

Scientific Report

EU COST Training School 2013: „Characterisation of biologically active secondary metabolites from endophytic fungi“

2-6 December 2013

Organisers:

Helmholtz-Centre for Infection Research (HZI), Braunschweig, Germany

Marc Stadler, Dept. Microbial Drugs (http://www.helmholtz-hzi.de/en/research/research_topics/anti_infectives/microbial_drugs/marc_stadler/)

Sabine Kirchhoff, HZI Graduate School for Infection Research (http://www.helmholtz-hzi.de/en/career/graduate_school/application/)

Trainers:

Marc Stadler, Kathrin Wittstein, Matthias Keck, Frank Surup (HZI)

Anja Schüffler, IBWF Kaiserslautern, Germany

Jesus Martín, Fundacion MEDINA, Granada, Spain

Special Guests:

Barbara Schulz, Technical University of Braunschweig, Germany (Opening lecture)

Russell Cox, Institute of Organic Chemistry, Leibniz University of Hanover, Germany (Closing lecture)

Eighteen Early Stage Researchers (ESR; i.e., PhD students and Postdocs) from 11 different countries and 13 different nationalities have convened at the HZI to become acquainted with basic techniques of analytical chemistry that are essential for e.g., secondary metabolome analyses and bioprospecting of endophytic fungi. The trainer team consisted of Postdocs of the HZI, complemented by experts from Fundación MEDINA (Spain; <http://www.medinadiscovery.com/>) and IBWF (Kaiserslautern, Germany; <http://www.ibwf.de/>). Hence, three of the Top European institutions of natural product research were represented.

The event started with a welcome reception in the afternoon of Dec. 2, when all participants had arrived. Carolin Schneider, Chair of the COST Action FA1103, as well as representatives of HZI, presented an overview on the training program and explained the modalities. Thereafter, **Barbara Schulz** of nearby TU Braunschweig, who is a member of COST Action FA1103 as well as a true pioneer in endophyte research, gave a talk, summarising her previous and current projects on the discovery of new bioactive secondary metabolites and the ecology of these intriguing organisms. The first day ended with a welcome dinner at a typical German restaurant where the trainees could get acquainted with their colleagues and the trainers. Even in the following days, similar social events were organised, following the practical work. The courses started on Dec. 3 and continued throughout the week. In the mornings, several lectures accompanying and introducing the practical work were presented by the trainers on various different elements of the workflow of natural products discovery.

Anja Schüffler (IBWF Kaiserslautern) presented the general workflow of bioprospecting and discovery of new drugs and agrochemicals based on fungal cultures and their secondary metabolites. She also had designed an experiment to illustrate the important process of **bioassay-guided fractionation** by High Performance Liquid Chromatography (HPLC).

Marc Stadler gave an overview on the process of large scale production and downstream processing to obtain bioactive microbial metabolites at multi-gram scale, which is essential for late preclinical drug research and development as well as for development of natural pesticides. His talk emphasised the importance of preparative chromatography methods, and the trainees were able to conduct an experiment involving **preparative chromatography** to obtain secondary metabolites from a fungal extract in pure state. The laboratory experiments were co-supervised by **Eric Kuhnert**, a PhD student at HZI.

