STSM Panel
COST action FA1103
Endophytes in Biotechnology and Agriculture

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

Title of the visit:
Quantification of diversity of fungal root endophytes using next generation 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:
17th of August till 3rd of September

Reference: COST-STSM-ECOST-STSM-FA1103-170814-048465

The primary aim of the proposed visit was to develop new protocols for quantifying the diversity of fungal root endophytes using next generation sequencing on MiSeq platform (Illumina). In conducted molecular analyses, we have used dried root samples collected from a long term Free-Air CO_2 Enrichment (FACE) Experiment conducted in Giessen (Germany) where we have focused on investigating the influence of elevated concentrations of atmospheric CO_2 on fungal root endophytes community composition.

During the first week (18th till 24th of August) of my stay at University of Essex, I was 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 KAPA HiFi HotStart ReadyMix. Obtained positive PCR products that were about 620bp long (Figure 1) were used in the next step of molecular analysis.

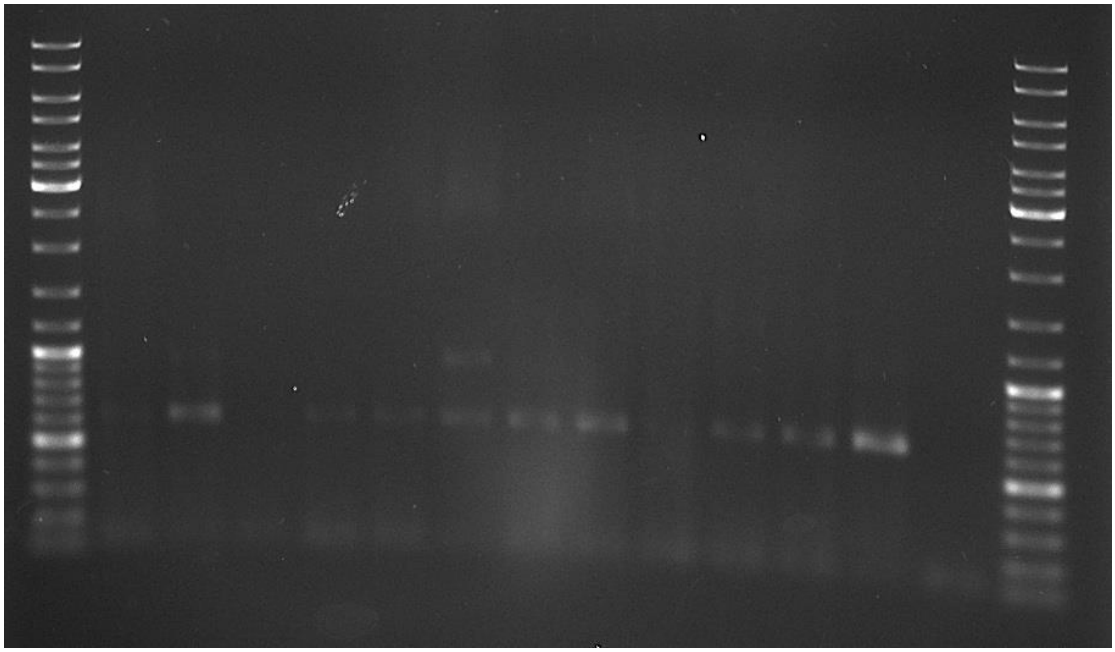

Figure 1: Gel electrophoresis of PCR products obtained in first PCR reaction. From left to right there are twelve samples and a negative control.

During the second week of my stay (25th till 31 of August) I have cleaned positive PCR products 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 KAPA HiFi HotStart 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 (see Figure 2). PCR products having more than one band were gel extracted using Qiagen QIAquick Gel Extraction Kit. The concentration of DNA in each library (final cleaned PCR product) was quantified using a fluorometric quantification method that uses dsDNA binding dyes. 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).

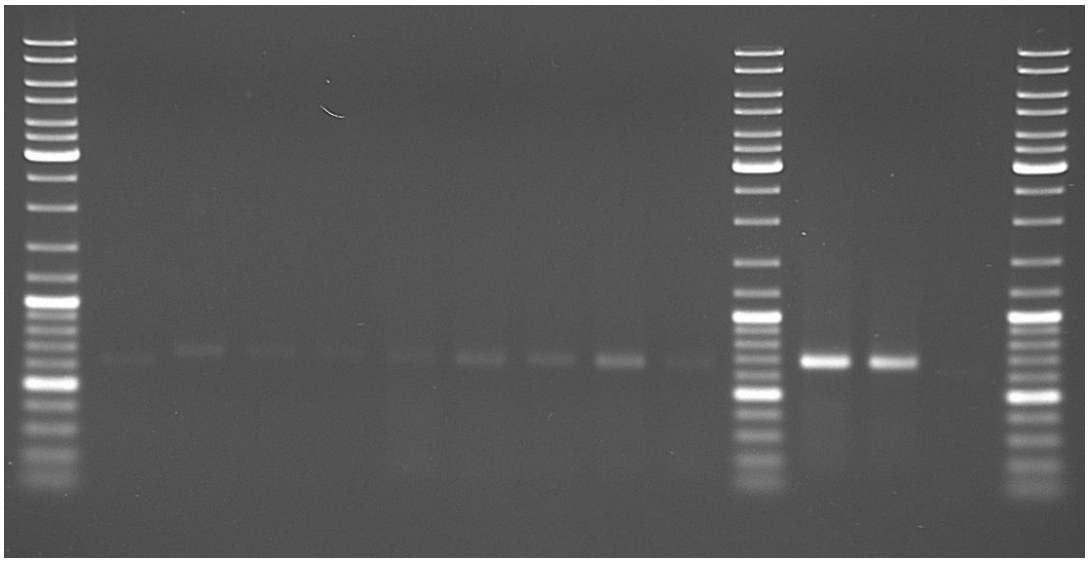

Figure 2: Gel electrophoresis of PCR products obtained in second PCR reaction. From left to right there is PCR product from first PCR followed by 8 samples from second PCR (longer fragment due to additional attached indiced), ladder, two PCR products from second PCR and one PCR product from first pcr.

After finishing with laboratory work, I have attended the Focused Meeting 2014: Emerging Challenges and Opportunities in Soil Microbiology (http://www.sgm.ac.uk/en/events/conferences/index.cfm/focused-meeting-emerging-challenges-and-opportunities-in-soil-microbiology) held from 1st till 2nd September at Loughborough University, UK.

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.

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