

Microbial Diagnostic Applications of Mass Spectrometry



Organised by
HPA Microbiology Services, Colindale, England



in collaboration with

Micoteca da Universidade do Minho, Braga, Portugal

ABSTRACT BOOK

London, Colindale
4th and 5th April 2012

ASSESSMENT OF FUNGI STABILITY BY MALDI-TOF ICMS FOLLOWING PRESERVATION ON ALGINATE ENCAPSULATED SAMPLES

M.F. Simões, C. Santos and N. Lima

IBB-Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Micoteca da Universidade do Minho, Campus de Gualtar, Braga, Portugal
e-mail: nelson@ie.uminho.pt

Alginate-encapsulation is a commonly used, simple and cost effective, method to preserve plant samples. Since alginate has proven to protect tissues against physical and environmental damage, minimising dehydration, it presents as a good preservation technique. The application of this method for the preservation of filamentous fungi was intended to present an alternative for the commonly used preservation methods, especially to be used on recalcitrant fungal strains.

Matrix-assisted laser desorption/ionisation time-of-flight intact cell mass spectrometry (MALDI-TOF) emerged in the late 1980s as a sound technique to investigate the mass spectrometry of molecular high-mass of organic compounds through a soft ionisation of molecules resulting in minimum fragmentation. This technique has demonstrated its high potentiality for identification of filamentous fungi species and, in some specific cases, for strain identification. One of the most interesting advantages of the technique is the possibility of analysing the intact fungal cell generating peptides and proteins profiles.

A novel technique was applied on the preservation of *Botrytis cinerea* (MUM 10.163, 10.165, 10.167), *Aspergillus ibericus* (MUM 04.68) and *Aspergillus brasiliensis* (MUM 06.181) using the methodology of alginate encapsulation, in two different conditions: distilled water (I) and a 10% glycerol solution (II), both at 4 °C for 1 year; the viability of these strains was studied. The assessment was made by comparison with the method of Castellani preservation in water (III), using morphologic and MALDI-TOF ICMS analyses for the analysis of the 3 preservation methods.

The encapsulated samples of the strains preserved in distilled water (I), presented lower viability than those preserved in 10% glycerol (II). However, when comparing the growth from the samples preserved in water (III) with the ones encapsulated and maintained in 10% glycerol (II), we noted that the last ones presented a healthier and faster growth.

From the MALDI-TOF ICMS analysis, it was observed that the strains were clustered according to species identification, and for *Botrytis cinerea* all presented small differences (< 5%) in the percentages of similarity, except for MUM 10.167 (>5%) when preserved in water (III).

From our evaluation we were able to conclude that the success of the preservation method is strain dependent.

Acknowledgements:

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7, 2007-2013), Research Infrastructures action, under the grant agreement No. FP7-228310 (EMbaRC project). M.F. Simões acknowledges FCT – Portugal for the scholarship SFRH/BD/64260/2009.