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Polyphasic identification of *Aspergillus* section *Nigri* preserved under mineral oil at URM Culture Collection

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The species of *Aspergillus* section *Nigri* are isolated from different environments. However, the main habitat of these species is the soil. According Samson *et al.* (2007) there are 19 species of *Aspergillus* section *Nigri* accepted. The species must be delineated based on a polyphasic approach, including morphology, physiology, profile of secondary metabolites and molecular biology (Samson and Varga, 2009). Additionally, according to Santos *et al.* (2010a, 2010b) it is clearer that spectral analyses add value to the polyphasic approach. It generates quality data which are accurate and useful when some of the methods described above presented limitations. Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight Intact Cell Mass Spectrometry (MALDI-TOF ICMS) is a spectral technique that analyses the chemical cellular composition of microorganisms providing rapid and discriminatory fingerprints for identification. The remarkable reproducibility of this technique is based on the measurement of constantly expressed and highly abundant proteins. The usually observable molecular mass range is between 2000 and 20000 Da where important ribosomal proteins appear, which is an advantage because these proteins can be easily used as biomarkers. A polyphasic approach consisting of morphological, biochemical and spectral analyses by MALDI-TOF ICMS was applied to 74 cultures of *Aspergillus* section *Nigri* deposited at University of Recife Mycology (URM) Culture Collection, for the characterisation and identification. Additionally, 12 type strains of *Aspergillus* section *Nigri* deposited at Micoteca da Universidade do Minho (MUM) were used as reference for the MALDI-TOF ICMS studies. For the morphological identification the biological material was grown on both media Czapek Dox Agar (CZ) and Malt Extract Agar (MEA) at 25 °C. Biochemical characterisation of each isolate was performed by HPLC and took into consideration the production of ochratoxin A and fumonisin B2. For the fungal identification by MALDI-TOF ICMS the fungal mycelia were grown for 3 days on solid MEA and then the mycelia were directly transferred from the culture plate to the MALDI stainless steel template and mixed with 0.5 µl MALDI matrix solution (75 mg/ml 2,5-dihydroxybenzoic acid in ethanol/water/acetonitrile [1:1:1] with 0.03% trifluoroacetic acid). The sample mixtures were air dried at room temperature. The analyses were performed at the laboratory of the Micoteca da Universidade do Minho on an Axima LNR system (Kratos Analytical, Shimadzu, Manchester, UK) equipped with a nitrogen laser (337 nm). The mass range was from m/z =

2000 to 20000 Da. *Escherichia coli* strain DH5α with known mass values of ribosomal proteins was used for external calibration. The fungi classification was performed on the SARAMIS software (AnagnosTec mbH, Potsdam-Golm, Germany). The results obtained from the polyphasic approach indicate that overall the MALDI-TOF ICMS results corroborate results obtained with classical morphology and biochemical analyses. In this case, from the 74 cultures of *Aspergillus* section *Nigri* analysed, 75% were finally identified as *Aspergillus niger*, 15% as *A. japonicus*, 5% as *A. carbonarius*, 4% as *A. aculeatus* and 1% as *A. foetidus*. Moreover, the biochemical analyses showed that of the overall population of *A. niger* analysed, 20% were ochratoxin A producers and 13% were fumonisin B2 producers. *A. carbonarius* and *A. foetidus* were 100% ochratoxin A producers. Additionally, with these results the holdings of URM belonging to *Aspergillus* section *Nigri* were deeply studied and requalified.

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