

## A POLYPHASIC APPROACH FOR BLACK *ASPERGILLUS* IDENTIFICATION USING MALDI-TOF MS

P. Rodrigues<sup>1,2</sup>, C. Soares<sup>1</sup>, C. Santos<sup>1</sup>, M. Fraga<sup>3</sup>, A. Venâncio<sup>1</sup>, N. Lima<sup>4</sup>

<sup>1</sup>IBB - Biological Engineering Centre, University of Minho, Braga, <sup>2</sup>CIMO - Escola Superior Agrária de Bragança, Bragança, Portugal, <sup>3</sup>Departamento de Microbiologia e Imunologia Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica RJ, Brazil, <sup>4</sup>IBB-Institute for Biotechnology and Bioengineering, Centro de Engenharia Biológica, Braga, Portugal

**Background:** 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 section. These new species were not only separated from their relatives in the section by morphological distinction but also from molecular point of view. 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 fingerprinting. This approach could be employed as an alternative to the molecular techniques.

**Objectives:** Validation of the MALDI-TOF MS analyses.

### Methods:

Molecular studies: DNA extraction and amplification of two sequences: ITS-5.8S rDNA and calmodulin gene.

MALDI-TOF MS: spectrum of protein masses in a range of 2000 to 20000 Da.

Mycotoxins: Immunoaffinity columns, HPLC.

### Results:

1. Morphologic characterisation of strains and cluster analysis was done.
2. Molecular characterisation of two DNA sequences was done.
3. Ochratoxin A production in *in-vitro* conditions was checked.
4. Protein spectrum (MALDI-TOF MS) characterisation of strains and cluster analysis was done.
5. Clustering of strains by morphologic analysis, molecular analysis and MALDI-TOF MS was done.

### Conclusions:

1. Morphologic analysis was in accordance with the identity of the strains.
2. Molecular analysis enables the separation of uniseriate from biseriata strains, and, within these, the separation of some biseriata species.
3. Neither the morphologic nor the molecular clustering was able to cluster separately the Ochratoxin A producing strains and the non-producing ones
4. Clustering of OTA producing strains was attempted with the Maldi-TOF MS.