterized and the results obtained thus far are described in the enclosed manuscript submitted to Biochemical and Biophysical Research Communications. The results of the preparation of P. purpurogenum mutants were given in the second-year report.

## Work plan and time schedule (originally envisaged)

First year:

Determination of optimal culture conditions and identification of enzyme activities produced. Initiation of the purification of the enzymes.

Second year:

Purification of cellulases and xylanases. Start work on the characterization of the enzymes. Start preparation of gene bank.

Third year:

Conclusion of the enzyme characterization work. Effect of supernatants and enzyme mixtures on straw and identification of the products formed. Conclusion of work on gene bank.

**Work plan and time schedule**
**(actual)**

Project landmarks, duration of individual tasks (use bar charts); evaluation criteria (publications, patents, services, training)

Project landmarks:

- Characterization of the conditions for optimal production of cellulases and xylanases by P. purpurogenum.

- Purification and characterization of two xylanase isoenzymes from P. purpurogenum.

- Purification and characterization of an acetyl xylan esterase from P. purpurogenum.

- Preparation of mutants of P. purpurogenum with enhanced xylanase production.

- Isolation of a yeast transformant capable of secreting P. purpurogenum xylanases.

Duration of individual tasks:

| year | 1991 | | 1992 | | 1993 | | 1994 | |
|---|---|---|---|---|---|---|---|---|
| month | 6 | 12 | 6 | 12 | 6 | 12 | 6 | 12 |
| optimization | ---------- | | | | | | | |
| ß-glucosidase purif. | ------ | | | | | | | |
| xylanase purification | ---------- | | ---------- | | | | | |
| esterase purification | | | ---------- | | ---------- | | | |
| mutant production | | | ---------- | | ---------- | | | |
| yeast transformants | | | | | ---------- | | ---------- | |
| evaluation of products | | | | | | | --- | |

Evaluation criteria:

A list of all publications, presentations to meetings, theses performed and students receiving training is enclosed. This list includes the total period of the project.

## NETWORKING

Prof. Jeannette Steiner. Department of Biochemistry and Molecular Biology, Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile, Santiago, Chile.

## PUBLICATIONS

A list of all publications and meeting presentations resulting from the work performed in this project is enclosed.

Photocopies of the publications appearing or submitted in 1994, and abstracts of presentations made during 1994 are enclosed.

Part +

## STATEMENT OF EXPENDITURES

The following information is for the third year of the project. Information for previous years was included in the respective reports.

| To be filled by ICGEB | | To be filled by the Affiliated Centre | |
|---|---|---|---|
| **Budgets as per original proposal** | | **Summary of expenditures** * | |
| 1) Capital equipment | US$ ..... | 1) Capital equipment | US$ 12,192.77 |
| 2) consumables | US$ ..... | 2) consumables | US$ 4,755.57 |
| 3) training | US$ ..... | 3) training | US$ 4,206.66 |
| 4) literature | US$ ..... | 4) literature | US$ 845 |
| 5) miscellaneous | US$ ..... | 5) miscellaneous | US$ ..... |
| **TOTAL GRANT** | **US$**..... | **TOTAL** | **US$** 22,000.00 |

**Please itemize the following budget categories (if applicable)**

**Capital equipment**

| | |
|---|---|
| Computer | $ 4 418.86 |
| network cards for above | $ 180 |
| vaccuum pump | $ 3 410.20 |
| fraction collector | $ 1 301.26 |
| desktop centrifuge | $ 2 500 |
| refrigerator | $ 382.45 |
| TOTAL : | **$12 192.77** |

**Training (provide names, duration of training, host laboratory)** Attendance to meetings :

Prof. Jeannette Steiner: attendance to the 94th General Meeting of the American Society for Microbiology, Las Vegas, U.S.A., May 23-27, 1994. $1 444.32.

Dr. Jaime Eyzaguirre: attendance to the 7th International Symposium on the Genetics of Industrial Microorganisms. Montreal, Canada, June 26-Julio 1, 1994, and the 8th Symposium of the Protein Society, San Diego, U.S.A., July 9-13, 1994. $2 762.34.

TOTAL : **$4.206.66**

Several students received training in the framework of the project. This did not represent a cost. A list of trainees is enclosed.

**Literature**

| | |
|---|---|
| ASBMB membership and subscrip. to Protein Science | $ 225 |
| Subscription to Biotech. Appl. Bioch. | $ 185 |
| Subscription to Enzyme Microb. Tech. | $ 170 |
| Subscription to Biochem. Educ. 1994 | $ 39 |
| Subscription to Biocnem. Educ. 1995 | $ 39 |
| ACS Membership and subscrip to Biotech. Progress | $ 187 |
| TOTAL : | $ **345** |

* Please do not send invoices, receipts etc.; these should be kept by the Affiliated Centre for future reference and sent to ICGEB upon request.