SAU-49 MALDI-TOF MASS SPECTROMETRY FOR IDENTIFICATION OF THE *Aspergillus niger* AGGREGATE

Santos, C.[1]; Souza-Motta, C.M.[2]; Neves, R.P.[2]; Araújo, J.M.[3]; Calazans, G.M.T.[3]; Carneiro-da-Cunha, M.G.[1,4]; Lima-Filho, J.L.[1,4]; Erhard, M.[5]; Kallow, W.[5]; Paterson, R.R.M.[6]; Venâncio, A.[6]; Lima, N.[6]

[1]Laboratório de Imunopatologia Keizo Asami, Universidade Federal de Pernambuco, Recife, PE, Brazil; [2]URM, Departamento de Micologia, Universidade Federal de Pernambuco, Recife, PE, Brazil; [3]UFPEDA, Departamento de Antibióticos, Universidade Federal de Pernambuco, Recife, PE, Brazil; [4]Departamento de Bioquímica, Universidade Federal de Pernambuco, Recife, PE, Brazil; [5]AnagnosTec GmbH, Am Mühlenberg, Potsdam/Golm, Germany; [6]IBB - Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Braga, Portugal.
cledirs@hotmail.com

INTRODUCTION: Erhard *et al.* [1] employed for the first time Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) fingerprinting in the characterization of toxic cyanobacteria. Subsequently, new developments and improvements of this technology enabled the characterisation a wide spectrum of microbial cells and typing by MALDI-TOF MS proved to have the potential to discriminate closely related *taxa*. The detection of large biomolecules up to 100,000 Da by MALDI-TOF MS is enabled by the soft ionization which largely avoids fragmentation. Thus, biomolecules give single and/or double charged ions and complex samples (*e.g.* whole cells) can be investigated. Employing unfractionated cell material, organism-specific signal patterns ("fingerprints") in the mass range of 2,000 – 20,000 Da are obtained. In filamentous fungi most signals correspond to membrane surface proteins so their highly characteristic masses can be used for identification and classification. Indeed, filamentous fungi have been studied in a preliminary manner using this approach [2]. Species of the *Aspergillus* section *Nigri* have been extensively used for various biotechnological purposes including production of enzymes and organic acids. *A. niger* is also amongst the best-studied fungi that cause biodeterioration of commodities including food. Related to this latter aspect, *A. niger* can produce ochratoxin A (OTA) which is one of the most important mycotoxins and it is considered to be nephrotoxic, immunotoxic, genotoxic, and teratogenic. Notwithstanding this, it has been classified as GRAS (Generally Regard as Safe) in biotechnological and food processes. Recent studies using restriction fragment length polymorphism (RFLP) has divided *A. niger* aggregate into two morphologically indistinguishable species: *A. niger* and *A. tubingensis* [3]. The ITS1-5.8S-ITS2 ribosomal DNA region gives 519 and 76 bp fragments for *A. niger* after digested with *Rsa*I and a single fragment of 595 bp for *A. tubingensis*. Accensi *et al.* [3] evaluated isolates of the *A. niger* aggregate and found that OTA producer strains were *A. niger* (termed the "N") and for strains termed "T" OTA could not be detectable. Samson *et al.* [4, 5] assumed in their last revisions that *A. tubingensis* is a non-OTA producer species. Perrone *et al.* [6] using strains isolated from grapes were able to demonstrate that *A. tubingensis* is also able to produce OTA. In the present work MALDI-TOF MS is used to study these *taxa* to assess the potentialities and limits of this new microbial identification technique since the taxonomy of the *A. niger* aggregate is not completely resolved.

