MALDI-TOF ICMS as a powerful technique for strain authentications in Culture Collections

Cledir Santos and Nelson Lima

IBB - Centro de Engenharia Biológica Micoteca da Universidade do Minho (MUM) Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal <u>cledir.santos@deb.uminho.pt; nelson@ie.uminho.pt</u>.

The identification of species is an important goal in taxonomic microbiology. Information about each microorganism (e.g. morphological and molecular descriptions, physiological and biochemical properties, ecological roles, and societal risks or benefits) is the key element in this process. Identifications can been a long and seemingly never-ended 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. It is also the ultimate goal for microbial culture collections (CC) quality control (QC) systems. This will enable CC to pass this knowledge on to their customers and as a result, improve the guidance and good services that they provide. On one hand, great emphasis is placed on this and with it CC demonstrate to users of their strains that they are delivering the quality that they promise and advertise. On the other hand, the users benefit of quality conformity and authenticity of biological material, and access to the related processes and procedures. These key elements underpin the changes to the paradigm which the traditional CC have undergone in relation to the new one of certificated/accredited biological resources centres (BRCs). OECD best practices guidelines for BRCs emphasise the importance of QC for the biological material. Each strain that arrives at a CC undergoes a battery of morphological, physiological and molecular tests for characterisation. These characteristics are comparing, according of the state-of-art of each taxonomic group, with the description of the species in order to determine the identification of the strains as an authentication process. The two approaches form the basis for the operation of the BRCs when linked to the conformity criteria and documentation specifying the quality management system. To perform a reliable strain authentication CC normally follow the polyphasic approach. This means that they combine the more traditional phenotypic and physiological approaches with modern techniques (e.g. molecular biology). Recently, microbial mass spectral analysis has been employed for microbial phenotype typing. Matrix Assisted Laser Desorption Ionisation – Time Of Flight Intact Cell Mass Spectrometry (MALDI-TOF ICMS) is a modern approach used for the spectral typing of the proteins contents in the microbial cells. MALDI-TOF ICMS technique uses a nitrogen laser to irradiated fresh microbiological cells mixed with a matrix (an aromatic compound such as 2,5dihydroxy benzoic acid acidified with trifluoroacetic acid) that gentle ionizes the above mentioned cellular proteic components. The spectra of protein masses in a range of 2 to 20 kDa 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) low cost of consumable; (b) simple sample preparation procedure, (c) short time for analysis and (d) reliability of the data. This technique has the potential to be used as part of quality control process in culture collections. The present work will report attempts to implement this technique in a CC and integrate these data into QC management system.

Acknowledgements: Research leading to these results received funding from the European Community's Seventh Framework Program (FP7, 2007-2013), Research Infrastructures Action, under grant agreement No. FP7-228310 (EMbaRC project).