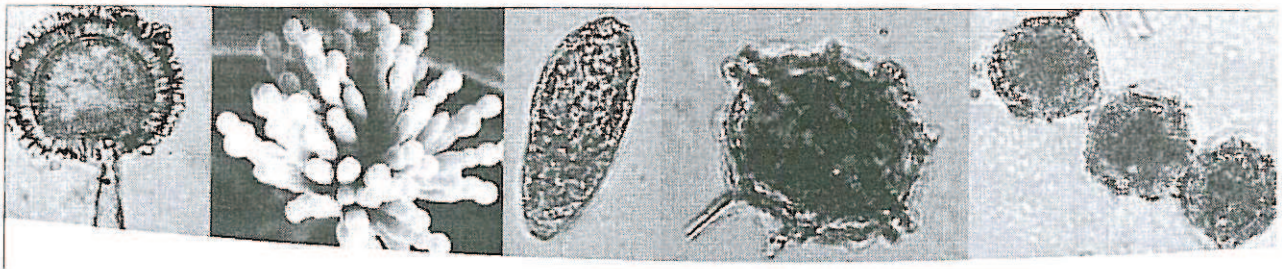

PROCEEDINGS

VI LATIN AMERICAN CONGRESS OF MYCOTOXICOLOGY
and
II INTERNATIONAL SYMPOSIUM
ON ALGAL AND FUNGAL TOXINS FOR INDUSTRY

June 27 to July 1, 2010

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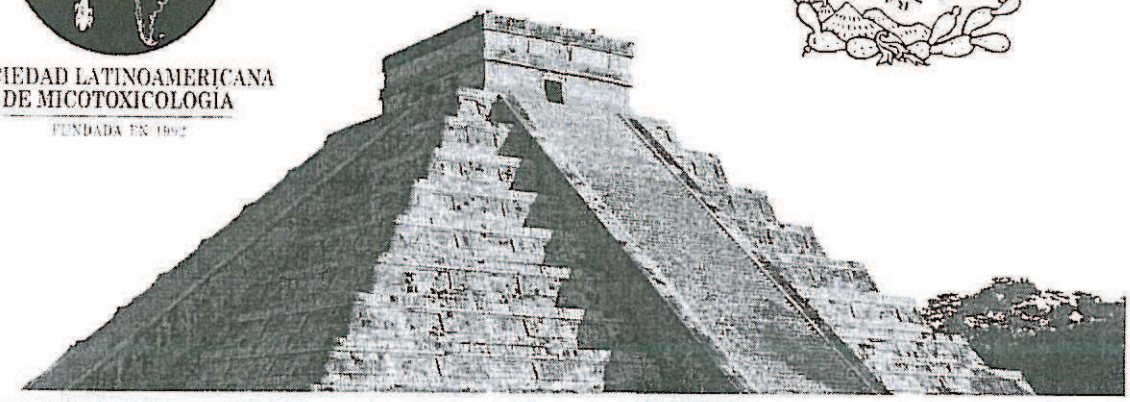

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11:10-11:40 MALDI-TOF ICMS AS A MODERN APPROACH TO IDENTIFY POTENTIAL AFLATOXIGENIC FUNGI

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Background: The *Aspergillus* section *Flavi* is among the best studied fungi, having different commercial applications, but also causing biodeterioration of commodities and food spoilage. Fungi from this Section are also responsible for the production of highly toxic secondary metabolites – the aflatoxins. They are morphologically and genetically very similar, and can be difficult to differentiate by both cultural and molecular biology methods. Besides that, new species are continuously being described in this Section. A reliable identification typically implies the analyses of a variety of morphological, biochemical and molecular traits. Recently, Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight Intact Cell Mass Spectrometry (MALDI-TOF ICMS) has been used to generate spectra of protein masses in a range of 2,000 to 20,000 Da that are a *taxa* specific fingerprinting. This technique has already shown high potentialities to discriminate very closely related *taxa* and, it has been used as a new tool in the polyphasic approach to identify potential aflatoxigenic fungi.

Aim: This work aims to validate the MALDI-TOF ICMS technique on *Aspergillus* Section *Flavi* identification and authentication. As a matter of consequence, obtained results by spectral analysis were compared to those obtained by morphological, biochemical and molecular biology methods.

Materials and Methods: 1. Morphological analysis: fungi were cultured on three different media (Malt Extract Agar [MEA], Czapek Yeast Autolysate [CYA] and CYA supplemented with 20% saccharose [CY20S]); 2. Biochemical analysis: Aflatoxins and Cyclopiazonic Acid analyses were performed by HPLC; 3. Molecular biology analysis: Partial calmodulin gene was sequenced; 4. MALDI-TOF ICMS analysis: spectra of protein masses, on 2,5-dihydroxybenzoic acid (DHB) in a range of 2,000 to 20,000 Da, were obtained using Shimadzu Axima-LNR equipment and treated for fungal identification using SARAMIS™ Package.

Results and Discussion: 1. A good agreement between methods on species level identification was obtained; 2. Molecular biology and spectral data analyses generated similar dendrograms with concomitant strains clustering; 3. Biochemical data analysis generated also a dendrogram which is compared with the previous ones; 4. Under the experimental conditions used spectral analyses were able to identify potential aflatoxigenic species.

Conclusion: MALDI-TOF ICMS has shown a very good resolution on the identification of *Aspergillus* Section *Flavi* species. Results obtained with MALDI-TOF ICMS were similar to those obtained by DNA sequence analysis, with the advantage of being (a) rapid, (b) inexpensive in terms of labour and consumables, and (c) reliable when compared with other biological techniques. Using MALDI-TOF ICMS the results showed a great potential to the fungal identification and it is another additional step for our polyphasic fungal identification approach. However, even with the polyphasic approach fungal identifications remain in some situations time-consuming and decisions regarding what represents a species tend to be subjective.

References:

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Santos C, Paterson RRM, Venâncio A, Lima N, 2010. Journal of Applied Microbiology 108, 375-385.

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