



# Polyphasic Approach Including MALDI-TOF Mass Spectrometry to Characterise Aflatoxigenic Species of *Aspergillus* Section *Flavi*



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## Introduction:

*Aspergillus* is a large genus, with a complex taxonomy. The genus is easily identified by its characteristic conidiophore, but species identification and differentiation is complex, mainly because it is traditionally based on a range of morphological features. *Aspergillus* subgenus *Circumdati* section *Flavi*, also referred to as the *A. flavus* group, has attracted worldwide attention for its industrial use and toxigenic potential. Section *Flavi* is divided in two groups of species. One includes the aflatoxigenic species *A. flavus*, *A. parasiticus* and *A. nomius*, which cause serious problems in agricultural commodities, and the other one includes the non-aflatoxigenic species *A. oryzae*, *A. sojae* and *A. tamarii*, traditionally used for production of fermented foods. Species from *A. flavus* group are morphologically and genetically very similar, and are therefore difficult to differentiate by both cultural and molecular methods. Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) Mass Spectrometry has already shown high potentialities in discriminating very closely related taxa.

## Aims:

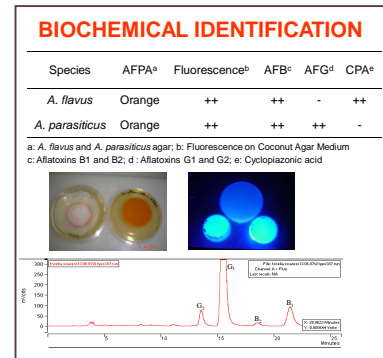
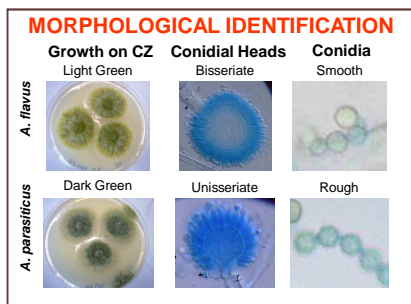
This work intended to discriminate *A. flavus* group strains using MALDI-TOF MS. Results are compared with those previously obtained by conventional and molecular methods.

## Material and Methods:

A group of 29 *Aspergillus* Section *Flavi* strains isolated from winemaking grapes were used.

The strains were characterised using polyphasic approach. The morphology with emphasis in conidial wall ornamentation was regarded as the primary diagnostic character for separation of *A. flavus* from *A. parasiticus*. The selective media *Aspergillus* Flavus and Parasiticus Agar (AFPA) and Coconut Agar (CCA) were used to complete this part. The aflatoxins (G1 and B1) as well as the cyclopiazonic acid (CPA) were analysed. The genes *afID* (*nor1*) and *afIQ* (*ord1*) were studied using the correspondent probes and amplified by PCR. The spectral analysis was done by Matrix Assisted Laser Desorption Ionization – Time Of Flight (MALDI-TOF-MS) and the spectra analyzed using SARAMIS software. The strains *A. flavus* MUM 92.01, 00.06, 00.06, 00.09 and "Brasil strain", *A. parasiticus* MUM 92.02 were used as reference strains. The *A. ochraceus* strain 06AAs01 was used as outgroup.

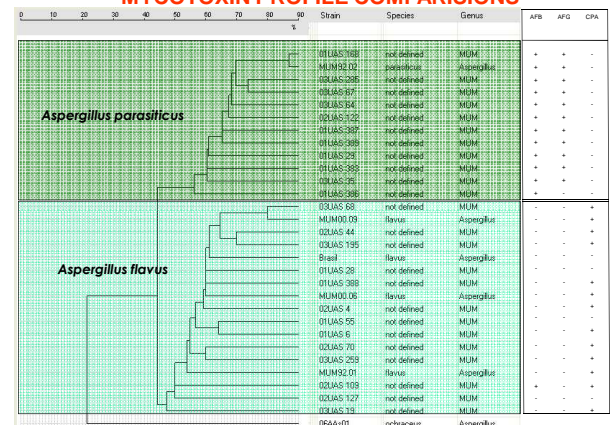
## Results:



