MALDI-TOF ICMS FOR BACTERIAL CLASSIFICATION AND IDENTIFICATION: HOW MANY CELLS ARE NEEDED?

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The identification of species is fundamental to taxonomy. In 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 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 specialised literature, terminology and characters. Analysis of microbial cells for their identification using Matrix Assisted Laser Desorption Ionisation – Time Of Flight Mass Spectrometry (MALDI-TOF MS) is a modern approach for these studies. MALDI-TOF MS has contributed to increasing scientific knowledge about the microorganisms and is now used as a reliable tool for rapid tests in hospitals and health centres. Taking into account MALDI-TOF MS is a sound technique for microbial classification/identification and authentication, it follows that it can be used for quality control programmes of culture collections. However, it is necessary to employ the correct number of cells per sample in the each analysis, as it is a critical issue that requires to be clarified. The main objective of this work was the establishment of the minimum number of cells of *Escherichia coli* strain DH5α required for identifications by MALDI-TOF MS. *E. coli* strain DH5α was grown in liquid Luria Bertani (LB) medium at 37°C with shaking for 20 h. The cells were centrifuged and washed with deionised water and subjected to dilution and counted with a Neubauer chamber. Final aqueous bacterial suspensions containing from ca. 10^3 up to 10^9 cells per sample were analysed by MALDI-TOF MS using two different matrices, namely 2,5-dihydroxybenzoic acid (DHB) and α-Cyano-4-hydroxycinnamic acid (CHCA). Spectra obtained were analysed by SARAMIS™ Software and final results indicated that for DHB and CHCA the optimal cell concentrations were 10^7 and 10^6 cells per MALDI sample, respectively.

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