SUMMARY

This thesis describes identification of the primary polygalacturonase protein secreted by *Aspergillus carbonarius*, its glycosylation resulting in higher molecular enzyme forms and the fungal growth physiology in relation to enzyme production.

Wild type *A. carbonarius* produced two polygalacturonases of molecular mass 61- and 42-kDa when grown in solid-state culture made of wheat bran. In submerged cultures, the fungus produced only very little enzyme. Polygalacturonase production by *A. carbonarius* in shake-flask could not be induced by pectin or pectin derivatives as carbon source.

Mutants of the fungus isolated after UV-treatment and temperature selection secreted polygalacturonase when grown in shake-flasks. These mutants grew as pellets due to increased hyphal branching during shake-flask cultivation. One of the mutants (UV-10046) isolated for fast growth rates, overproduced (300 U ml^{-1}) polygalacturonase in shake-flask cultures. This mutant exhibited compressed colony morphology on agar plates. In solid-state cultures made of wheat bran, the mutant strains behaved like the parent in the quantity of enzyme secreted. In the wheat bran medium containing 2 % glucose, enzyme secretion was affected in the parent but not in the mutant UV-10046. No appreciable difference in polygalacturonase production was observed when the mutant strain (UV-10046) was grown in shake-flask containing pectin and other simple sugars as carbon source.

Kinetics of polygalacturonase production by the mutant of *A. carbonarius* (UV-10046) during shake-flask growth showed that the enzyme production was

induced by acidic culture pH. Overproduction of the 42 kDa polygalacturonase by the mutant was observed by SDS-PAGE analysis of the culture filtrate.

The 42 kDa polygalacturonase purified by alginate affinity precipitation demonstrated optimum activity at temperature 40-50ºC and pH 4.3. However, the enzyme was stable only at temperatures below 30ºC. A high specific activity of 6350 U mg^{-1} was estimated with polygalacturonate substrate using the purified enzyme. Low affinity of the enzyme to the polygalacturonic acid substrate was evidenced by high K_M (2.5 %). Antibodies raised to the purified enzyme cross reacted with the 61 kDa polygalacturonase produced by the fungus in solid-state cultures and several high molecular mass enzyme forms that were present in the commercial samples of *A. niger*.

Studies on enzyme production during growth of the fungus in the shake-flask revealed pH induced enzyme secretion. pH controlled cultivation confirmed induced enzyme synthesis at pH 3.0. Basal level enzyme secretion at pH 5.0 was also observed. Rapid acidification of the culture broth and induced enzyme secretion at pH 3.0 resulted in the occurrence of a protein of molecular mass 40.5 kDa in the culture filtrates. The evolution of the lower molecular mass enzyme protein was not due to proteolytic hydrolysis by the acid proteases present in the culture filtrates since, the 40.5 kDa protein was also identified when the culture was grown in presence of protease inhibitors. The above results suggested differential glycosylation as the cause for the secretion of multiple enzyme forms by the fungus. In order to evidence differential glycosylation of the proteins, the 61- and 42-kDa enzymes were deglycosylated using PNGase F. The molecular mass of the deglycosylated protein was estimated by SDS-PAGE and western blot analysis of the products. Upon deglycosylation, the 61- and 42-kDa enzyme resolved as 40.5 kDa proteins in SDS-PAGE gels. The glycans were found to have a role in enzyme conformation for stability to pH.

The *N*-terminal of the 42 kDa protein had a sequence GS(C)TF. Only an indicative *N*-terminal sequence XVTXXF was deciphered in the 61 kDa polygalacturonase.

Highest yields of the enzyme in shake-flasks using the mutant UV-10046 were obtained when the fungus was grown in a medium containing corn flour. The conditions standardized resulted in the up scaling the batch fermentation process to 125 L for enzyme production.

pH induced enzyme secretion during shake-flask growth of the fungus also resulted in the description of a fed-batch fermentation protocol for increasing enzyme yield during submerged fermentation process.

Growth of the fungus as pellets in the fermentor aided in easy separation of the fungal mycelia from culture filtrate after fermentation using plate and frame filter. The raw culture filtrate could be concentrated 30 fold by membrane filtration. Stability of the enzyme during storage at 4ºC and ability of the enzyme preparation to degrade apple and banana fruit pulps to yield juice, described a process for industrial polygalacturonase production using the mutant strain of *A. carbonarius*.

The results are discussed in relevance to literature pertinent to this investigation.