Cellulolytic and Xylanolytic Enzymes of *Trichoderma* Spp. and *Aspergillus phoenicis*

A Thesis submitted for the degree of Master of Philosophy in the University of Colombo, Sri Lanka

by Indrani Silva

Ceylon Institute of Scientific and Industrial Research

Colombo.

1990

A B S T R A C T

The enzymic hydrolysis of cellulosic materials need the synergistic action of several cellulases and hemicellulases to bring about a complete hydrolysis. One of the main drawbacks in the hydrolysis of cellulosic materials with microbial enzymes is the deficiencies observed in enzyme preparations, obtained from any single microorganism.

The aim of this study was to obtain a more balanced microbial enzyme preparation, that is capable of complete hydrolysis of cellulose, using a proven cellulolytic fungus; *Trichoderma reesei* QM 9414 and four other cellulolytic fungi (*Trichoderma sp.* isolated from rice straw; IMBTr and three strains of *Aspergillus phoenicis).*

Research work included in the thesis are characterization and comparison of enzyme production and enzyme activities of *T.reesei*, IMBTr and *A.phoenicis* strains, some studies on cultivation conditions for improved cellulase production and cellulose saccharification by using enzyme preparations of *Trichoderma sp.* and *A.phoenicis* strains.

IMBTr and *A.phoenicis* showed cellulolytic and xylanolytic enzyme production, which is comparable to those produced by *T.reesei* QM 9414. IMBTr produced nearly 3 and 2 times, respectively greater β-glucosidase activity compared to *T.reesei* QM 9414, when grown on cellulose and rice straw. The maximum β-glucosidase activity of *A.phoenicis* TISTR 3252 on wheat bran medium was 2.4 and 9.3 times greater than the maximum activities observed in IMBTr and *T.reesei* respectively.

A.phoenicis produced significant β-glucosidase activities on a number of cellulosic substrates, when grown in liquid culture with Mandels Reese salts and also in surface culture on wheat bran. When the environmental conditions were worked out for producing higher levels of β-glucosidase, the maximum activity of 4260 mu/ml was produced by *A.phoenicis* TISTR 3252 in Mandels Reese medium with 3% (w/v) wheat bran after nine days of growth.

Two *Trichoderma* strains and *A.phoenicis* TISTR 3252 strain produced a number of glycosidase activities in considerable levels, on a range of growth substrates. Therefore in *Trichoderma* and *A.phoenicis* strains,overall action of cellulase, hemicellulase and glycosidase enzymes may be responsible for efficient breakdown of cellulosic materials.

Attempts were made to enhance the enzyme levels by growing these organisms as co-cultures (*T.reesei* with IMBTr and *T.reesei* with *A.phoenicis*). However significant enhancement of totalcellulase or β-glucosidase was not observed.

Percentage saccharification increased with increase in the NaOH concentration used in the pre-treatment of rice straw and with the increase of enzyme concentration used in the hydrolysis. When a combined enzyme preparation from *Trichoderma* and *A.phoenicis* was used, appreciable degree of synergism was observed. The time course of reducing sugar production, with the combination of enzyme of *T.reesei* and *A.phoenicis*, produced 77% saccharification after 48 h, and it could be considered as the optimum period. The reducing sugar produced by enzymic hydrolysis were mainly glucose, xylose and cellobiose. Saccharification studies indicated that

a combination of *T.reesei* and a *A.phoenicis* enzymes has been very effective in saccharifying NaOH treated rice straw upto almost 100% of the reported holocellulose content.