Automated FT-IR Microspectroscopy on Microbial Microcolonies

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Infrared spectra of intact microbial cells are fingerprint-like signatures which provide multi-dimensional information on cell composition and structure. These spectral signatures are already used in practice to identify divers microbial species and strains, to characterize cell compounds *in situ*, and to monitor cell-drug interactions [1]. However, the infrared spectra currently used are based on microorganisms cultured for 24 h or longer prior to the measurement. Our interest was focused on the development of new rapid, and easy to use characterization and identification techniques for microbial pathogens, which are requested as important decision factors in clinical microbiology and therapy with respect to the increasing prevalence of infectious diseases. Infectious diseases are a particularly serious health care problem due to the continuous development and spread of bacterial resistance to antimicrobial drugs over the past decades. We report on a FT-IR microscopic technique which allows the rapid differentiation of clinically relevant microorganisms, potentially providing diagnostic results within one working day [2]. The FT-IR microscope combines a light microscope with a FT-IR spectrometer thus providing a versatile instrument for rapid biological microanalysis. High quality IR-spectra can be obtained from microcolonies containing a few hundred cells after only 6 to 8 hours of culturing on agar plates (depending on the type of organisms) by a special stamping device. With this stamping technique it is possible to transfer spatially accurate microcolonies growing on solid culture plates to a special IR-transparent sample holder. Using a computer controlled x,y stage together with spectral mapping and video techniques, detection, enumeration, and differentiation of microorganisms are integrated in one single apparatus.
The high differentiation capacity of the microspectroscopic approach will be illustrated on a selection of clinically important *Staphylococcus* species and strains. An important additional advantage of the technique is the investigation of mixed cultures. This will be exemplary demonstrated on the analysis of a mixed *Candida* culture which could be discriminated into three distinct clusters corresponding to three different strains. The unknown isolates could be then identified with an existing classification scheme based on microcolony spectra of a representative number of different *Candida* species and strains. Another interesting item of the new approach is to rapidly test microorganisms for their antibiotic susceptibility behaviour based on the investigation of microcolonies grown in the presence of an antibiotic agent on the agar plates. The influence of a typical β-lactam on the cell growth of sensitive and resistant strains will be presented.

References:

1. Naumann, D., Helm, D., Labischinski, H., Nature, 351 (1991) 81-82.
2. N.A. Ngo Thi, C. Kirschner and D. Naumann, 'FT-IR Microspectrometry: A new tool for characterizing micro-organisms' in: A. Mahadevan-Jansen, G.J. Puppels (eds.) Biomedical spectroscopy: Vibrational spectroscopy and other novel techniques, Proceedings of SPIE vol. 3918, pp 36-44, Bellingham, Washington, (2000).