RAMAN SPECTROSCOPIC ANALYSIS OF MICROORGANISMS ON A SINGLE CELL LEVEL

P. Rösch[1], K.-D. Peschke[2], O. Ronneberger[2], H. Burkhardt[2] and J. Popp[1*]

[1]Institut für Physikalische Chemie, Friedrich-Schiller-Universität Jena, Helmholtzweg 4, D-07743 Jena, Germany, E-Mail: juergen.popp@uni-jena.de
[2]Lehrstuhl für Mustererkennung und Bildverarbeitung, Institut für Informatik, Albert-Ludwigs-Universität Freiburg, Georges-Koehler-Allee Geb. 052, 79110 Freiburg, Germany

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Abstract: This contribution reports on Raman spectroscopic characterisation of bacteria and yeast on a single cell level. The cells were measured under different cultivation condition and Raman mappings over single bacteria and endospores were performed. The identification was done using a support vector machine.

Microbial contamination is not only a medical problem, but also plays a large role in pharmaceutical production and food processing technology. For all these fields a fast and non-ambiguous identification of pathogenic and non-pathogenic microorganisms is required. Most of the hitherto applied identification methods are based on the microorganism's ability to grow in various media or the fermentation of different substances. These tests however require not only a certain amount of cells but also an axenic culture, which makes several isolation and breeding steps necessary. All these steps together require up to 24 h. In order to shorten the identification time new analytical methods need to be developed. The analysis of microorganisms by means of vibrational spectroscopic techniques (IR and Raman spectroscopy) has a long tradition, since the vibrational spectrum displays a fingerprint of the chemical composition of each bacterium.

IR and Raman spectroscopy yields good results for the identification of bacteria and yeast by using micro colonies (several hundreds of cell) of axenic cultures.[1,2] However for an online monitoring of clean room production the situation varies from normal environmental conditions. Here only a few particles and within them only a limited amount of bacteria or yeast species can be found. For this application micro Raman spectroscopy with a spatial resolution in the sub micrometer range is most suitable because here single bacterial cells can be investigated. [3]

For the investigation of bacterial contamination in clean rooms no information about the origin of the microorganisms are known. Therefore a huge dataset was established considering different culture conditions such as nutrition, age, light, O_2/CO_2 concentration and temperature. In addition to these variations also the different cell types (vegetative cells and endospores) were investigated. In order to test the heterogeneity of the cell types various 2D and 3D Raman mappings of vegetative cells and endospores have been performed.

Additionally to the above mentioned variations there are some particular changes when going from bulk material to single bacteria investigation. Especially coloured bacteria show a totally different behaviour when measured in micro colonies or as single cells. Most of the pigments belong to the carotenoids and this substance is photo bleached in single cells. In Fig. 1 Raman spectra of a single bacterial cell of *Micrococcus luteus* DSM 348 are shown. The inset shows a microphotograph of single *M. luteus* bacteria. The cell from which the Raman spectra have been taken off is marked by a circle. Spectrum A shows the first Raman spectrum of the cell whereas spectrum B was recorded right afterwards on the same bacteria. As can be seen spectrum A shows mainly signals of sarcinaxanthin the carotenoid of *M. luteus* (marked with asterisk). Carotenoids are characteristic for various microorganisms but this pigments varies in intensity and are very common throughout the coloured bacteria. So a carotenoid signal is not sufficient for a distinct identification.

Spectrum B shows photo bleaching of the carotenoid signal. Here the signals of the cell matrix are more pronounced. This behaviour is highly reproducible and can therefore be applied as additional information for the identification of microorganisms.

