Scientific Report – Short Term Scientific Mission (STSM)

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STSM Topic: Identification and bioactivity potential of endophytic fungi from *Astragalus* plants

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1. **Purpose of the STSM**

   Fungal endophytes are a rich source of bioactive naturals product with medicinal, agricultural and industrial potential. Many novel compounds with antimicrobial, insecticidal, immunomodulatory, antiviral, anticancer activies have been obtained from the endophytic fungi.

   The endophytic fungi used in this study were isolated from two *Astragalus* species *(Astragalus angustiflorus, Astragalus condensatus*) collected from Manisa, Turkey, in 2013 and fungal isolates were maintained on potato dextrose agar (PDA) plates. The antimicrobial activity of the selected strains was tested against pathogenic organisms (Methicillin-resistant *Staphylococcus aureus* (ATCC 43300) (MRSA), vancomycin-resistant *Enterococcus faecium* (DSM 13590) (VREF), enteropathogenic *Escherichia coli* O157:H7 (RSKK 234), *Pseudomonas aeruginosa* (ATCC 27853) and *Candida albicans* (DSM 5817) using disk diffusion assay (1). 15 fungal isolates showed bioactivity at least one of the test organism.

   Therefore, the purpose of the STSM was identification of the endophytic fungi from *Astragalus* plants and to investigate their bioactivity potential. The studies were conducted in the context of the STSM at the Helmholtz Centre for Infection Research in the lab of Prof. Stadler, who is an expert in the field of fungal natural product research.

2. Description of the work carried out during the STSM

a) Molecular phylogenetic analyses: The endophytic fungi isolated from different parts of *Astragalus* species were characterised by molecular phylogeny analysis based on ITS region using ITS4 and ITS1F (2,3).

b) Cultivation, extraction and isolation: 5 isolates have been selected for further analysis according to the molecular phylogenetic analysis and agar diffision assay against test strains (*Bacillus subtilis, Mucor plumbeus*). The selected strains were cultivated in two different liquid media (YM, ZM1/2) in Erlenmeyer flasks (250 ml) filled each with 100 ml media and incubated at 23°C, 140 rpm. After that, the mycelia were separated from the culture broth by filtration under vacuum and extracted with acetone. The culture filtrates were extracted with ethyl acetate and concentrated under vacuum using rotary evaporator to obtain crude extracts.

Minimum inhibitory concentrations (MIC) of the crude extracts were determined in a serial dilution assay using test strains (*Bacillus subtilis, Mucor plumbeus*). Extracts with high antimicrobial activity were analysed by high performance liquid chromatography coupled with a diode array and mass spectrometric detection (HPLC-DAD/MS) and then fractionated by semi-preparative HPLC into 96-well plates. The bioactive fractions were purified via preparative HPLC and structures of the compounds were elucidated by spectroscopic and spectrometric methods (NMR, HRMS).

3. Main Results Obtained

The crude extracts obtained from mycelia and culture filtrates of submerged cultures (5 strains) showed antimicrobial activity against *Bacillus subtilis.* Only one strain also active against *Mucor plumbeus.*

1E2AR1-1 (*Neosartorya hiratsukae*) was the most active isolate in YM and ZM1/2 media (Table 1).

Table 1. Bioactivity of crude extracts of *N. hiratsukae* against *Bacillus subtilis*

Extract type	***Bacillus subtilis***
1E2AR1-1 - YM-mycelia	18.75 µg/ml
1E2AR1-1 - YM-supernatant	9.37 µg/ml
1E2AR1-1 - ZM1/2-mycelia	4.68 µg/ml
1E2AR1-1 - ZM1/2-supernatant	9.37 µg/ml

1E2AR1-1 - YM-supernatant (18.8 mg) was fractionated by prep-HPLC and 4 active fractions were obtained (Fr.40, Fr.41, Fr.63-64, Fr.75-76) (**Fig 1**).

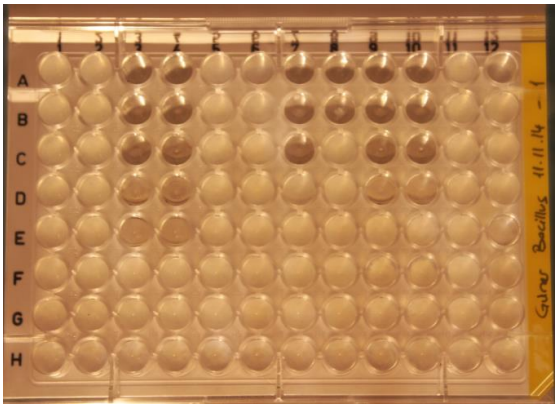


Fig 1. Fr. 40 (3), Fr. 41 (4), Fr. 63-64 (7), Fr.75-76 (8)

The active fractions were not pure enough for NMR analysis. To isolate sufficient material for structure elucidation, *N. hiratsukae* will be cultivated in larger scale.

