Microbial Single Cell Detection with Raman Spectroscopy: Taxonomic Resolution and Data Accuracy

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Micro-spectroscopy by means of FTIR or Raman gives very promising perspectives in microbiology among which the possibility to analyze a sample without prior growth [1-3]. This condition offers the advantages of:

(i) shorter analytical times, due to the lack of the 24-48 h necessary for growth. This aspect can be crucial when the species identification is part of diagnostic process, normally requiring short times.

(ii) possibility of detecting VNC (viable non culturable) microorganisms that grow in the natural condition, but not in laboratory media.

(iii) unbiased estimation of the alpha-diversity, which can be significantly altered during the growth due to the different growing rates of the various strains and species.

However, this technique is challenged by the difficulty of obtaining reliable spectra from single cells, due to their inherent variability. Moreover, eukaryotic cells, as fungi, algae and protozoa have the additional inconvenience of undergoing a four phase life cycle, meaning that the metabolome of the same cell in each phase can be significantly different.

In order to define the level of variability among different measures of the same cells, with a Raman microspectroscopy apparatus, we developed a simple taxonomic model consisting of four certified strains belonging to four pathogenic species*Candida albicans, C. tropicalis, C. parapsilosis*and *C. glabrata*. Cells where grown with and without shaking and spectrawere recorded in both polarized and non-polarized configuration. Twelve independent readings for each combination of strain and experimental condition were carried out. Two different analytical approaches were employed, one considering the distance of the spectra from the most representative (central) spectrum of the set and one calculating the distance of the spectra from the average spectrum. All analyses were performed for the whole spectrum and for the different spectral regions separately.

Results showed that indeed a great level of variability can be detected among the spectra of the same strain and that sometimes this variability is comparable with the distance between cells of different species. This condition reduces the taxonomic resolution (i.e. the possibility to discriminate between species) and requires a number of countermeasures that will be presented and discussed.

References

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