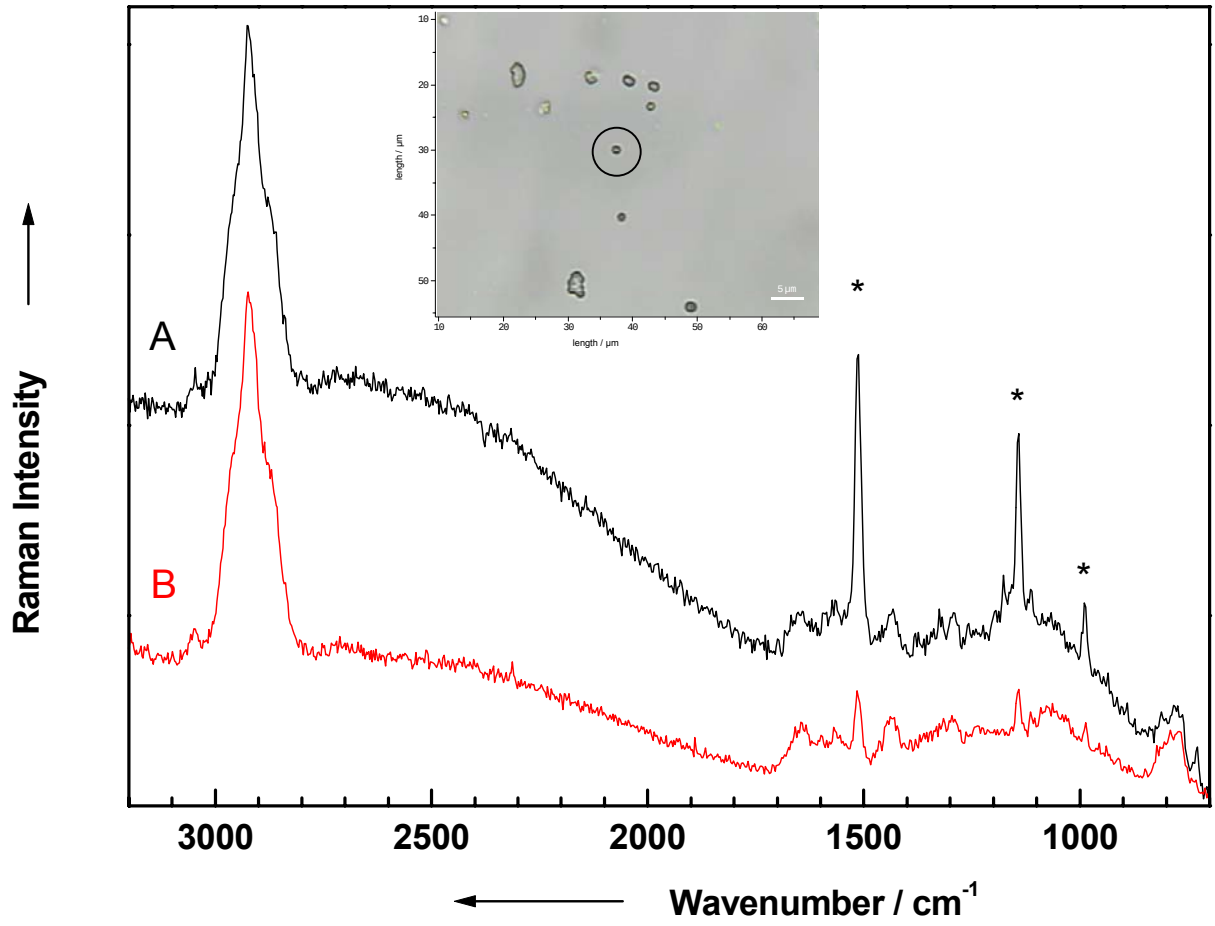

Fig. 1. Micro Raman spectra of a single *Micrococcus luteus* DSM 348 cell recorded after irradiating the single *M. luteus* cell for 60 (A) and 120 s (B). The sarcinaxanthin signal is marked with asterisk. Inset: Microphotograph of various single *M. luteus* bacteria. The cell from which the Raman spectra have been taken off is marked by a circle.

With this knowledge the identification of the single bacteria was done with a support vector machine yielding an overall recognition rate of 86.7 % on strain level and 88.6% on species level.

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