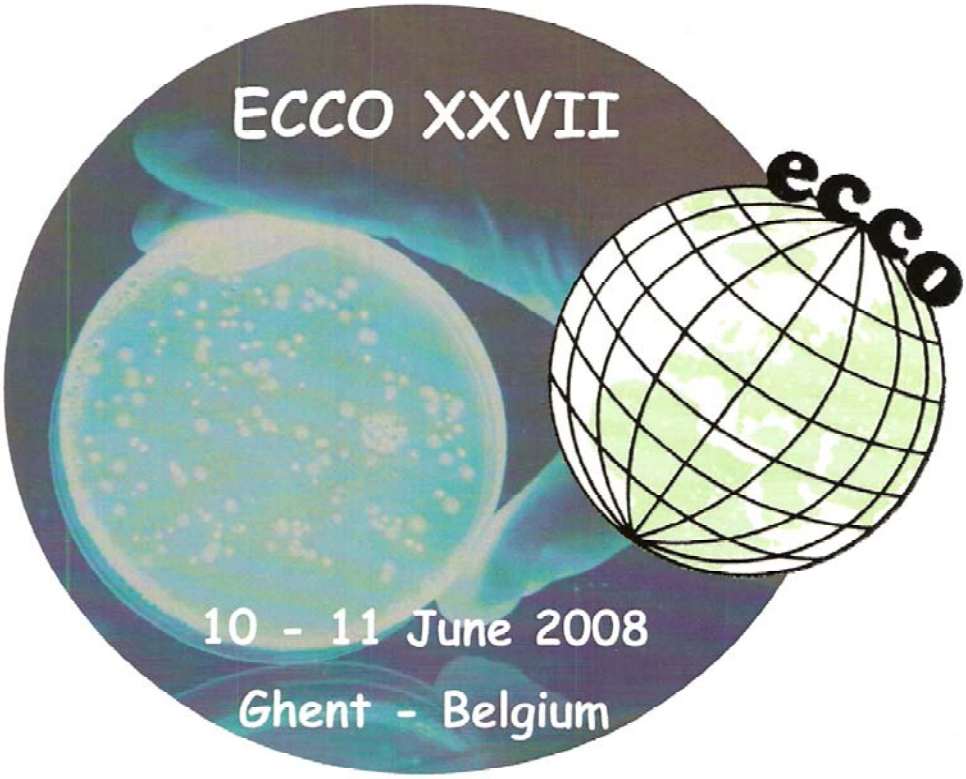
Proceedings of the 27th Annual General Meeting of the European Culture Collections' Organization



HARMONISATION & VALIDATION OF METHODS

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MODERN POLYPHASIC METHODS THAT INCLUDE MALDI-TOF ANALYSES FOR FUNGAL IDENTIFICATIONS AND AUTHENTICATIONS

N. Lima[1,2], P. Rodrigues[1,3], C. Soares[1], C. Santos[4], R.R.M. Paterson[1,5] and A. Venâncio[1]

[1]Micoteca da Universidade do Minho, IBB-Institute for Biotechnology and Bioengineering, Biological Engineering Centre, University of Minho, 4710-057 Braga, Portugal
nelson@iec.uminho.pt
[2]Biochemistry Department, Federal University of Pernambuco, Recife-PE, Brazil
[3]CIMO–Escola Superior Agrária de Bragança, Campus de Santa Apolónia, 5301-855 Bragança, Portugal
[4]Fundamental Chemistry Department, Federal University of Pernambuco, Recife-PE, Brazil
[5]IOI Professorial Chair, Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia

The identification of species is an important goal in taxonomic mycology. Information about each fungus (*e.g.* morphological description, physiological and biochemical properties, ecological roles, and societal risks or benefits) is the key element in this process. Identifications can be a long and seemingly never-ending process with frequent revisions of the taxonomic schemes. These changes make identifications even more complicated for the non specialised researchers as each taxonomic group has specialized literature, terminology and characters. This occurs to the extent that identifications can only be undertaken by a narrow group of scientists especially skilled in the "art", which can make the procedures appear to be subjective.

The species concept is clearly abstract and delimitations are very difficult, and often not consensual. Taking this into account, fungal taxonomy, and their associate data can often be best applied at the moment where the data are used for a specific purpose: A pragmatic definition is "data fit for use". Moreover, databases have no actual value (or even quality); they only have potential value. Data have actual value when it is used to do something useful.

It is gradually becoming clearer that fungal identifications and authentication require a polyphasic approach to generate quality data that are accurate and useful. In reality this means that it is necessary to combine the more traditional phenotypic and physiological approaches with modern molecular biology. Restriction fragment length polymorphism (RFPLs), random amplification of polymorphic DNA (RAPDs), amplified fragment length polymorphism-PCR (AFLPs-PCR), and DNA fingerprinting have all been used to distinguish fungal *taxa* that are difficult to characterize by traditional morphological means. On the other hand, identical ITS sequences have been obtained for morphologically distinguishable species (*e.g.* the mushroom genus *Armillaria*). *A. gallica* is a circumboreal, largely saprophytic species occurring in a variety of hardwood forest types, but particularly is common in oak forest. In the birch-beech-maple forest type of North-Eastern North America, a sister species of limited distribution, *A. calvescens*, replaced *A. gallica*. These two species differ in ecology and morphology but intergenic spacer region (IGS) sequences do not separate them adequately. Incongruences between molecular markers (ITS and IGS) and phenotypic markers have been noted in the fungi although it is often assumed that the

molecular characters are the most discriminating. We assume that the genotype of the species is only an indirect indication of phenotype and ecological adaptation because we work with fungal species definitions, which incorporate the phylogenetic species concepts of population, lineage, and phenotype. In other words, fungal species are the smallest aggregation of population with a common lineage that share unique diagnosable phenotypic characters.

Recently, microbial mass spectral analysis has been employed for phenotype typing. Matrix Assisted Laser Desorption Ionization Time Of Flight (MALDI-TOF) mass spectroscopy microbial analysis uses a nitrogen laser to irradiated freeze dried fungal mycelia mixed with a matrix (an aromatic compound such as 2,5-dihydroxy benzoic acid acidified with trifluoroacetic acid) that gently ionizes the cellular proteic components. The spectrum of protein masses in a range of 2000 to 20000 Da are used as *taxon* specific fingerprints, after archiving in a database. The advantages of this novel approach as a microbial identification and authentication method are the (a) simple sample preparation procedure, (b) short time for analysis and (c) reliability of the data. In the present study, results from yeast and filamentous fungi using different modern methods and with a polyphasic philosophy will be presented.

P. Rodrigues and C. Soares acknowledge the FCT Portuguese grants SFRH/BD/28332/2006 and SFRH/BD/37264/2007, respectively. C. Santos acknowledges the financial support of Facepe/CNPq, Brazil.