Identification of aflatoxigenic ***Aspergillus flavus*** and ***A. parasiticus*** by Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

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Introduction

- aflatoxins are secondary metabolites produced by ***Aspergillus flavus*** and ***A. parasiticus:*** AFB_1, AFB_2, AFG_1, AFG_2

- AFB_1 among the genotoxic agents most potent in nature

- severe effects on human and animal health: immunosuppression, liver necrosis, pulmonary aedema, etc.

Biosynthetic pathway of aflatoxins in ***Aspergillus*** spp.

(from Hicks ***et al.***, 2002)

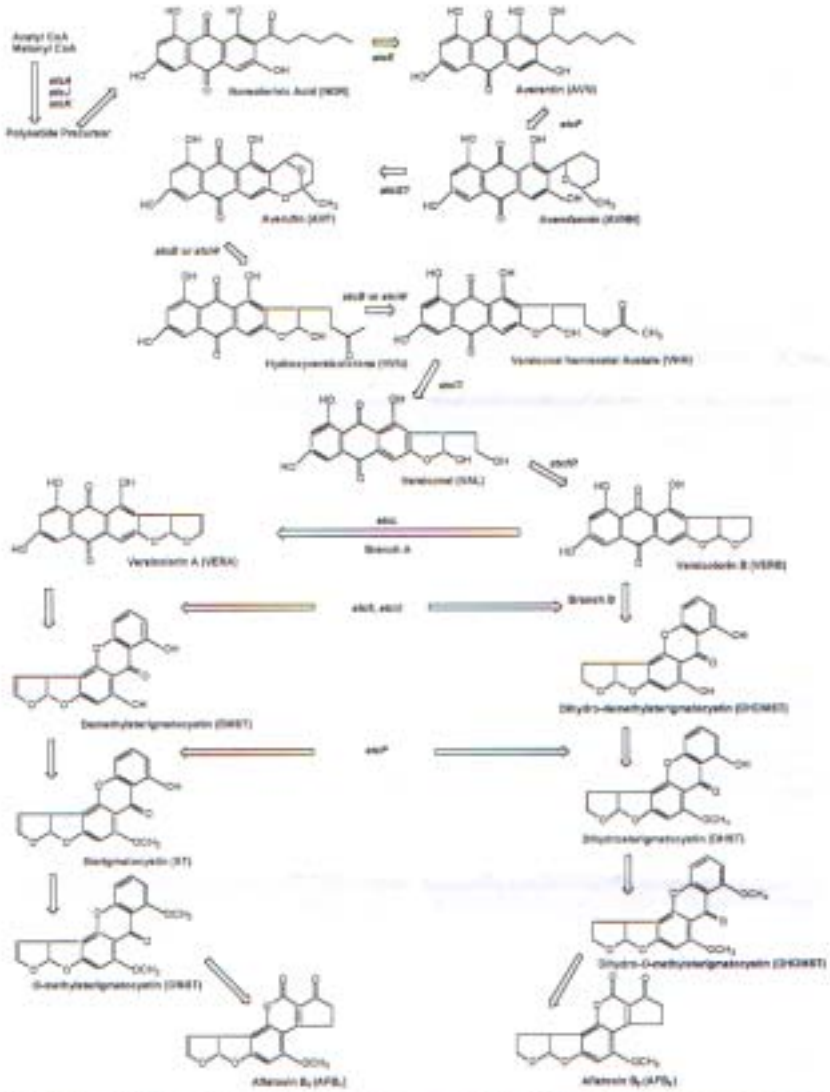


Fig. 4.1. Aflatoxin/sterigmatocystin biosynthetic pathway. Malonyl CoA and acetyl CoA are converted into a polyketide precursor by the combined activities of a fatty acid synthase and polyketide synthase. The polyketide precursor undergoes a series of conversions to result in sterigmatocystin (*A. nidulans*), AFB_1/AFB_2 (*A. flavus*) or $AFB_1/AFB_2/AFG_1/AFG_2$ (*A. parasiticus*). The *A. nidulans* or (aflatoxin/sterigmatocystin cluster) genes responsible for producing various enzymes in the pathway are indicated. A *question mark* following the gene indicates that function has not been demonstrated and activity is only postulated

