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Calmodulin gene as good voucher as MALDI-TOF ICMS to identify Portuguese isolates of *Aspergillus* section *Flavi*

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Aspergillus is a large genus, with a complex and ever evolving taxonomy. Section *Flavi* is one of the most significant Sections in the Genus. Taxonomy and species identification is subject of great interest for scientists aiming to clarify the species concept and limits within the section. Furthermore, this Section comprises both toxigenic and non-toxigenic species/strains, with great interest to biotechnology and food industry.

Various genes, namely the rRNA (internal transcribed spacer (ITS) and partial LSU-rRNA regions), calmodulin and β-tubulin genes, have been widely reported as good markers for *Aspergillus* species identification, because they are rapid and cost-effective.

In the present study, we evaluated the discriminatory power of the ITS region and the calmodulin gene to distinguish closely related *taxa* within *Aspergillus* Section *Flavi*. For this purpose, 24 isolates of *Aspergillus* Section *Flavi* obtained from Portuguese almonds were characterized at various levels: i) phenotypic, regarding various aspects of morphology (colony diameters and colours on CYA, MEA, CY20S and CYA at 42ºC media, colony reverse colour on AFPA, conidia ornamentation and size, and seriation), physiology (fluorescence on coconut agar medium and production of aflatoxins B_1, B_2, G_1 and G_2 as well as cyclopiazonic acid), and spectral, using Matrix Assisted Laser Desorption/Ionisation Time-Of-Flight Intact Cell Mass Spectrometry (MALDI-TOF ICMS) to obtain protein spectra in the range 2 to 20 KDa; and ii) genotypic, by sequence analysis of a 730 bp segment of the calmodulin gene generated using CL1 and CL2A primers and a 908 bp segment of the ITS region generated using V9D and LS266 primers. For the various methods, dendrograms were created and results were compared.

Both genotypic and spectral analyses divided the isolates in 3 groups, corresponding to *taxa* closely related to *A. flavus*, *A. parasiticus* and *A. tamarii*, respectively. Except for the ITS region, all sets of analysis positioned 5 of the 24 isolates in two unidentified clades close to *A. parasiticus*, and divided the *A. flavus* group in two distinct clades. Furthermore, the calmodulin dendrogram was strongly supported by the phenotypic, including spectral, analyses.

These results confirm that ITS gene was not able to resolve differences at the species-level on this particular taxonomic group. In contrast, the calmodulin gene is a robust and reliable genomic marker for this group of fungi providing good DNA barcoding potential. MALDI-TOF ICMS results confirm that this technique is as good as calmodulin gene analysis for fungal identification. MALDI-TOF ICMS technique is rapid, reliable and inexpensive in terms of labour and consumables when compared with other biological techniques. At present, it adds an additional step for polyphasic identification which is essential when there is a paucity of characters for defining many fungal species. Finally, the problematic strains are now under further study using multilocus sequence typing (MLST) approach, and to perform this 5 additional housekeeping genes were selected to enlarge the analysis.