1E2AR1-1 - ZM1/2-supernatant and **1E2AR1-1 - ZM1/2-mycelia** were combined and bioactivity-guided fractionation by prep-HPLC, with B*acillus subtilis* as indicator organism, yielded 9 active fractions (Fr.80-81, Fr.82, Fr. 84-86, Fr.90-91, Fr. 93-95, Fr. 99-100, Fr. 101-102, Fr. 114-115, Fr.116-117)(**Fig 2**).

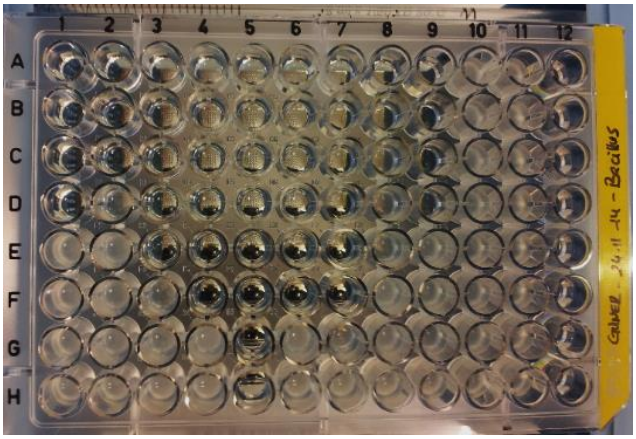
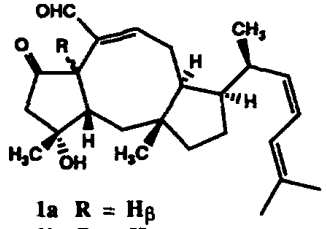


Fig 2. Fr. 80-81(1), Fr. 82 (2), Fr. 84-86(3), Fr.90-91 (4), Fr. 93-95 (5), Fr. 99-100 (6), Fr. 101-102 (7), Fr. 114-115(8), Fr.116-117(9)

The structure of purified compound (Fr. 93-95) was identified as **ophibolin k** (Fig. 3) on the basis of NMR and MS data by comparison with the literature (4). The structure elucidation of the other active compounds is still in progress.



1a R = H_β
1b R = H_α

Fig. 3. Ophibolin K (1a)

1E4BS-1 (*Penicillium roseopurpureum*) was also active against *Bacillus subtilis* (Table 2).

Table 2. Bioactivity of crude extracts of *P. roseopurpureum* against *Bacillus subtilis*

Extract type	***Bacillus subtilis***
1E4BS-1 - YM-mycelia	300 µg/ml
1E4BS-1 - YM-supernatant	75 µg/ml
1E4BS-1 - ZM1/2-mycelia	300 µg/ml
1E4BS-1 - ZM1/2-supernatant	37.5 µg/ml

1E4BS-1 - ZM1/2-supernatant (48.94 mg) was fractionated by prep-HPLC and fraction 43 (Fig. 4) contained 8.06 mg active compound. The structure of this compound was elucidated as **αβ-dehydrocurvularin** (Fig. 5) by 1D, 2D NMR and HR-MS techniques (5).

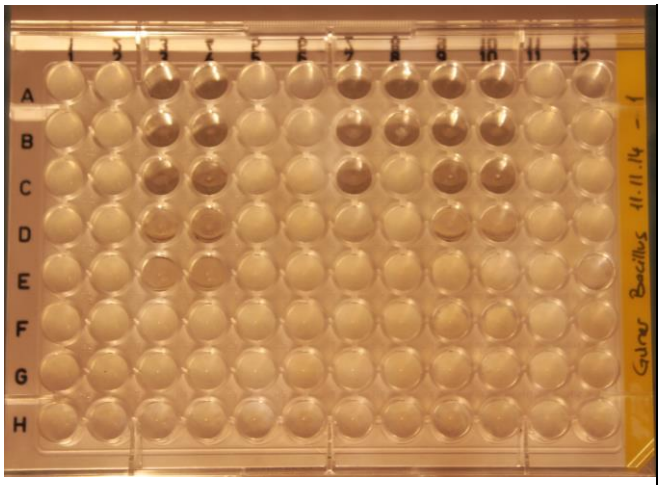


Fig 4. Fr. 43 (10)

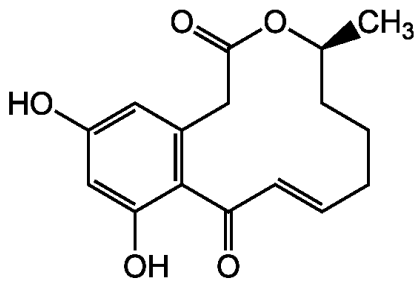


Fig 5. αβ-dehydrocurvularin

4. Future collaboration with institution

This STSM has opened the door to future collaborations with the group of Prof. Stadler. After purification of the active fractions in my home institution, structure elucidations of the active compounds will continue at the HZI.

5. References

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