Hyperspectral imaging of hyphae and spores of *P. chrysogenum* using confocal Raman spectroscopy and SERS

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**INTRODUCTION**

*Penicillium chrysogenum* is a filamentous fungus very well known for its capability to produce penicillin. In order to improve a fermentation process, detailed knowledge of the biochemical processes as well as the physiological state of the fungus during fermentation are essential and strongly linked to cell morphology. Therefore, the hyphae which are present as pellets in the bioreactor were measured with the confocal Raman imaging method in order to investigate any chemical differences indicating the physiological state of different parts of the pellet. The spectral data was analyzed applying non-supervised classification algorithms. Spores of *P. chrysogenum* which were also investigated do not only exhibit high autofluorescence masking the weak Raman signal, they are also very thermolabile. Thus, three different lasers (532 nm, 633 nm, 785 nm) were used to overcome carbonization of the sample during measurement. Finally, first spore spectra were generated with the SERS technique using silver nanoparticles. Furthermore, spore spectra were obtained with the 785 nm Raman laser which enabled us to differentiate between living and dead spores in a PCA score plot.

Three different Raman spectrometer were used for performing Raman measurements: Horiba Jobin-Yvon LabRam 800HR (Vienna University of Technology), Renishaw inVia Raman Microscope (Carnibian Tech Research, Villach), Thermo Scientific DXR Raman Microscope (testing device provided by Thermo Fisher Scientific).

*P. chrysogenum* HYPHAE

Raman measurements performed with two different lasers (532 nm, 633 nm) clearly indicate the 532 nm Raman laser provides the optimal excitation wavelength for the hyphae of *P. chrysogenum*.

Band assignments according to [1].

- 532 nm laser
- 10 x 2s acquisition time
- 0.01% laser power

**Problem solving**

Washed spore samples were placed onto a microscopic glass slide and dried at room temperature. Raman measurements were performed using the green (532 nm) and red (633 nm) laserline applying 0.01- 1 % laser power each. No Raman signal could be collected using a laser power below 1 %. However, graphite bands recorded with 1 % laser power indicate carbonization of the biological sample due to thermolability.

**Hyperspectral imaging**

Raman mapping was performed covering a sample area of 25x38 µm². Different groups of biological components with their characteristic bands could be detected. Molecule specific information such as the CH str. vibration is visualized in an intensity map.

**Hyperspectral imaging**

PCA (principal component analysis) and HCA (hierarchical cluster analysis) was applied to the data set in order to extract additional chemical information using ImageLab software (© Epina Software Labs).

**Dead / alive study**

Sample preparation according to [4].

- 50x objective
- 0.1% laser power

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