Scientific Report – Short Term Scientific Mission (STSM)

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STSM Topic: Endophyte watching: analysis of tagged strains and evaluation of their plant growth promoting effect

Purpose of the STSM

The main goal of this STSM was the evaluation of the endophytic behaviour of some bacterial strains known as plant growth promoting bacteria or biocontrol agents and the effect of these strains on the native endophytic community of tomato.
The effects of these strains on plants physiology and morphology were already demonstrated; we selected 20 strains, isolated from different plants and we studied the colonization of tomato plants and the impact of these bacteria on the natural endophytic community using a HTS approach.

The group of Leo van Overbeek has a strong experience on bacterial endophytes and community analysis and all the facilities needed for bacteria transformation, plant cultivation and microbiology.

Description of the work carried out during the STSM

In this STSM I worked with a collection of 20 putative endophytes isolated from different plants and with different beneficial properties in plant growth promotion and biocontrol. The strains are listed in the table. Strains highlighted with green colour are bacteria isolated from grapevine, *Vitis vinifera cv. Glera*; the other strains were isolated within the EU project “Biofector”.

Strain
Verrucomicrobia IRVE
Verrucomicrobia CHC8
Pseudomonas sp. DSMZ 13134
Bacillus amyloliquefaciens FZb42
Pseudomonas P9
Pseudomonas jessenii
Bacillus licheniformis GL174
Pantoea sp. GL83
Micrococcus sp.
Bacillus pumilus
Bacillus megaterium
Microbacterium testaceum
Bacillus licheniformis
Micrococcus sp.
Bacillus subtilis GL420
Bacillus subtilis GL452
Paenibacillus polimyxa GL24
Agrobacterium tumefaciens
Bacillus cereus
Staphylococcus epidermis

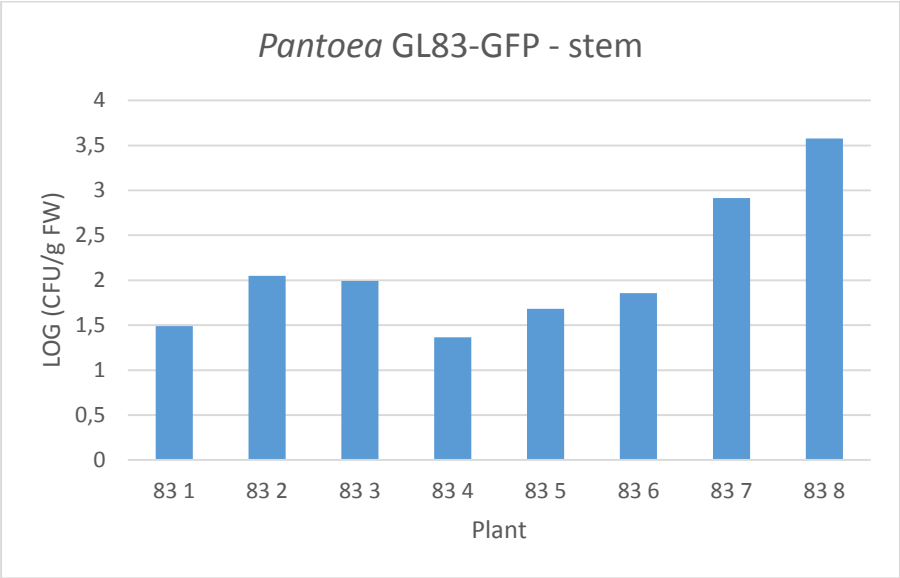
The antibiotic resistances of these strains were also determined during this STSM.
Using electroporation technique, some strains were transformed using integrative vectors (Tn5 transposon like) containing the GFP gene in order to obtain green fluorescent bacteria.
The colonization of tomato plants after seeds inoculation (10^6 CFU) with these 20 strains was evaluated plating on R2A medium surface sterilized plant material from roots, stem and leaves; in this way we selected 7 best endosphere colonizers for the analysis of the impact of these strains on the natural tomato endophyte community.

Strain	GFP transformation
Pseudomonas sp. DSMZ 13134	
Pantoea sp. GL83	*This work*
Pseudomonas jessenii	*This work*
Pseudomonas P9	
Bacillus licheniformis GL174	
Bacillus amyloliquefaciens fzb42	*Fan et al., J Biotechnol (2011) 151, 303–311*
Bacillus subtilis GL452	

In this experiment, we inoculated 8 tomato seeds with each selected strain and the seedlings were afterwards moved in pots with horticultural soil; 4 plants were sampled after 3 weeks post germination and 4 plants after 8 weeks.
Stem, roots and some leaves were sampled for each plants; roots were carefully washed to remove all the soil particles, roots and leaves were surface sterilized, grinded and plated on nutrient and selective medium to re-isolate and quantify the inoculated strain; stems were surface sterilized, peeled aseptically in order to exclude the epiphytic bacteria and grinded. One part of the grinded material was plated and from the remaining material the total DNA was extracted for the amplification of the 16S rRNA of the endophyte bacteria.

Description of the main results obtained

In this work we obtained useful information about the antibiotic resistance these endophytic strains; moreover we obtained 3 GFP tagged strains: *Pantoea ananatis GL83, Pseudomonas jessenii, and Pseudomonas P9*. Analysing the plant colonization after inoculation of tomato seeds we demonstrated the ability of some "bio-effector" strains to enter into the plant, survive and thrive inside plant tissues. The colonization was also quantified counting the colonies of the inoculated bacteria in plates: the colonization of root was up to 4.07 log(CFU/g FW) by *Bacillus subtilis GL452*, the colonization of stem up to 4.4 log(CFU/g FW) by *Bacillus amyloliquefaciens* FZb42 and the colonization of leaves 2.29 log(CFU/g FW) by *Pseudomonas jessenii*. From these results we demonstrated that the colonization of different plant tissues is non consistent: plant inoculated with the same inoculum didn't show the same level of colonization. Figure 1 is an example of colonization level of stem of plants (plants 1-4: 3 weeks after inoculation; plants 5-8: 5 weeks).



From the peeled stems the total DNA was extracted that will be used for the 16S rRNA amplification of the endophytes present in the plants. These amplicons will be sequenced and analysed to visualize if the community of endophytes has been changed by the inoculum of the endophytic bio-effector strain.

Future collaboration with host institution

The collaboration with the group of Leo van Overbeek is not concluded; sequences obtained by next generation sequencing will be processed together in order to analyse the impact of the inoculated strains on the native endophytes of tomato.

Foreseen publications/articles resulting or to result from the STSM

All the results of this work will be used for a publication; moreover the transformation of endophytic strain pave the way for many studies of localization, inoculation, and observation of fluorescent bacteria *in vivo in planta.*

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

Sebastiano Nigris successfully performed in the host lab the community analysis after endophyte colonization of tomato plants and the strain transformation with a GFP tag. Besides the scientific results, Sebastiano had the opportunity to improve his knowledge in microbiology and ecology of endophytes, the use of molecular biology techniques and fluorescence microscopy. He also presented his preliminary results during the scientific meeting of The Royal Netherlands Society of Plant Pathology.

The short stay of Sebastiano in our lab was satisfactory; he is a carefully working, highly dedicated student, quickly learning and integrating different concepts and we are looking forward to further cooperation with him and his home institution.