Raman spectroscopic analysis of microorganisms, finding the limits of sensitivity

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In optimising Raman spectroscopy as a diagnostic tool for medical microbiology, we are evaluating how different culturing methods influence the accuracy of our analysis. For instance, while analysing abdominal infections from intensive care patients, we noticed that infections with mixed *Candida* species had a higher incidence than expected. Due to the high similarity in colony morphology of the species involved, recognising these mixed cultures was sometime a problem. Choosing chromatic culture media, on which the colonies of different species have different colors, helped in the initial detection of mixed *Candida* cultures. However, a clear distinction is not always present between all species, therefore additional methods are often used to confirm this presumptive identification. Preliminary data shows that Raman spectra could be obtained, directly from suspected colonies on the chromatic medium, with enough differences between the species.

The choice of the right culture medium is one of the oldest tricks in the microbiology book. Additionally, is also well known that varying culture conditions can influence the outcome of commercially available identification methods. Because varying culture conditions lead to variance in the molecular composition of microbial cells, we were interested to see how this influenced the identification of *Bacillus* species by Raman spectroscopy. A collection of 30 strains from 3 *Bacillus* species was cultured under various conditions (3 media, 2 temperatures, 2 culture times per strain). In spite of this variation, it was still possible to accurately identify the strains up to 92% at species level. While these extreme variations will not often occur in a diagnostic setting, due to standardised operating procedures, it did show that Raman-based identification is not as sensitive to culture conditions as one would think.

Finally, a brief introduction will be given to recent studies that were aimed at strain-level identification. Our preliminary results on *Acinetobacter* species clearly show that classification based on the Raman spectra, is similar to those achieved with elaborate molecular genetic methods.