UV-RAMAN STUDIES ON SACCHAROMYCES CEREVISIAE

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***Keywords**: UV Resonance Raman spectroscopy, yeast, photo degradation*

Abstract: Rotation of the sample under the laser beam is a well-known method to prevent sample degradation by the laser light. We have applied UV micro Raman spectroscopy on the model eukaryote *S. cerevisiae* (bakers/brewers yeast) in a systematic approach to assess the maximum laser power applicable on microorganisms in order to record reproducible Raman spectra with minimal signal contributions from UV-damaged cells.

The well-studied model eukaryote *S. cerevisiae* was the first eukaryote to have its genome entirely sequenced. Widely used as an agent of fermentation, in beverage production and as a food supplement it is commonly known as bakers yeast or brewers yeast. Yeasts are amongst the most widely used microorganisms in industry, where large scale fermentation processes permanently require rapid and reliable quality controls.

Yeast cells are larger than bacterial cells and can be readily distinguished from bacteria under the microscope by the size and by the particular internal cell structures. The cells are thick-walled with an oval shaped size, around 10 μm long by 5 μm wide [1]. Ease of handling and the availability make this microorganism a suitable candidate for spectroscopic studies in UV resonance Raman experiments.

In UVRR spectroscopy on microorganisms the exciting laser line lies under the electronic absorption band of the chromophores. Raman scattering thus gets strongly enhanced and provides characteristic selectively excited resonance Raman bands that may be used as a fingerprint for the identification of different yeast cells [2]. Spectra are free of fluorescence und provide information rich spectral features for rapid and accurate identification.

We have applied UV Raman spectroscopy on yeast cells using either active dry yeast (‘Bavarian bottom fermenting’, Arauner/Kitzingen) or compressed yeast cakes, which are commercially available in food or drug stores. Deep UV laser light (229, 244, 257 nm) was obtained from the frequency doubled lines of an argon ion laser (Innova300C, Coherent) generated by second harmonic generation via BBO crystals. The Raman scattered light was analysed by a micro Raman spectrometer (HR800, Jobin Yvon/Horiba) equipped with a cryogenically cooled CCD camera and a UV/VIS sensitive video camera for visual observation of the samples. A broadband anti-reflection coated, UV micro spot objective (LMU UVB, 40x/0.50, WD 1 mm, OFR) and a deep UV 5x objective (LMU UVB, 5x/0.13, WD 35 mm, OFR) were employed to focus the laser light on the sample and to collect 180° backscattered light. Recording of reproducible spectra was achieved within less than 4 min total measurement time using ~ 4 mW or less of laser power on the sample.

Aqueous solutions of the samples were circulated through the laser beam within a closed loop consisting of a peristaltic pump and a flat quartz capillary. The flow rate through the quartz capillary was varied systematically in steps from 1 – 10 ml/min in order to study the peak power of the excitation source applicable on biological samples. Additional experiments were performed on a liquid nitrogen cooled stage (Linkam Scientific Instr., LTS350) to prevent thermal damage of the samples during extended exposure to the focused laser light (focus diameter: 3 μm).

The UVRR spectra exhibit strongly enhanced, characteristic bands that allow for the discrimination of the different microorganisms (Fig. 1). Main spectral features were obtained at room temperature from the 1613 and 1476 cm^{-1} bands that can be assigned to the contributions from aromatic amino acids and from nucleic acids (Guanine, Cytosine), respectively. Note the sharp Raman bands indicated by the dashed lines in Fig. 1, which are not contributed from the samples, but originate from the vibrational modes of nitrogen (2323 cm^{-1}) and of oxygen (1555 cm-^{1}) from the ambient air in the laboratory. Inspection of the varying Raman band intensities observed on fresh bakers yeast, after 4 and 7 days revealed that UVRR is extremely sensitive to changes in the amount of nucleic acid in the microorganisms.

It is noteworthy that the contributions from UV damaged yeast cells in the Raman spectra is considerably reduced by arbitrarily moving the samples under the UV light. We will report on first UVRR experiments where the microscope objective is agitated via a piezo-controlled device describing Lissajoux figures on the sample. The new setup allows for the accumulation of Raman scattered light and simultaneously reduction of sample damage caused by the UV light. The UVRR experiments are designed to provide further insights into the laser power needed to investigate sample volumes containing a small number of cells.

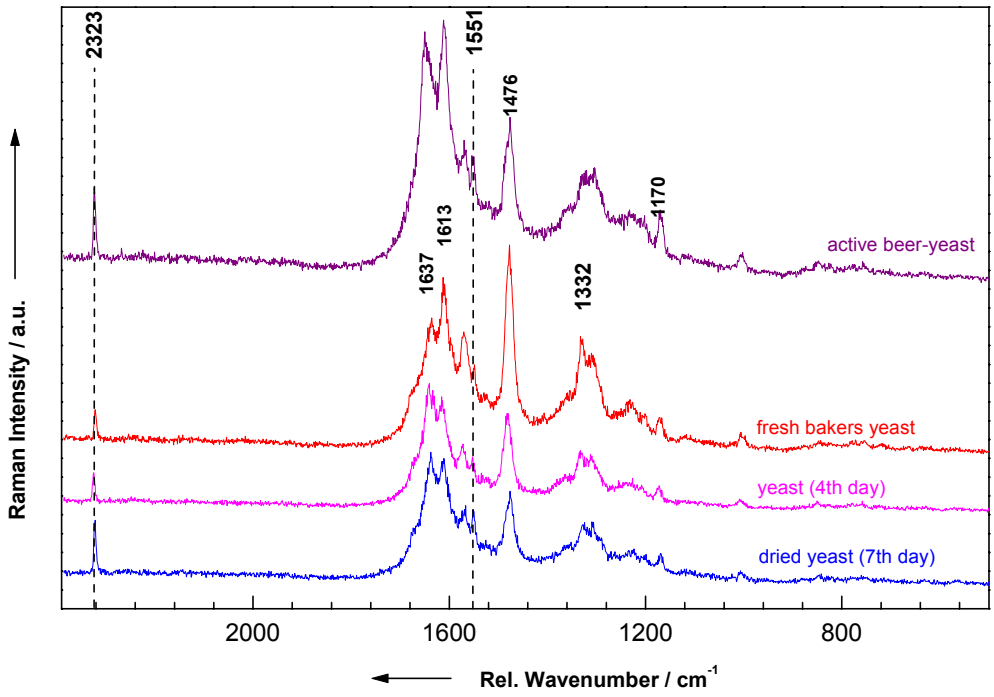

Fig. 1. Representative UVRR spectra of brewers yeast (top) and bakers yeast (bottom), excited at 244 nm. The band intensities at 1476 cm^{-1} (nucleic acids) were signify-cantly reduced on aged (4 and 7 days) bakers yeast samples. All spectra are presented as obtained and displaced vertically on the intensity axis.

Acknowledgements:
The authors are grateful for financial assistance of this project by the Federal Ministry of Education and Research, Germany (BMBF) within the framework 'Biophotonik' (13N8369).

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