

THE COMPLEX CHITINOLYTIC SYSTEM OF
ASPERGILLUS FUMIGATUS

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Kolej Universiti Sains Dan Teknologi Malaysia (KUSTEM)

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The complex chitinolytic system of aspergillus fumigatus /
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**THE COMPLEX CHITINOLYTIC SYSTEM OF
*ASPERGILLUS FUMIGATUS***

by

MARIAM TAIB

**Submitted in accordance with the requirements for the
degree of Doctor of Philosophy**

**The University of Leeds
School of Biochemistry and Microbiology**

May 2005

The candidate confirms that the work submitted is her own and
that appropriate credit has been given where reference has
been made to the work of others

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Abstract

Aspergillus fumigatus is one of the most important fungal pathogens of humans and there is an urgent need for new drugs to counter infections caused by *A. fumigatus* and other pathogenic species. Enzymes of chitin metabolism, and their regulators present novel targets for antifungal agents. During the work described here, the patterns of expression of the *chiA1* and *chiB1* chitinase genes of *A. fumigatus* during batch culture were investigated using real-time, reverse-transcription PCR. The *chiA1* gene, encoding the fungal/plant chitinase ChiA1, was expressed at significant levels throughout the six days of culture. However, the level of expression of *chiB1*, encoding the fungal/bacterial chitinase ChiB1, was only just detectable on day one but had been induced 1280-fold, to a level similar to that detected for *chiA1* expression, by day 6. The results suggest markedly different roles for these enzymes. The gene encoding the transcription factor CreA was cloned and expressed, as a glutathione S-transferase (GST) fusion protein, in *Escherichia coli*. In electrophoretic mobility shift assays purified GST-CreA, or an *A. fumigatus* cell extract, bound specifically to putative CreA binding sites upstream of the *chiB1* gene. CreA may therefore have a role in the regulation of chitinase activity in *A. fumigatus*. The effects of a range of compounds on *A. fumigatus* chitinase activity were determined. The cyclopentapeptides, argadin and argifin (each at 0.6 μM), were potent inhibitors of enzyme activity. The cyclic dipeptides, D-Leu-D-Pro, cyclo-(D-Leu-D-Pro) and cyclo-(L-His-L-Pro) (each at 300 μM), did not inhibit chitinase activity, while the methylxanthines, pentoxifylline and theophylline, caused significant inhibition at concentrations of 75 μM and 300 μM , respectively. In preliminary expression studies, ChiA1 was fused with GST or maltose-binding protein (MBP) and expressed in *E. coli*. In addition, ChiA1-His₆ peptide was expressed in *Pichia pastoris*. These constructs will be used in future work which will further explore the complex chitinolytic system of *A. fumigatus* and which may lead to the exploitation of this system as a target for antifungals.

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Abbreviations

The following abbreviations are used:

| | |
|--------------------|---|
| ATCC | American Type Culture Collection |
| ddH ₂ O | Double distilled water |
| DIG | Digoxygenin |
| DMSO | Dimethylsulfoxide |
| DTT | Dithiothreitol |
| EDTA | Diaminoethane tetraacetic acid, disodium salt |
| EMSA | Electrophoretic Mobility Shift Assay |
| GlcNAc | N-acetylglucosamine |
| GST | Glutathione S-transferase |
| HEPES | N-[2-Hydroxyethyl]piperazine-N'-(2-ethylsulfonic acid) |
| IPTG | Isopropyl β-D thiogalactoside |
| LB | Luria-Bertani |
| MBP | Maltose-binding protein |
| MMS | Minimal Medium with 1 M sucrose |
| MOPS | 3-(N-morpholino)propanesulfonic acid |
| MU | Methylumbelliferone |
| NBT/BCIP | Nitro blue tetrazolium chloride / 5-Bromo-4-chloro-3-indoyl phosphate |
| NCCLS | The U.S National Committee of Clinical Lab Standard |
| NTP | Nucleoside Triphosphates |
| PAGE | Polyacrylamide gel electrophoresis |
| PBS | Phosphate-buffered saline |
| PEG | Polyethylene glycol |

| | |
|------|---|
| PMSF | Phenylmethanesulphonyl fluoride |
| rpm | Revolutions per minute |
| SDS | Sodium dodecyl sulphate |
| TBS | Tris-buffered saline |
| Tris | Tris(hydroxymethyl)aminoethane |
| TTBS | Tween Tris-buffered saline |
| YNB | Yeast Nitrogen Base with ammonium sulfate without amino acids |
| YPD | Yeast Peptone Dextrose |

Chapter 1

General Introduction

1.1 Human Mycoses

Fungi can cause a variety of diseases of humans and other animals ranging from minor superficial skin and mucous membrane infections to life threatening, systemic involvement of the internal organs. There are three major types of disease caused by fungi: allergies, poisonings and fungal infections (Schroeder et al., 1998). Allergic reactions to fungi are caused by sensitivity to fungal proteins, such as those present in dried fungal spores, while poisonings result from the ingestion of fungal toxins in contaminated food or poisonous mushrooms. Fungal allergies and poisonings are important concerns in agriculture and other industries where fungal contamination is common. Fungal infections, or mycoses, result from the invasion of living tissue by a fungus and they represent the most common form of fungal disease.

Chapter 1

General Introduction

There are more than 100,000 recognized species of fungi, but only about 400 are known to infect humans (de Hoog & Guarro, 1998). Some species of fungi, called primary pathogens, cause disease regardless of the individual's state of health. Other species that infect individuals with a weakened immune system are described as opportunistic pathogens. However, among patients with severe immunosuppression, almost any type of fungus can exhibit pathogenic potential, which may have devastating consequences for the host (Dunn, 2000). The most common fungal infections in immunocompromised hosts are candidiasis, aspergillosis, cryptococcosis, pneumoconiosis (cryptococcosis) and *Pneumocystis carinii* pneumonia (pneumocystisosis).

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