Scientific Report of COST STSM
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STSM Topic: Deep insights into classical and molecular techniques to study plant endophytic bacteria
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1. State of the art

Plants are naturally associated with microorganisms in various ways. The soil environment attached to the root system is, for example, rich of microorganisms due to the presence of root exudates and rhizodeposits. Some rhizosphere microorganisms may be neutral or deleterious in regard to plant growth, whereas other microbes support their hosts. Such plant growth-promoting bacteria can stimulate plant growth, increase yield, reduce pathogen infection, as well as reduce biotic or abiotic plant stress, without conferring pathogenicity (Compant et al., 2010). The rhizosphere is well known to host a variety of PGPB and some rhizosphere colonizers can enter plants. In the past, healthy plants were considered to be free of microorganisms but in the last decades it has been repeatedly demonstrated that the plant interior is colonized by a range of endophytes mostly deriving from the rhizosphere and many of them have been reported to improve plant growth and to act as plant pathogen antagonists (Ryan et al., 2008; Hardoim et al., 2008). Following rhizosphere colonization endophytes may colonize various plant organs (Compant et al., 2005, 2008). Distinct microbial communities have been found in various plant organs such as roots, stem, leaves, flowers as well as fruits and seeds indicating different capacities of bacterial strains to colonize various plant compartments (Compant et al., 2010, 2011).

Classical studies on the diversity of bacterial endophytes have focused on characterization of isolates obtained from internal tissues following disinfection of plant surfaces. Studies that make use of both culture-based and culture-independent techniques can be particularly useful (Ryan et al., 2007; Sessitsch et al., 2002).

2. Purpose of the STSM

The main goal of this Short Term Scientific Mission focused on the comprehension of classical and molecular techniques employed for the characterization of plant endophytic communities and endophyte isolates.

The stay focused on techniques for isolation and characterization of new endophytic bacteria from plant material and on molecular characterization of endophytic bacterial communities after treatment of wheat plant with specific strains. One of the goals of the STSMs is the learning of a new technique to be applied once back in your own laboratory, and considering that T-RFLP analysis requires a specific apparatus, the characterization of an endophytic community was accomplished through the construction of a 16S rDNA library, a technique that requires common laboratory facilities.

3. Description of the work carried out during the STSM

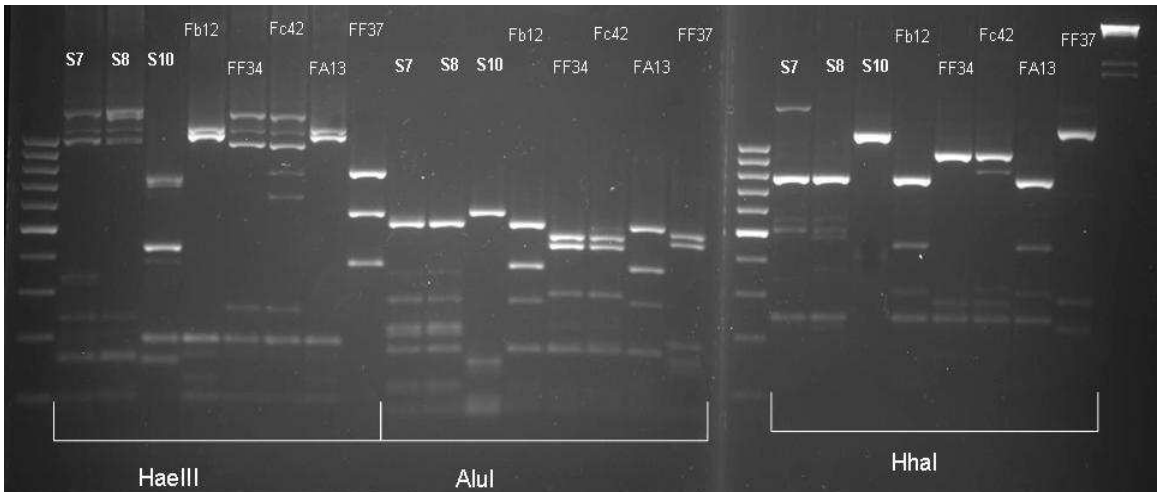
Learning of:

1. Techniques for endophytes isolation from plant material. Protocol for sterilization of plant material, extraction and plating of endophytes on suitable media (both rich and poor media should be used).
2. Techniques for endophytes characterization. DNA extraction, IGS amplification and RFLP (using three different restriction enzymes) for clustering analysis. 16S rDNA amplification, purification and sequencing of the different isolates.
3. 16S rDNA library formation from environmental samples. 18 samples of wheat material were extracted and gDNA quantified on Nanodrop. 2 different wheat varieties: Monsun and Collada, treated with 2 different endophytic bacteria (extractions were made in triplicate). Construction of the 16S rDNA library from three Collada samples (the control and two treated samples) to assess the possible colonization of the plant. 16S rDNA amplification of the gDNA, band cutting and purification. Cloning of the 16S amplicons. Colony screening and sequencing of positive clones.
4. IGS library formation from environmental samples. One sample of Collada treated with an endophytic bacteria was also used to create an IGS library. Two bands were present after amplification with IGS primers. Both bands were excised and amplicons cloned. Colony screening and sequencing of positive clones.

4. Description of the mail results obtained

The most important result obtained were the following:

- Grouping of endophytic isolates through RFLP on IGS sequencing



- Sequencing of the 16S rDNA of the isolates

Strain	bp	RDP classifier	RDP match	Identity (blast)	GeneBank
S7	1422	*Pantoea* 100%	*P. ananatis*	99%	AP012032.1
S8	1407	*Pantoea* 100%	*P. ananatis*	99%	AP012032.1
S10	1434	*Paenibacillus* 100%	*P. taichungensis*	99%	NR_044428.1
Fb12	1418	*Enterobacter* 99%	*E. cloacae*	99%	JF772064.1
FF34	1421	*Pantoea* 100%	*P. agglomerans*	99%	FJ756354.1
Fc42	1377	*Pantoea* 100%	*P. agglomerans*	99%	AY395010.1
FA13	1421	*Enterobacter* 100%	*E. cloacae*	99%	JF772064.1
FF37	1405	*Burkholderia* 100%	*B. phytofirmans* PsJN	99%	CP001053.1

RESULTS: Considering RFLP analysis and 16S rDNA sequencing results, strains S7/S8 and strains FF34/Fc42 were different strains of the same species; while strains Fb12 and FA13 that displayed the same 16S sequence and the same RFLP pattern were probably the same strain.

- 16S rDNA library for 3 wheat samples. 50 colonies were considered in each sample
- IGS library for 1 wheat sample. Two bands were excised from gel and cloned. 50 colonies were considered in each cloning.

RESULTS: 50 colonies have been isolated from each sample (250 colonies). 96 clones have been selected, at the end, for sequencing (about 20 for each cloning). Sequencing results displayed 95/96 good sequences, however Blast analysis revealed only chloroplast and plastidial DNA. It is possible to suppose that colonization did not occur.

5. Confirmation by the Host Institution of the successful execution of the STSM

The aim of the stay of Mrs. Loredana Baffoni at the AIT Austrian Institute of Technology was to learn standard techniques, both classical and molecular techniques for the characterization of plant endophytic communities and endophyte isolates. Loredana Baffoni was very active, highly motivated and dedicated to learn everything for the detection of inoculants strains within the schedule as planned. We are confident that Loredana will successfully apply the techniques in her own work and will establish the protocols in her home institute in Bologna.

References

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