## IDENTIFICATION SCHEME REVISION

Strain	Fluorescence (CAF)	AF G1		AF B1		CPA (mg/L)		Species	DNA	
		YES	NO	YES	NO	afID (nor1)	afIQ (ord1)			
01UAS 1	N	N	N	N	N	N	N	<i>A. flavus</i>	+	+
01UAS 6	N	N	N	N	N	Y	N	<i>A. parasiticus</i>	-	-
01UAS 23	N	N	N	N	N	N	N	<i>A. flavus</i>	+	+
01UAS 29	Y	Y	Y	Y	N	N	N	<i>A. parasiticus</i>	+	+
01UAS 55	N	N	N	N	N	N	N	<i>A. flavus</i>	+	+
01UAS 168	Y	Y	Y	Y	N	N	N	<i>A. parasiticus</i>	+	+
01UAS 383	Y	Y	Y	Y	N	N	N	<i>A. parasiticus</i>	+	+
01UAS 386	N	N	Y	Y	N	N	N	<i>A. parasiticus</i>	+	+
01UAS 387	Y	Y	Y	Y	N	N	N	<i>A. parasiticus</i>	+	+
01UAS 388	N	N	N	N	Y	N	N	<i>A. flavus</i>	+	+
01UAS 389	Y	Y	Y	Y	N	N	N	<i>A. parasiticus</i>	+	+
02UAS 4	N	N	N	N	Y	N	N	<i>A. flavus</i>	+	+
02UAS 44	N	N	N	N	N	N	N	<i>A. flavus</i>	+	+
02UAS 70	N	N	N	N	N	N	N	<i>A. flavus</i>	+	+
02UAS 109	N	N	N	Y	N	N	N	<i>A. flavus</i>	+	+
02UAS 122	Y	Y	Y	Y	N	N	N	<i>A. parasiticus</i>	+	+
02UAS 127	N	N	N	N	N	N	N	<i>A. flavus</i>	+	+
02UAS 13	N	N	N	N	N	N	N	<i>A. flavus</i>	+	+
02UAS 22	N	N	N	N	N	N	N	<i>A. flavus</i>	+	+
02UAS 35	Y	Y	Y	Y	N	N	N	<i>A. parasiticus</i>	+	+
02UAS 64	Y	Y	Y	Y	N	N	N	<i>A. parasiticus</i>	+	+
02UAS 67	Y	Y	Y	Y	N	N	N	<i>A. parasiticus</i>	+	+
02UAS 69	N	N	N	N	N	N	N	<i>A. flavus</i>	+	+
02UAS 195	N	N	N	N	N	N	N	<i>A. flavus</i>	+	+
02UAS 259	N	N	N	N	Y	N	N	<i>A. flavus</i>	+	+
02UAS 285	Y	Y	Y	Y	N	N	N	<i>A. parasiticus</i>	+	+
MUM02.01	N	N	N	N	N	N	N	<i>A. flavus</i>	+	+
MUM03.06								<i>A. flavus</i>	+	+
MUM03.09								<i>A. flavus</i>	+	+
MUM 92.02	Y	Y	Y	Y	N	N	N	<i>A. parasiticus</i>	+	+
<i>A. nidulans</i> strain	N	N	N	N	N	N	N	<i>A. flavus</i>	-	-

## DENDROGRAM OF SPECTRAL ANALYSIS AND MYCOTOXIN PROFILE COMPARISONS



## Conclusions:

- The different methods used to identify *A. flavus* and *A. parasiticus* were well correlated.
- MALDI-TOF MS analysis shows potential to discriminate between these two taxa and the spectral profiles produced for each organisms were consistent.

## Acknowledgments:

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