Introduction

- 17 genes are responsible for the complete biosynthetic pathway of aflatoxins
- production of aflatoxin is not directly correlated with the presence of ***all*** known biosynthetic genes
- importance of developing rapid diagnostic tools for toxigenic isolates

Objectives

- characterising gene expression in aflatoxin-producing and non-producing ***Aspergillus*** spp. by RT-PCR

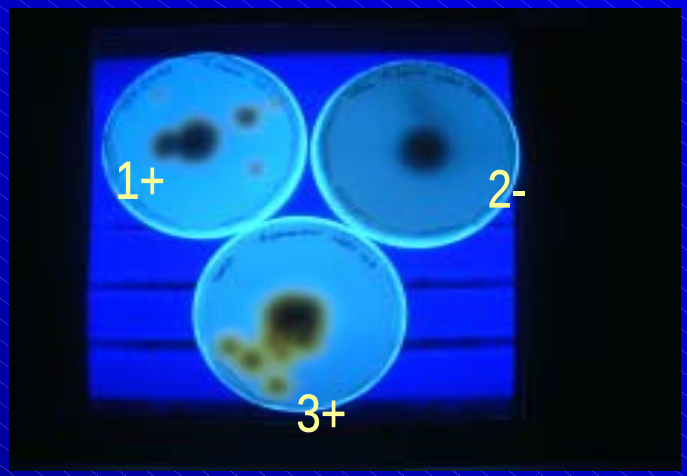
- identifying specific primers targeted at key genes in the aflatoxin pathway (diagnostic approach)

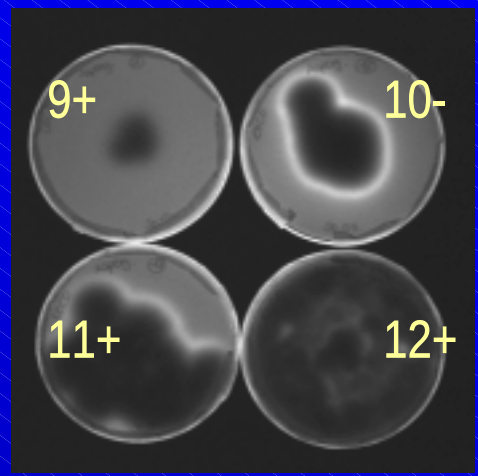
- confirming molecular-based analysis with cultural methods and HPLC/MS-based analysis

