Microscopy-based Raman Spectroscopy of Fungal Melanins in a Genetically Amenable Ascomycete

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Fungal melanins are distinctive markers of animal (including human) and plant pathogenic fungi as well as their environmental relatives. These complex polyphenols play important roles in pathogenicity and stress tolerance while being essential components of fungal cell walls and useful biomarkers. Accordingly, it is important to clarify melanin function in black yeasts, a group of clinical and environmental importance [1].

Here we report signatures of melanins and carotenoids in single and double knockout strains of the model environmental black yeast *Knufia petricola* A95 [2]. This genetically amenable strain is an ancestor of many plant and animal pathogenic fungi. Knock-out mutants of protective pigment genes Kp*PKS* (polyketide synthase), Kp*SDH* (scytalone dehydratase) and Kp*PDG* (phytoene desaturase) were studied using Raman spectroscopy.

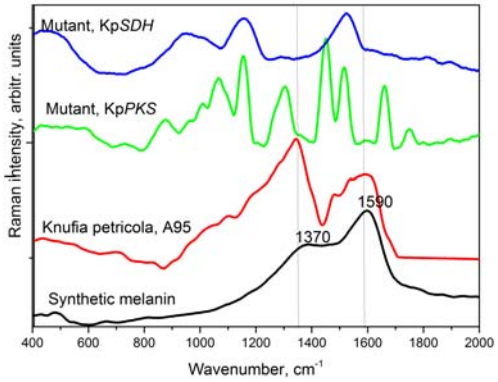

Figure 1. Raman spectra of synthetic melanin, wild type strain *Knufia petricola* A95 and its mutants

Mutants allow discrimination between the various pigments and elucidation of melanin structure. Hence interactions between natural fungal melanins (as well as other protective pigments) and complex environmental/material matrices can be characterised on a range of spatial and temporal scales. A library of Raman spectra of natural fungal melanins will be created to serve as an exploration tool to detect and study pathogenic and environmental fungi in clinical samples and on material surfaces, especially in extreme environments.

We used an NTEGRA Spectra system from NT-MDT (Zelenograd, Moscow 124460, Russia) that combines atomic force microscopy (AFM) and Raman microscopy. The system is equipped with an inverted optical microscope, an upright optical microscope and laser beams of 532, 633 and 785 nm wavelengths. Individual Raman spectra were obtained for reference substances, mutant strains and the wild-type strain. In addition, we applied tip- and surface-enhanced techniques [3] to collect Raman spectra.

References

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