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Purpose of the STSM

Rhizosphere, the zone of soil surrounding the root, is believed to be one of the richest bacterial ecosystems on Earth. The rhizosphere-inhabiting microbes can promote growth of their hosts, as well as increase their resistance to wide range of biotic and abiotic stresses. Additionally, a fraction of rhizospheric bacterial populations is capable of colonising root interior without inducing host defence response. Those bacteria can be called endophytes.

Previous work done on *Arabidopsis* (Lundberg et al. 2012, Bulgarelli et al. 2012, Schlaeppi et al. 2014) indicated that plant roots are capable of shaping bacterial communities in their surrounding, as well as within their interior.

In order to broaden our understanding of plant-endophyte interactions, current project involved application of another model organism in plant genetics – *Lotus japonicus* ecotype Gifu, as well as its symbiotic mutant – *nfr5*, described below. Such approach enabled investigation of three goals:

- Identification of differences in rhizosphere/root interior microbiome composition between unrelated species – *Lotus* and *Arabidopsis.*
- Investigation of how do different belowground organs of Gifu shape microbiome populations. *Lotus* is a legume, thus it is capable of involving into nitrogen fixing symbiosis with rhizobia, which takes place within the nodules. Presence of both roots and nodules enables for comparisons between their internal microbiota composition.
- Study of requirement for Nod factor perception in shaping microbial composition of belowground organ interior. To accomplish that aim, *L. japonicus nfr5* mutant was used. It lacks a functional LysM receptor kinase, required for perception of nodule-inducing rhizobia. The mutant is unable to involve in nitrogen-fixing symbiosis, thus comparison to Gifu would allow to study the role of such symbiotic interactions in shaping the microbiome community.

Description of the work carried out during STSM

The workflow applied in this study followed one presented by Bulgarelli et al. (2012), with minor changes towards optimisation for *Lotus*. It could be separated into two stages:

- Isolation of microbiome DNA from different belowground compartments, followed by microbiome library sequencing
- Bioinformatical analysis of microbial composition based on OTUs (Operational Taxonomic Units)

The former stage involved growth of plants in two batches of natural soil, collected from the same spot during different seasons (spring and fall). Such approach could give information on dynamics of microbial populations throughout the vegetation season. Plants from both soil batches were processed separately, but according to common protocol. In both cases, plants were incubated in greenhouse for 11 weeks. Afterwards, belowground organs were extracted, washed and surface sterilised. Concomitantly, rhizosphere samples were obtained for each plant organ compartment. Apart from Gifu/*nfr5* belowground organ samples and corresponding rhizosphere specimens, 3 additional samples were included into data set. Bulk soil, not being influenced by presence of belowground plant organs, represented the default microbial taxa composition for tested soil type, while Gifu/*nfr5* axenic controls originated from plants grown in sterile conditions (on agar slopes in petri dishes) and were included to verify possibility for endophyte transfer via seeds. Overview of all tested compartments is given in Table 1. For each compartment, 3 biological replicas were made.

***nfr5* genotype samples:**	*nfr5* root rhizosphere	*nfr5* root interior	
Gifu genotype samples:	Gifu root and nodule rhizosphere	Gifu root interior	Gifu nodule interior
Additional samples:	Bulk soil	*nfr5* axenic control	Gifu axenic control

Table 1: List of samples (compartments) included into sequenced libraries for both spring and fall soil batches. Gifu is a wild type genotype of *Lotus*.

For each of presented compartments, microbiome DNA was isolated, followed by PCR amplification of 16S rDNA with barcoding primers. The barcoding allowed for identification of individual samples within the library. After that step the samples were purified, equal concentration of DNA from each of them were combined into library, which was sent for sequencing (454 Next generation sequencing approach). In total, 5 libraries were created – 3 for spring, and 2 for fall soil batches.

Description of the main results obtained

The bioinformatical analysis of sequencing results is still undergoing. That is why it is not possible to present results or final conclusions of this work.

Future collaborations with host institution

The screening could be extended by *Lotus japonicus* symbiotic accomodation or symbiotic signalling mutants. Additionally, root/nodule microbiome study could include spontaneously nodulating mutants (*snf1* or *snf2*, Tirichine et al, 2007).

Foreseen publications/articles resulting from STSM

After *in silico* analysis is finished, the results are planned to be published in a single manuscript.