PO088

USE OF NEXT GENERATION SEQUENCING IN SOIL MICROBIOM ANALYSIS

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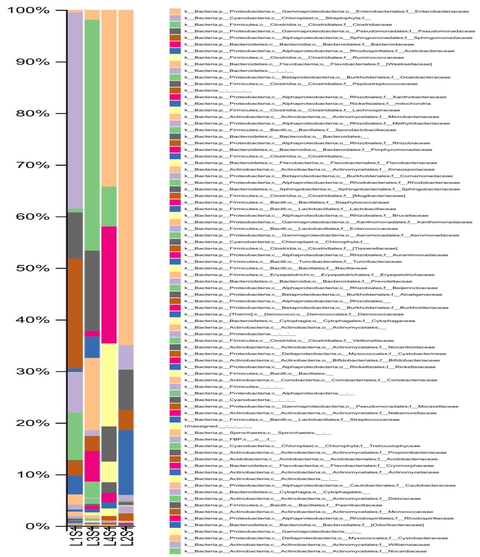
Results

Our results showed distinct bacterial community composition between samples, as well as specific composition fingerprint of each sample type. The most abundant in plant sample were bacteria from *Sphingomonadales* and *Pseudomonadales* orders. Comparison between polluted and unpolluted soil samples showed that *Enterobacteriales* and *Clostridiales* orders were highly present in polluted samples.

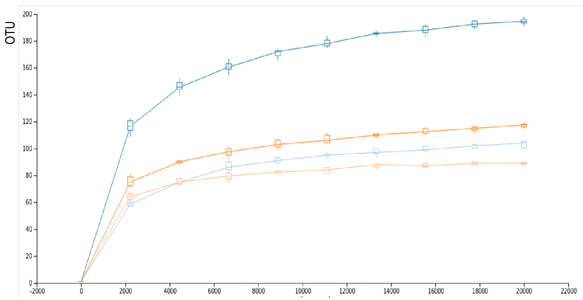


LEGEND: L1S4 – unpolluted soil, L2S4 – polluted soil , L3S4 – leaf sample, L4S4 – polluted soil.

Relative abundance of tested samples. Only first 6 most frequent taxa are showed.
L1 – unpolluted soil, L2 – polluted soil , L3 – leaf sample, L4 – polluted soil.

L1		L2		L3		L4	
Taxon	Percentage	Taxon	Percentage	Taxon	Percentage	Taxon	Percentage
pasteurianum	**0.410102**	Enterobacteriaceae	**0.643383**	Streptophyta	**0.386047**	Morganella	**0.239791**
Azorhizophilus	**0.152638**	Gluconobacter	**0.124878**	Sphingomonas	**0.170046**	coprosuis	**0.187703**
meningoseptica	**0.117034**	Pseudomonas	**0.065219**	Bacteroidetes	**0.104465**	guilliermondii	**0.162176**
Azorhizobium	**0.058065**	Streptophyta	**0.050713**	Pseudomonas	**0.053156**	butyricum	**0.077746**
Sporolactobacillus	**0.029838**	Sphingomonas	**0.027525**	Massilia	**0.041784**	Proteus	**0.073368**
Acetobacter	**0.028799**	Rhizobiaceae	**0.014838**	asaccharolytica	**0.041056**	Peptostreptococcus	**0.07274**

Alpha rarefaction of tested samples. OUT- operational taxonomic units. Dark blue line – unpolluted soil; Light blue and orange lines – polluted soil; Yellow line – plant leaf.



Introduction

Microbiome is a complex of bacteria, archaea, viruses and fungi in particular environmental niche. The complexity of microbiome investigation refers to interpretation of biological interactions and technical limitations. Biological interactions within microbiome community or with other organisms and environment are crucial for understanding microbiome influence on agriculture and potential benefits from it. One of the benefits could be the estimation of sample origin and pollution by microbiome composition convergence assessment. However, less than 2% of bacteria can be cultured in laboratory and this technical limitation calls into a question the representation of microbiome and taxon abundance assessment. This technical limitation can hide changes in microbe diversity which consequently hinders biotechnological development and application in agriculture as well as in many other scientific fields. The last major breach in technical limitations came by introduction of massive parallel sequencing technology known as next generation sequencing. This technology overcomes culturing limitations and provides direct assessment of microbiome diversity, taxon identification and quantification. In bacteria, sequencing and analysis of variable domain in conservative genes enables bacteria taxon identification and quantification. Therefore, we assessed deep sequencing approach in soil and plant samples.

Materials and Methods

Pilot sequencing was conducted by choosing representative samples from polluted (two samples from different locations), unpolluted soil, and *Pyrus communis* cultivar 'Williams' leaf grounded on unpolluted soil. DNA was isolated directly from samples using commercial silicate columns (Zymo Research). Highly variable V3-V4 region of rDNA was amplified with universal primers 341F and 805R. Amplicons were pair-end sequenced (2x300bp) on MiSeq V3 platform after 600 cycles (Illumina, San Diego, USA). Average sequence coverage was approximately 100 000 reads per sample. Raw data were processed according to both QIIME 2 and DADA2 pipeline. The whole procedure included quality control, filtering and trimming, pair ends merging, sequence table construction, taxonomy assignment.

Conclusion

The massive parallel sequencing technology provides striking technological opportunity to investigate microbiome composition and identify bacteria from different sample types, but also to estimate sample origin by bacterial composition. The depth of sequencing could be reduces after pilot study and alpha rarefaction determination, which provides resource balancing in favor of the increasing sample size. The plant upper parts microbiome (without fungi and viruses) seems to be distinctive in comparison to the ground where plant is rooted, while microbiome is uniquely distinguished by high percentage of chloroplast sequences similar to members of Cyanobacteria phylum.

Future perspective

In future our study will encompass soil samples from different locations known to be polluted, but also samples of the unpolluted soil from different locations known to be agriculturally suitable for specific fruits and vegetables. In addition, fungi will also bi included in analysis to investigate both possible influence of fungi to other members of microbiome and pollution fingerprints in fungi diversity. The goal of further microbiome studying is to investigate and define microorganisms that could influence plant culturing and prevent unnecessary economical loss.