

Scientific Report of COST STSM Reference Number: COST-STSM-ECOST-STSM-FA1103-011112-023160

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STSM Topic: Antibiotic secondary metabolites of Capnodiales (Ascomycota)

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Place: Braunschweig (Germany)

1. Purpose of the STSM

The Capnodiales is rich in so called fruit colonizing sooty blotch and fly speck fungi (SBFSF). Initially assumed to present a complex of only 5 species, DNA barcode based species typing revealed that the species diversity of SBFSF was underestimated by more than ten-fold and several genera and species have been described only recently. The life cycle of many of them is not yet fully understood and little is known about their bioactive secondary metabolites (SM) and the functions of these compounds in the ecology of SBFSF although they do not seem to be specifically plant pathogenic. Because they interfere with each other on the plant surface, for example, by forming demarcation lines, our hypothesis is that some of the SBFS fungi can interfere with and possibly inhibit other fungi, for example, plant pathogens, or that they form interesting antibiotic secondary metabolites. Some of their potentially beneficial compounds could have novel biological activities.

The inhibitory effect of selected SBFS strains on plant pathogenic fungal strains were tested in dual culture assays at Agricultural institute of Slovenia. Within all potential antagonistic fungal isolates only strain *Microcyclospora tardicrescens* had shown inhibitory effect on mycelial growth of *Colletotrichum sp.* with a reduction of 46% after 7 days of incubation and 56% after 14 days of incubation.

The purpose of the STSM was therefore to study secondary metabolites of a selected SBFS fungus, to learn isolation methodologies and to use instruments not available at the Agricultural Institute of Slovenia (home institution). These studies were then conducted in the context of the STSM at the Helmholtz-Zentrum für Infektionsforschung, Inhoffenstraße 7, D-38124 Braunschweig in the working group of Dr. M. Stadler, who is an expert in fungal natural product research. Methodologies such as preparative HPLC, high resolution mass spectrometry and nuclear magnetic resonance spectroscopy (NMR) were performed in the context of the STSM.

2. Description of the work carried out during the STSM

The selected strains were cultivated in solid (CYA) and three liquid media (YM, ZM, Q6) from where metabolites were extracted with organic solvents. Preparation of mycelium extract was done separately from the culture fluid extract. Bioactivity tests were done using crude extracts against several bacterial and fungal test strains including human and plant pathogens in agar diffusion and serial dilution assays. Extracts showing antimicrobial activity were analysed by high performance liquid chromatography coupled with diode array and mass spectrometric detection (HPLC-DAD/MS) and subsequently fractionated by semi-preparative HPLC on 96 well plates. The biologically active fractions were correlated with their metabolite UV peaks by HPLC profile. Metabolites which were assumed to be potentially active compounds were purified through preparative (RP) HPLC and analyzed again by HPLC-DAD combined with electrospray time-of-flight mass spectrometry (ESI-TOF-MS). This procedure allowed the determination of their accurate masses and empirical molecular formulae. To isolate sufficient material for structure elucidation, *Microcycluspora tardicrescens* was cultivated in YM medium in a 5L scale and 10L YM medium in a biofermenter. Subsequently molecular structures of pure compounds are being elucidated by 1D and 2D NMR spectroscopy.

3. Main results obtained

Extract of mycelium from liquid medium (YM) of antagonistic strain *Microcycluspora tardicrescens* had the highest bioactivity potential against the phytopathogenic strain *Mucor hiemalis*. By a bioactivity-guided isolation strategy using *Mucor hiemalis*, which was determined as the most sensitive indicator organism, two metabolites were isolated from liquid cultures of *Microcycluspora tardicrescens*. NMR spectra identified the bioactive compounds as fatty acids. Final structure elucidation of these active compounds is still in progress. Fatty acids partially inhibited *Mucor hiemalis* with MIC up to 16.7 µg/mL.

From extracts of solid media, two antifungal metabolites were isolated by preparative HPLC.

4. Future collaboration with institution (if applicable)

Isolation and structure elucidation is ongoing at the moment at the HZI. The mentor of this project at the home institution (Agricultural Institute of Slovenia) will continue collaboration on novel bioactive natural components with the host institution. In the context of the current project, the PhD student stays in close contact with the Stadler lab so that the analyses can be completed and has an interest and wish to continue researching after finishing doctoral study on this scientific field as post doctoral student.

5. Foreseen publications/articles resulting or to result from the STSM

Fatty acids are known to possess antimicrobial activity, however, only few reports are currently available. The results obtained seem to be specifically interesting because little is known about the interaction of Capnodiales with other organisms and the functional basis of these interactions. The selected Capnodiales are particularly challenging because secondary metabolites of slow growing fungi are comparably badly studied. Eventually, slow growing fungi might be more often specialists, highly adapted to a specific ecological niche.

6. Confirmation by host institution of the successful execution of the STSM

I confirm that Ajda has performed excellently and superseded our expectations, She was working hard to accomplish her goal and integrated immediately into our research team. We were very sad that she had to leave again so soon, and I am presently trying to find some funding to re-integrate her as a postdoc after her PhD.

It is certainly not normal that students at Ajda 's level can learn how to do large scale fermentations and even preparative and analytical HPLC from scratch in such a brief period of time. Moreover, her work was also finally rewarded. Even though the bioactive compounds turned out to be notorious mycotoxins, we have found a new natural product among them and the project will definitely result in an interesting publication.

Marc Stadler, Braunschweig 14 Feb 2013