



A polyphasic approach for black *Aspergillus* identification using MALDI-TOF MS



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Introduction

The *Aspergillus* section *Nigri* is among the best studied fungi, having different commercial applications, but also causing biodeterioration of commodities and food spoilage. Although being well studied, their identification is not straightforward, and, recently, new species have been described in this section¹⁻³. These new species were not only separated from their relatives in the section by morphological distinction but also from molecular point of view. The concept of species is clearly abstract and delimitations are very difficult, and often not consensual. Recently, Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) has been used to generate spectrum of protein masses in a range of 2000 to 20000 Da that is a *taxon* specific fingerprinting⁴. This approach could be employed as an alternative to the more tedious molecular techniques.

Aim

Validation of the morphological and molecular biology data through MALDI-TOF MS analyses.

Strategy

All strains (Table 1) were morphologically characterised based on various macro and microscopic features. Molecular characterisation was performed for two different DNA regions: the ITS1-5.8S-ITS2 region (data not shown) and partial calmodulin gene (Fig. 1).

Protein spectrum (MALDI-TOF MS) characterisation of strains and cluster analysis was done (Fig. 2) enabling a comparative study among clustering of strains by morphologic, molecular and MALDI-TOF MS analysis.

Table 1: List of strains used for MALDI-TOF Mass Spectrometry analysis.

Species	Isolate number	Geographical origin	Source
<i>A. ibericus</i>	MUM 03.49 (HIMI 391429, ITEM 4776) (T)	Portugal	Wine grapes
	MUM 03.50 (HIMI 391430, ITEM 4401)	Portugal	Wine grapes
	MUM 03.51 (HIMI 391431, ITEM 4402)	Portugal	Wine grapes
<i>A. carbonarius</i>	MUM 03.04 (HIMI 016136, NRRL 3491) (T)	Unknown	Paper
	MUM 03.18 (HIMI 387223)	Portugal	Wine grapes
	MUM 03.59 (HIMI 387242)	Portugal	Wine must
<i>A. niger</i>	MUM 03.01 (HIMI 050546, NRRL 326) (T)	USA	Tamrin-gallic acid fermentation
	MUM 03.37 (molecular pattern N)	Portugal	Wine grapes
	MUM 05.13 (molecular pattern T)	Portugal	Wine grapes
<i>A. sclerotioriger</i>	CBS 115572 (MUM 04.151) (T)	India	Aromatic coffee, green
<i>A. lacticoffeatus</i>	CBS 101883 (MUM 04.150) (T)	Indonesia	Coffee robusta, surface sterilized beans
<i>A. tubingensis</i>	CBS 134.48 (MUM 04.152) (T)	Unknown	Unknown
<i>A. vadensis</i>	CBS 113365 (MUM 04.153) (T)	Unknown	Dead plant tissue
<i>A. ellipticus</i>	MUM 03.12 (HIMI 172283, NRRL 5120) (T)	Costa Rica	Soil
<i>A. japonicus</i>	MUM 03.02 (MATCC 1042) (T)	Puerto Rico	Soil
<i>A. aculeatus</i>	MUM 03.11 (HIMI 211388) (T)	Unknown	Topical soil
<i>A. phoenicis</i>	MUM 03.05 (HIMI 211365)	Unknown	Unknown
<i>A. flavus</i> (outgroup)	MUM 00.06	Portugal	Cheese ripening chamber

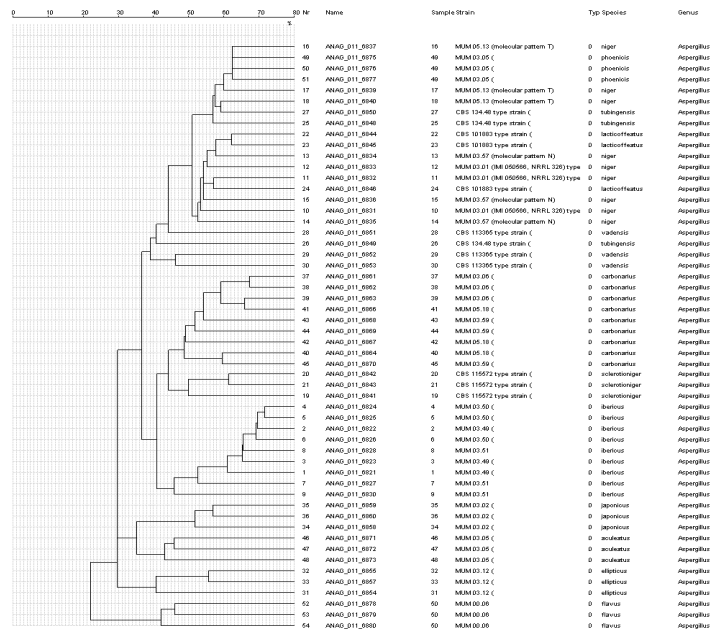


Fig. 2: Dendrogram of relatedness between members of section *Nigri* based on MALDI-TOF MS analysis. *Aspergillus flavus* was used as out group.

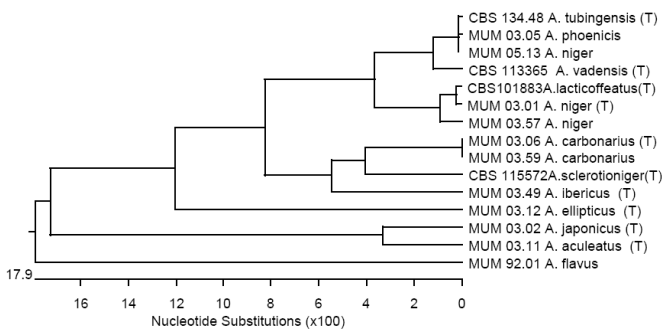


Fig. 1: Dendrogram of relatedness between members of section *Nigri* based on molecular analysis of the partial calmodulin gene sequence. The tree is rooted with *Aspergillus flavus*. The sequences were aligned with Clustal W using the Software DNASTar v. 5.00.

Conclusions

The obtained results showed that morphologic analysis was in accordance with the identity of the strains. The molecular analysis enables the separation of uniseriate from biseriata strains and, within these, the separation of some biseriata species. Furthermore, the CBS MALDI-TOF MS shows high reproducibility and was able to give results faster than the molecular biology.

References

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