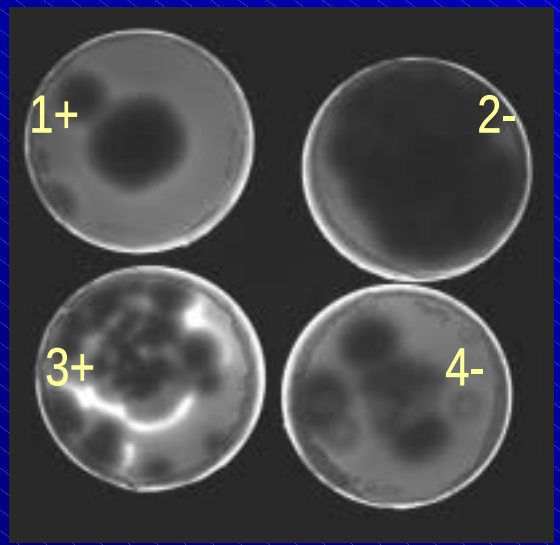
Materials & Methods

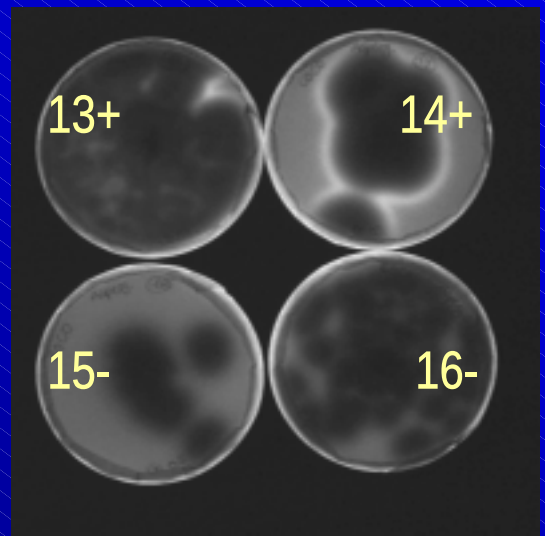
- growth on coconut agar and observation of UV fluorescence
- extraction of aflatoxin from culture filtrates and HPLC/MS
- Total RNAs extracted from 16 strains of ***A. flavus*** and ***A. parasiticus*** grown in inducing and non-inducing medium
- RT-PCR with 12 primer pairs matching biosynthetic and regulatory genes + β tubulin ***tub1*** gene as control

Fluorescence produced by toxigenic (+) and non toxigenic (-) ***Aspergillus*** spp. colonies grown on coconut agar and exposed to UV radiation