MATERIALS AND METHODS: Fungi were grown for 3 days in liquid medium (GYM) and then the mycelia were washed with distilled water and lyophilised. The dried mycelia were transferred as a thin film to the MALDI stainless steel template and mixed with 1 ml MALDI matrix solution (10 mg/ml 2,5-dihydroxybenzoic acid in water/acetonitrile [1:1] with 0.03% trifluoroacetic acid). The sample mixtures were air dried at room temperature. The analyses were performed on a Voyager DE-PRO system (Applied Biosystems, Foster City, CA) equipped with a nitrogen laser (337 nm) employing a linear mode with delayed positive ion extraction with a mass accuracy of at least 200 ppm. Routinely, the mass range from m/z = 2,000 to 20,000 Da was recorded. *Escherichia coli* strain DH5α with known mass values of ribosomal proteins was used for external calibration. Following smoothing, baseline correction and peak detection steps, the peak lists of studied fungal strains were directly transferred into the Spectral ARchiving And Microbial Identification System (SARAMIS) software where it is matched against the SARAMIS database for spectra comparison. This software allows the classification of microorganisms and the computing of theoretical consensus spectra (superspectra) to increase the efficiency of microbial identifications and the grouping of samples. The fungal strains used in this study are listed in Table 1.

Table 1 - List of *Aspergillus* strains included in this study for MALDI-TOF MS analysis.

Species	Isolate number	Substratum	Geographical origin
A. niger	MUM 03.01 (**T**) (=IMI 050566)	Tannin-gallic acid fermentation	USA
	MUM 03.57 (molecular pattern N)	Wine grapes	Portugal
	MUM 05.13 (molecular pattern T)	Wine grapes	Portugal
A. tubingensis	CBS 134.48 (=MUM 06.152) (**T**)	Unknown	Unknown
	MUM 00.06	Cheese repining chamber	Portugal
A. flavus			

T=*ex*-type strain; MUM=Micoteca da Universidade do Minho

RESULTS AND DISCUSSION: A SARAMIS-dendrogram computed for mass spectra in a range from 5,000-20,000 Da shows 3 distinct clades for the strains studied (Fig. 1). *A. flavus* MUM 00.06 form a clear out-group as expected since is not a related species from this *Nigri* section. The *A. niger* MUM 05.13, which is a T-type strain, is closely related to *A. tubingensis*. In contrast *A. niger* MUM 03.57, which is a N-type strain, is clearly related and aggregated in the *A. niger* clade.

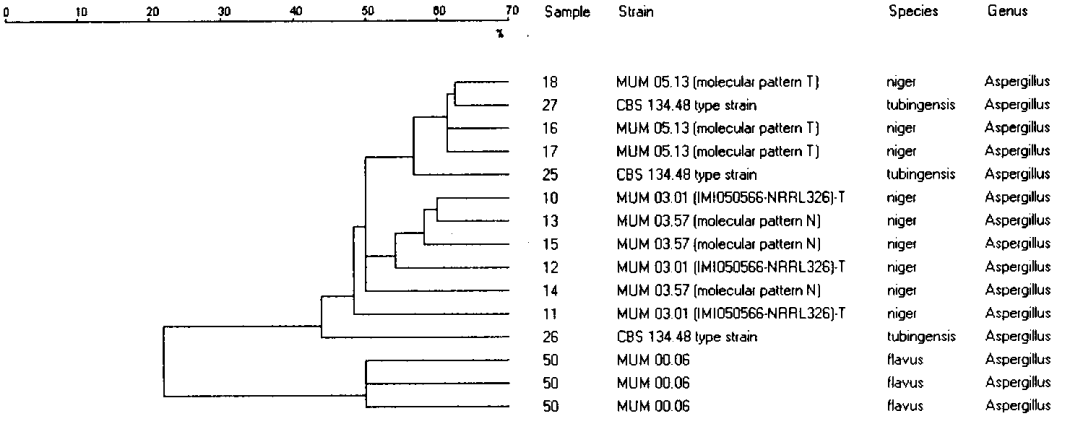


Figure 1 - Dendrogram of relatedness between strains based on MALDI-TOF MS analysis. For each strain 3 independently obtained mass spectra are considered.

