Bacteria identification by infrared imaging spectroscopy

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Fourier transform infrared (FTIR) spectroscopy has been demonstrated within the past two decades to provide a rapid means for the identification of bacteria at the genus, species, and serotype/strain levels. In our research, the spectra of bacterial films are acquired by employing an infrared imaging spectrometer equipped with an IR microscope and a 32×32 focal-plane-array (FPA) MCT detector. While infrared imaging is normally employed for the purpose of obtaining spatially resolved information about the composition of samples that exhibit heterogeneity on the micron scale, its utility in the present context derives from the simultaneous acquisition of thousands of spectra with a nominal spatial resolution of 5.6 μm. The resulting capability of analyzing multiple samples simultaneously, thereby increasing the sample throughput of FTIR microbial analysis, is not readily exploited except with the use of microarray deposition technology, which is costly at the present time. However, infrared imaging also offers a less evident but more immediate benefit. Our examination of infrared images of bacterial films deposited on an IR optical window revealed variations in (i) the distribution of the cells on the window, (ii) the thickness of the bacterial film, and (iii) the moisture content of the film within the microscope's field of view (~200 μm^2). The extent of this inhomogeneity was found to vary from species to species, owing to inherent differences in surface adhesion properties. In principle, filtering of the 1024 spatially resolved spectra constituting the image on the basis of spectral quality and similarity criteria provides a means of compensating for this inhomogeneity, and the spectra that are retained after filtering may then be co-added to increase the signal-to-noise ratio (SNR), yielding a spectrum virtually equivalent to the ideal spectrum of a perfectly homogeneous sample. The validity of this approach is dependent on a high degree of pixel-to-pixel reproducibility, and its practical utility is also dependent on sufficient instrument-to-instrument image reproducibility to allow for database transferability. We verified that these requirements could be met by developing "whole-spectrum" PLS calibrations for quantitative analysis of multicomponent solutions and assessing their pixel-to-pixel and instrument-to-instrument transferability. Furthermore, we found that satisfactory SNR for bacteria identification was attained by co-addition of only ~50-100 of the 1024 spectra constituting the image of a sample. Accordingly, 8-15 sets of co-added spectra could be generated from the image of each sample and a confidence level was assigned to the identification of the sample on the basis of the concurrence of the results for these sets, with a low confidence level possibly indicating a mixed culture. In this manner, infrared imaging spectroscopy has been shown to afford a means of increasing the reliability of FTIR bacteria identification and is currently being employed to develop a high-quality spectral database and methodology for the routine identification of foodborne pathogens.