The extracellular proteases of *Aspergillus nidulans*: a study of structural genes and their regulation.

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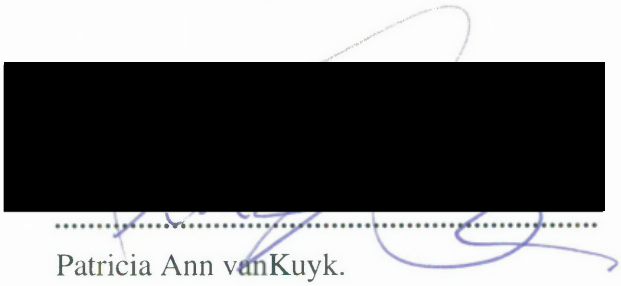
I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.



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Patricia Ann vanKuyk.

With the exception of the plasmid pAlk/Arg2 (section 5.3.1), which was constructed by M. Katz, the entire contents of this thesis are the sole work of Patricia Ann vanKuyk.

Abstract.

Polyacrylamide gel electrophoresis and specific protease inhibitors were used to identify three serine proteases and one metallo-protease in the culture filtrate of *A. nidulans* cultures subject to nutrient-limiting conditions.

A gene encoding a putative acid protease was isolated from an *A. nidulans* gene library using a heterologous probe. Sequence analysis showed that the *A. nidulans* aspergillopepsin gene, designated *prtB*, shows similarity to the *A. fumigatus* and *A. niger* aspergillopepsin genes, with nucleotide identities of 67.7% and 54.8% respectively. When the putative PrtB protein sequence was compared to the protein sequences of other aspergillopepsin genes, it was found that although the active sites were conserved, there was an 8 amino acid deletion prior to the second active site. It is unknown what effect this deletion has on the function of the PrtB protein. No *prtB* transcript was detected, using Northern analysis or RT-PCR, in RNA obtained from cultures subjected to 4 hours nitrogen limitation at pH 3 and pH 8. It is possible that the *A. nidulans prtB* gene is not expressed, or *prtB* may be expressed under conditions other than those tested.

Transformation was used to generate strains in which the *prtA* gene, encoding an extracellular serine protease, was disrupted. Data from protease assays showed that the *prtAΔ*strains produced significantly lower levels of protease activity. Analysis of culture filtrate using native polyacrylamide gel electrophoresis determined that the band of protease activity absent in the *prtAΔ* strains corresponded to a band which had been identified as a serine protease. The serine protease gene, *prtA*, was mapped to the region between the *hxA* and *riboD* genes on chromosome V. Examination of the promoter region of *prtA* showed that there was a region of approximately 100 bp which was highly conserved in a similar position in the promoters of other *Aspergillus* extracellular serine proteases. Preliminary experiments using gel mobility shift assays

identified proteins in nuclear protein extracts which bound in a specific manner to DNA fragments which contained this highly conserved region.

Two mutants which did not produce extracellular protease in response to sulphur limitation, were isolated in a mutagenesis screen. Haploidisation analysis showed that one mutation, designated *xprI1*, was linked to a translocation event involving chromosomes II and VII. Further genetic characterisation of the second mutant, suggested that the phenotype of interest was the result of two mutations. Characterisation of strains carrying the *xprI* mutation have shown that it did not produce extracellular protease under sulphur-, nitrogen-, or carbon-limiting conditions. It was also shown, that the secretory process in strains carrying the *xprI* mutation was not impaired. The ability of strains carrying the *xprI1* mutation to utilise a variety of carbon, nitrogen, and sulphur sources was examined. No pleiotrophic effects were observed, suggesting that *xprI* may play a role in the regulation of the extracellular proteases of *A. nidulans.*

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