STSM Panel
COST action FA1103
Endophytes in Biotechnology and Agriculture

Dr Natasa Sibanc
University of Ljubljana
Biotechnical Faculty
Jamnikarjeva 101
SI-1000 Ljubljana
Slovenia

Ljubljana, 26th of October 2015

Scientific report for Short Term Scientific visit in the frame of COST Action COST Action FA1103 Endophytes in Biotechnology and Agriculture

Title of the visit:
Quantification of root fungal endophyte diversity from pristine forests and secondary succession area of Balkan Peninsula using Illumina sequencing

Applicant:
Dr Natasa Sibanc, University of Ljubljana, Biotechnical Faculty, Slovenia

Host:
Dr Alex J. Dumbrell, University of Essex, School for Biological Sciences, United Kingdom

Time of visit:
September 2015 (8 September till 3 October 2015)

Reference:
COST-STSM-ECOST-STSM-FA1103-070915-067470

Arbuscular mycorrhizal (AM) fungi are plant root endosymbionts and important, ubiquitous organisms in soils belonging to the phylum Glomeromycota. They improve plant mineral nutrition, maintain plant health, increase pathogen resistance and water availability, and are involved in several other important ecosystems services (e.g. carbon and nutrient cycling, soil aggregation). Despite the fact that they have been proven as an important component of forest ecosystems, research into their communities in different forest types is still very scarce, especially within Eastern Europe, including the Balkan Peninsula. Alongside Iberia, this area harbours the richest flora in Europe, possessing not only the largest number of species, but also hosting most endemics.

The Balkan Peninsula, represents an unstudied region regarding research on any aspect of AM fungal ecology or biodiversity. Within proposed STSM mission, we have studied biodiversity and community ecology of AM fungi in selected biodiversity hot spots of the Balkan Peninsula (mainly Slovenia and Serbia). We have used high resolution amplicon sequencing (Illumina) of AM fungal DNA from plant roots in order to study the changes in AM fungal community composition across a range of secondary succession areas.

The primary aim of the proposed visit was to implement protocols for quantifying the diversity of root fungal endophytes in the area of Balkan Peninsula using next generation sequencing MiSeq platform (Illumina).

During my stay in University of Essex, I have analysed a total of 240 plant root samples. Samples were collected from Slovenia and from Serbia in August 2014, November 2014, March 2015 and May 2015. We have collected soil core samples from meadow, transition and forest sites (succession) with mixed plant species and individual plant species. Plant roots were washed from soil cores, dried and homogenised using Retsch mill. Total DNA was extracted from homogenised root samples using MO BIO Power Plant Isolation kit.

Molecular work started with optimising the polymerase chain reaction (PCR) protocol, using specific primers for arbuscular mycorrhizal (AM) fungi targeting 18S ribosomal RNA gene (18S rRNA) with added llumina adapter with overhang nucleotide sequences. The PCR reaction used to amplify template out of a DNA sample was carried out in 25 µl reaction using SIGMA REDtaq ReadyMix. Obtained positive PCR products that were about 620bp long (Figure 1) were used in the next step of molecular analysis.

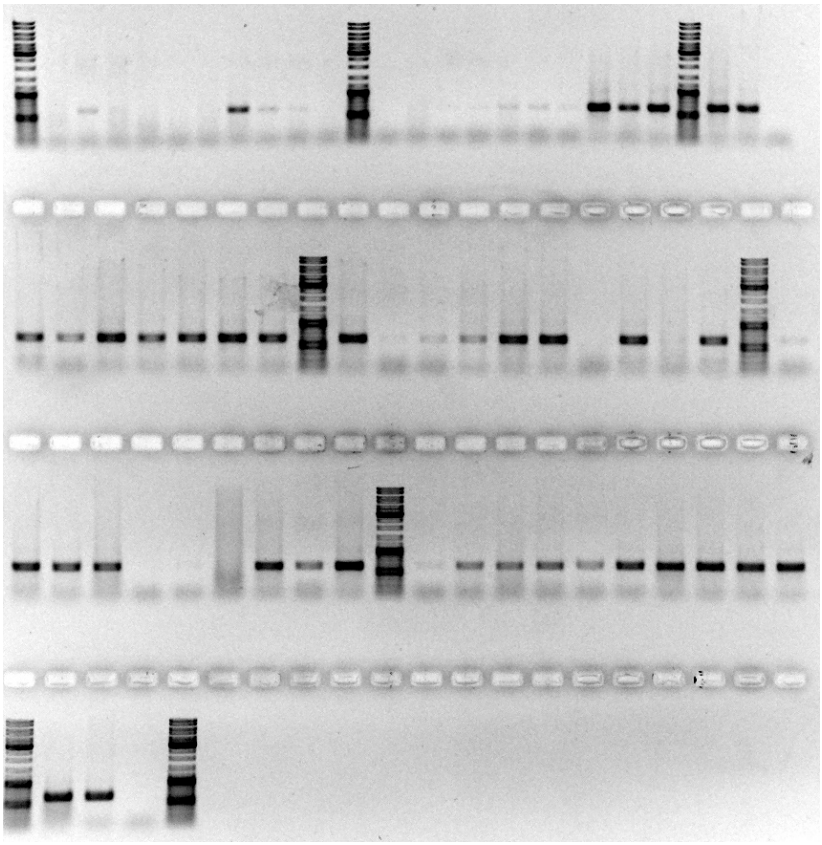


Figure 1: Gel electrophoresis of PCR products obtained in first PCR reaction. From left to right there are 62 samples and a negative control.

Positive PCR products were cleaned using AMPure XP beads according to manufacturer protocol to remove free primers and primer dimer.

After the PCR clean-up step, I have performed the second PCR, called also Index PCR. This step attaches dual indices and Illumina sequencing adapters using the Nextera XT Index Kit and SIGMA REDtaq ReadyMix on a TruSeq Index Plate Fixture.

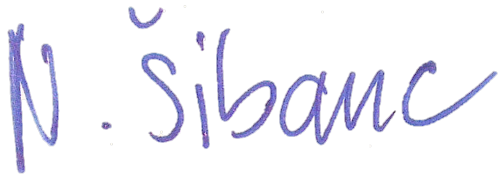
PCR products were cleaned once more using AMPure XP beads according to manufacturer protocol and visualised on agarose gel.

The concentration of DNA in each library (final cleaned PCR product) was quantified on a Bioanalyzer. Calculated aliquot of each library containing equal amounts of DNA were pooled into one sample. The samples are now waiting to be sequenced on MiSeq platform (Illumina) at GATC Biotech Ltd., UK.

When we will have sequencing results, we will test if observed patterns in AM fungal communities are seasonally stable, and try to determine the main predictors of the spatial and temporal AM fungal community compositional shifts. Research into this valuable genetic pool is not only important from a biodiversity view point, but also represents a resource for site-directed reclamation of disturbed soils (ecosystems), sustainable forestry, nature conservation, and agriculture that has not been used as such in Balkans so far.

Future work will be in collaboration with Dr Alex J. Dumbrell in a forthcoming visit to University of Essex and it will include analysing DNA sequences, aligning amplicon sequences, use of molecular systematics to construct phylogenetic trees and fungal community structure analyses. Analysing the raw sequences is an important final step in molecular work and there are several approaches to do this. It is important to have good collaboration established between University of Essex and University of Ljubljana to understand the statistics and computing science behind it to be able to interpret the results.

I would kindly like to thank STSM Panel, for award me with this STSM.



Dr Nataša Šibanc
University of Ljubljana,
Biotechnical Faculty
Slovenia