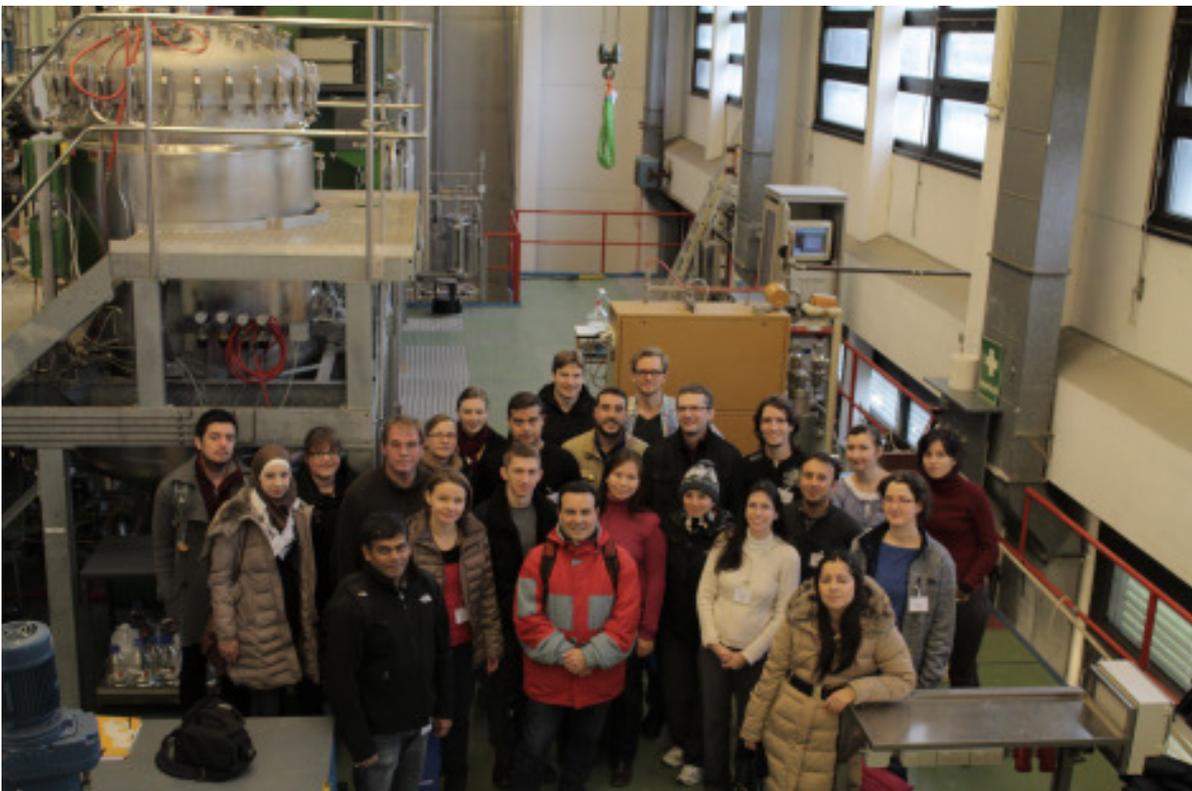
Kathrin Wittstein explained the principles of Nuclear Magnetic Resonance (**NMR**) spectroscopy and demonstrated this technique with the pure compounds obtained in the aforementioned experiment. The trainees were not only challenged with the evaluation of NMR data, but even managed to obtain an acetyl derivative of one of their compounds by means of a standard derivatisation reaction.

Matthias Keck presented the principles of gas chromatography/mass spectrometry (**GC-MS**), based on the example of endophytic Xylariaceae that can combat the newly arising, notorious ash dieback pathogen *Hymenoscyphus pseudoalbidus* in dual culture. The trainees were asked to analyse and evaluate the profiles of volatile organic components produced by both the pathogen and the endophytes.

Jesús Martin introduced the trainees into the secrets of HPLC-MS and high resolution (**HR-MS**) and showed them how to identify unknown compounds from fungal crude extracts by comparison with public and proprietary spectral libraries.

Frank Surup gave a general overview of secondary metabolites and demonstrated differences in the secondary metabolism between cultures and corresponding stromata (fruit-bodies) of the same species, using the xylariaceous genera *Hypoxylon* and *Annulohypoxylon* as example.

In the closing lecture, **Russell J. Cox** finally presented a wonderful overview on fungal secondary metabolite biosynthesis gene clusters and innovative methods based on molecular biology, genomics and epigenetics to characterise fungal metabolites.



The participants were also given a guided tour to several HZI departments, including the microbial strain collection and the NMR spectroscopy facilities in the Structural Biology department. They were particularly impressed by the in-house fermentation platform with bioreactors and matching downstream processing equipment for fermentations in up to 3.000 litre scale (see group photo).

The PhD students of the HZI Graduate School and of universities associated with HZI had the opportunity to attend the lectures of the framework program and some of them also joined the social program in the evenings.

From the feedback obtained by the participants during and after the Training School, we feel that this event has been a great success. Of course, the courses could only provide a very general overview on the highly interdisciplinary workflow of natural product discovery from endophytes, and it was not possible for the trainees, who were mostly biologists, to become proficient with the presented chemical techniques. Several trainees have expressed their great interest to become better acquainted with the presented methodology, e.g., through a Short Term Scientific Mission (STSM) in the course of the COST FA1103 Action plan at the institutes of the trainers. Through intensive discussions with the guests, the trainers and students of the host institute, however, also learned a great deal about the methods that are being established in the home institutes of the trainees, and in summary the Training School was a great success.

Unfortunately, the return trip of some of the participants was hampered by a severe storm that hit Germany in the night of Dec. 5. Therefore, some of the trainees got stranded on their way back home, as many flight connections on Dec. 6 were cancelled or delayed.

Finally, we wish to thank all colleagues who helped us to arrange the Training School as well as the trainees for their very active participation.

Kathrin Wittstein, Matthias Keck & Marc Stadler