Investigating Colony Heterogeneity by Raman Spectroscopy

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The application of confocal Raman microspectroscopy for the rapid identification of clinically relevant microorganisms is a new approach currently under development. This method involves measuring Raman spectra directly from microcolonies (cultured for as little as 6 hours) still growing on the culture plate. An important aspect of this method is the non-subjective identification of microorganisms through the use of multivariate statistical analyses such as hierarchical cluster and linear discriminant analyses. These methods rely on having spectral libraries/databases in order to compare unknown spectra to arrive at an identification. Therefore, it is critical that reproducible representative spectra are contained within the database. Culture conditions and spectrometer acquisition parameters need to be rigidly standardized so that variation in growth conditions, medium composition or spectral quality do not influence the identification results. Furthermore the database spectra should be comprehensive such that it captures any intrinisic biological variance of the microorganisms.

In order to investigate the biological heterogeneity of microorganism growth, Raman measurements were acquired from various positions and different depths within (micro)colonies cultured for 6 h, 12 h and 24 h. The studies reveal that there is little spatial heterogeneity in the molecular composition of 6 h microcolonies. In contrast, the 12 h and 24 h cultures exhibited a significant amount of heterogeneity. Hierarchical cluster analysis of the spectra from the various positions and depths reveals the presence of different layers in the colonies. Further analysis indicates that spectra acquired from the surface of the colonies exhibit higher levels of glycogen when compared to the deeper layers of the colony. Additionally, the spectra from the deeper layers present with higher RNA levels than the surface layers. Therefore, the 6 h colonies with their limited heterogeneity are more suitable for inclusion in a spectral database. These results also demonstrate that confocal Raman microspectroscopy can be a useful tool for studying the physiology and biochemistry of colony development. Such studies can provide insight into understanding the nature of biofilm formation and structure.