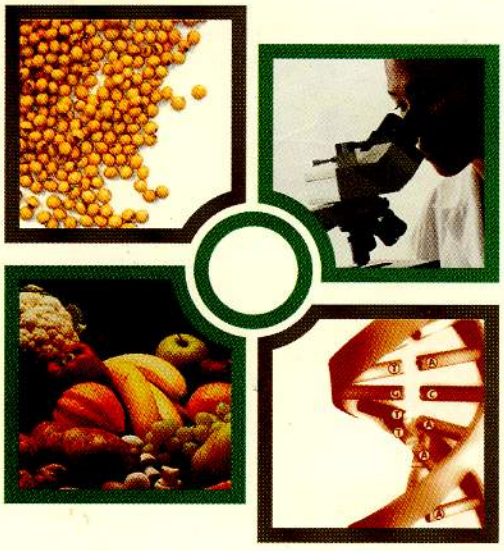
The test strains are closely related to the corresponding *ex*-type strains and only sample 26 of *A. tubingensis* did not group closely. Additionally, *A. niger* MUM 05.13 (T-type) and *A. tubingensis* were not OTA producer. In contrast, *A. niger* MUM 03.57 and MUM 03.01 were OTA producer (unpublished data).

CONCLUSIONS: MALDI-TOF MS analysis shows potential to discriminate these two relevant *taxa* which was feasible otherwise only by molecular (nucleic acid) techniques. Further work will be undertaken in order to increase the number of strains studied and to validate the method.

ACKNOWLEDGMENTS: FACEPE/Brazil for financial support [CS].

REFERENCES

1. Erhard, M.; von Döhren, H.; Jungblut, P. 1997. Nature Biotechnol. 15: 906-909.
2. Kallow, W.; Erhard, M.; Lima, N.; Santos, I.M.; Serra, R.; Venâncio, A.; Friedl, T.; Müller, J.; de Hoog, G.S.; Verkley, G.J.M. 2006. Proceedings of the Annual G. ECCO Meeting, Budapest, NCAIM, pp.: 146-159.
3. Accensi, F.; Cano, J.; Figuera, L.; Abarca, M.L.; Cabañes, F.J. 1999. FEMS Microbiol. Lett. 180: 191-196.
4. Samson, R.A.; Houbraken, J.A.M.P.; Kuijpers, A.F.A.; Frank, J.M.; Frisvad, J. C. 2004. Studies in Mycology 50: 45-61.
5. Samson, R.A.; Noonim, P.; Meijer, M.; Houbraken, J.; Frisvad, J.C.; Varga, J. 2007. Studies in Mycology 59: 129-145.
6. Perrone, G.; Mulè, G.; Susca, A.; Battilani, P.; Pietri, A.; Logrieco, A. 2006. Appl. Environ Microbiol. 72: 680 -685.



I WORKSHOP INTERNACIONAL EM

Biotecnologia

III Encontro III Jornada

ALFA-Valnatura Científica do LIKA

Programação e Resumos

05 A 07

DE MAIO DE 2008

Editoração Científica:

Ana Lúcia Figueiredo Porto
Camila Souza Porto
Carolina de Albuquerque Lima
Cledir Santos
Cynthia de Oliveira Nascimento
Maria das Graças Carneiro da Cunha
Nelson Manuel Viana S. Lima
Norma Buarque de Gusmão

Design Gráfico:

Fernandes Beckman

Diagramação:

Sérgio Siqueira

Revisão:

Comissão organizadora

Impressão:

EDUFPE

Workshop Internacional em Biotecnologia (1.: 2008 : Recife)
I Workshop Internacional em Biotecnologia. III Encontro Alfa-Valnatura, UFPE. III Jornada Científica do LIKA / organizadores Maria das Graças Carneiro da Cunha... et al. – Recife : Ed. Universitária da UFPE, 2008.
435 p.: il., fig., tab.

Inclui bibliografia
ISBN 978-85-7315-484-9 (broch.)

1. Ciências biológicas – Congressos. 2. Biotecnologia – Congressos. 3. Ciências ambientais – Congressos. I. Carneiro da Cunha, Maria das Graças, org. II. Título.

57 CDU (2.ed.) UFPE
660.6 CDD (22.ed.) BC2008-033

Maria das Graças Carneiro da Cunha
Maria Elizabeth Cavalcante Chaves
José António Teixeira
José Luiz de Lima Filho
Norma Buarque de Gusmão
Ana Lúcia Figueiredo Porto
Camila Souza Porto
Carlos Alberto da Silva Júnior
(Organizadores)

I WORKSHOP INTERNACIONAL EM
Biotecnologia

III Encontro ALFA-V Valnatura

III Jornada Científica do LIKA

Editora Universitária UFPE

Recife - 2008