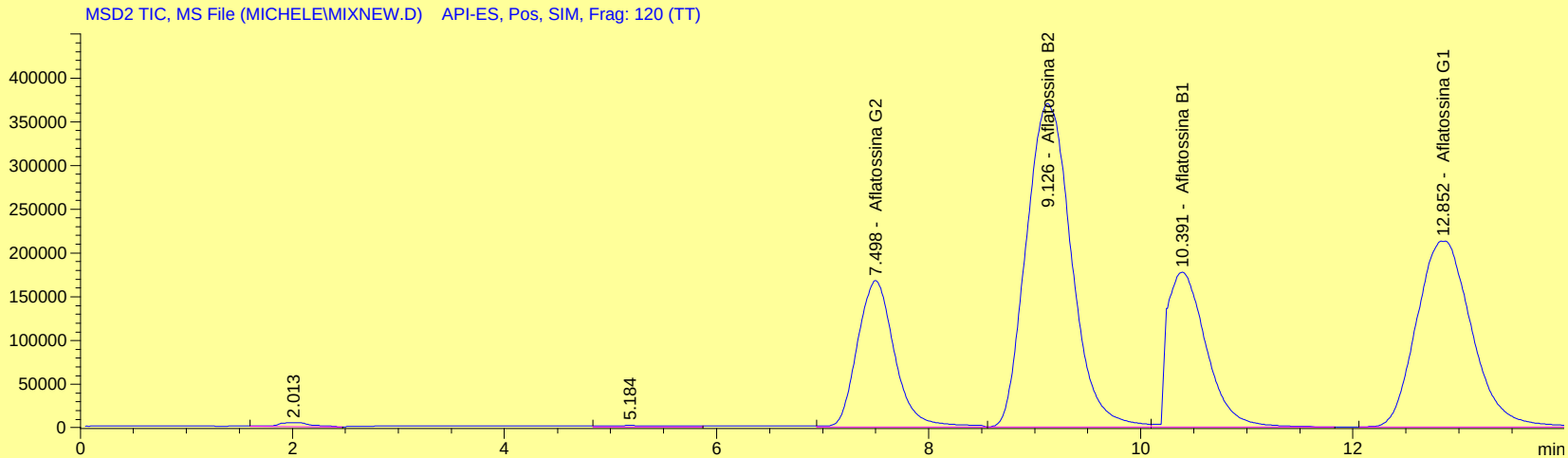


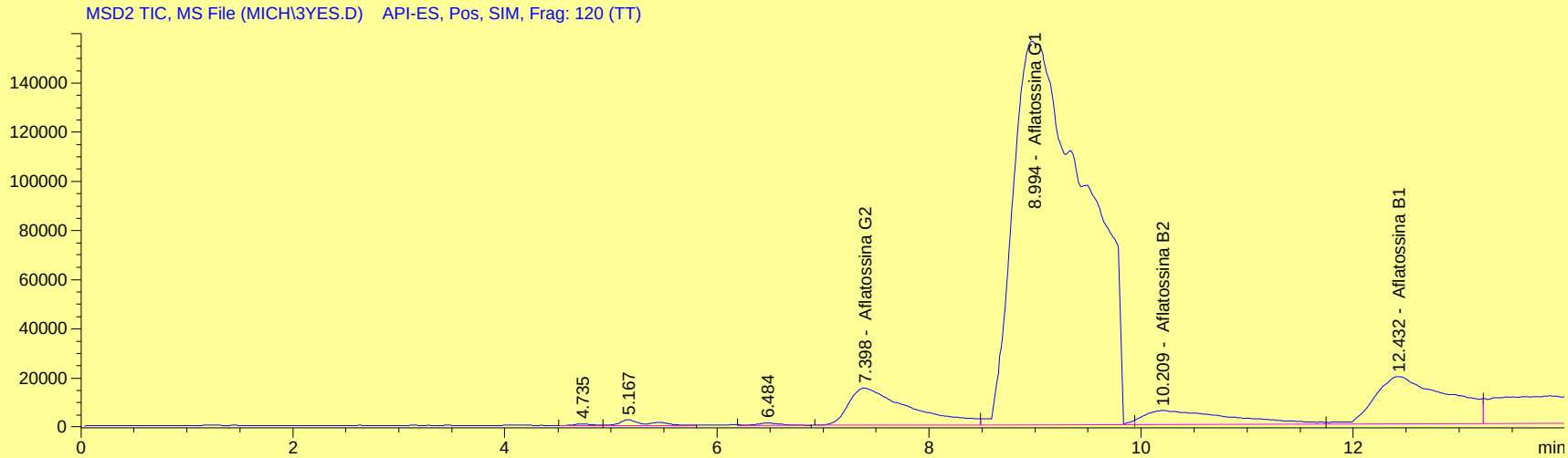






HPLC/MS analysis of isolates *A. flavus* SRRC285 (tox-) and *A. parasiticus* SRRC143 (tox+), grown in inducing medium and aflatoxin standards AFB_1, AFB_2, AFG_1 and AFG_2





	Isolates of ***Aspergillus*** spp.	Tox	Tox Coconut agar	Tox HPLC	***nor1***	***avnA***	***adhA***	***ver1***	***omtB*** F	***omtB*** P	***omt1***	***ord1***[8]	***alfR***[8]	***aflR***	***aflJ***	***tub1***
1	*A. flavus* Tx118-2[1]	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
2	*A. flavus* SRRC285[1]	-	-	-	-	-	+	-	-	-	-	-	+	+	+	+
3	*A. parasiticus* SRRC143[1]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	*A. flavus* MAM 03[2]	-	-	+	-	-	-	+	-	-	-	-	-	-	-	+
5	*A. flavus* MAM 11[2]	+	+	+	-	-	+	+	-	-	-	-	-	-	-	+
6	*A- flavus* MAM 13[2]	+	-	+	-	-	+	+	-	-	-	-	-	-	+	+
7	*A. flavus* MAM 16[2]	-	-	-	+	-	+	+	+	+	+	+	+	-	+	+
8	*A. parasiticus* SRRC2036[4]	+	-	-	+	-	+	+	+	-	+	+	-	+	+	+
9	*A. parasiticus* BN009-E[5]	+	+	+	+	-	+	+	+	-	+	+	+	-	+	+
10	*A. parasiticus* SRRC2043[6]	-	+	-	+	-	+	+	+	-	+	+	+	+	+	+
11	*A. flavus* NRRL3357[7]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	*A. flavus* AF13[5]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	*A. flavus* SRRC2550[3]	+	+	+	+	-	+	+	-	-	+	+	-	+	+	+
14	*A. flavus* SRRC2042[3]	-	-	+	-	-	+	+	-	-	-	-	+	+	+	+
15	*A. flavus* SRRC284[3]	-	+	-	-	-	+	+	-	-	-	-	-	+	+	+
16	*A. flavus* SRRC283[3]	-	-	-	-	-	+	+	-	-	-	+	+	+	+	+

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Conclusion

- test on coconut agar does not allow unequivocal distinction between toxigenic and non-toxigenic isolates
- RT-PCR may be applied for rapid identification of aflatoxigenic isolates
- some genes were identified whose expression appears essential in the biosynthetic pathway of aflatoxin
- potential application of microarray technology?

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