SHORT TERM SCIENTIFIC MISSION (STSM)
SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator.

Action number: CA16107
STSM title: Effect of plant genotype, plant niche and environmental conditions on the diversity and structure of microbial communities in cultivated olive trees
STSM start and end date: 21/09/2020 to 29/11/2020
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PURPOSE OF THE STSM

Olive tree is one of the most important cultivar in Mediterranean agriculture since olive oil exhibits numerous beneficial health properties and plays a central role in Mediterranean diet. However, nowadays its viability is seriously threatened by phytopathogenic organisms such as Verticillium dahliae and Xylella fastidiosa. These pathogens colonize the vascular bundles of the xylem obstructing the sap flow, leading to wilting of plant tissue, and ultimately causing the death of the olive tree resulting in heavy environmental and economic losses worldwide. Recent studies indicate that plant-associated microbial communities play an important role in controlling vascular wilt diseases and could form the basis of sustainable biocontrol strategies for crop production. NGS approaches together with advances in bioinformatics and statistical analyses of generated data represent valuable tools for exploring, identifying, and characterizing the diversity of these plant-colonizing microorganisms which will lead to a better understanding of the interactions between microorganisms and plants. Therefore, the purpose of the STSM was to decipher the olive microbiome from endophytic and epiphytic plant ecological niches taking into account the effects of soil type, the olive genotype and the environmental and agronomic conditions in order to understand the principal factors that governs its assemblies.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSM

Raw fastq sequences obtained from MiSeq Illumina platform underwent quality control and adapter trimming in order to increase the quality of the reads. We used FastQC, a tool designed to spot potential problems in high throughput sequencing datasets. It runs a set of analyses on fastq sequence files to highlight any areas where the library looks unusual. Then, we applied Trim Galore tool which performs quality trimming of low-quality base calls from the 3' end of the reads and also removes adapter sequences from Illumina sequencing. The output results were summarized through the bioinformatics tool MutiQC which searches a given directory for analysis logs and compiles a HTML report. We visualized a Phred quality score (Q) higher than 30 which means 1 error per 1.000 bases (99.9% base call accuracy). These quality reads were used for ASVs (Amplicon Sequence Variants) identification instead of the traditional OTUs (Operational Taxonomic Units) in an innovative workflow analysis based on the incorporation of the quality scores and sequence frequencies in a probabilistic noise model for nucleotide transitions incorporated into the assignments. After filtering the sequences and removing the chimeric reads, the data were compared to Silva v.138 and UNITE databases for bacteria and fungi identification, respectively. In this workflow, denoised reads were labeled and assembled into ASVs without imposing any arbitrary threshold, and thereby resolving variants that differ by as little as one nucleotide in contrast to the conventional OTUs classification which group sequencing reads that differ by less than a fixed dissimilarity threshold. De novo

phylogenetic tree was built using MAFFT for multiple alignment and Fasttree in QIIME2 because it uses sequences profiles to implement neighbor-joining to reduce the length of the tree and produce a more curated phylogenetic tree. Finally, the ASV table, environmental sample metadata, taxonomy affiliation, phylogenetic tree and reference sequences were combined into a phyloseq object to perform the exploratory analysis of the microbiome data. Subsequently, R software and R packages such as dada2, phyloseq, Deseq2 and vegan were used to identify microbial taxa enriched in specific treatments/locations and to detect significance differences between the different variables in the study: season, olive genotype, location (soil type and environmental conditions) and plant niche. In addition, correlations of microbial communities against physicochemical and environmental data and co-occurrence network inference analysis between all microbial taxa was carried out using CoNet and Cytoscape software. Key network properties, such as keystone species, node degrees, centrality and density were calculated to assess differences in potential microbial interactions taking place in the microbiome of olive trees under different treatments and locations.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

Sequence analysis reported 5.891 ASV in bacteria (29 phyla, 157 classes, 639 orders, 1.343 families and 2.906 genera) and 3.055 ASV in fungi (13 phyla, 324 classes, 464 orders, 870 families and 1.511 genera). Globally, we obtained a higher number of bacteria than fungi at all taxonomic ranks. In bacteria the most abundant phylum were Proteobacteria and Actinobacteria whereas at genus level Pseudomonas and Sphingomonas stood out above the others. In fungi, the greatest abundance was represented by the phylum Ascomycota and Basidiomycota and the genus Aureobasidium. Interestingly, when ecological niche, field location, genotype and seasonality were considered, the 15 most abundant genera varied in their relative abundance. Thus, we observed in bacteria the low level of Cutibacterium in soil and rhizosphere ecological niches; the increase of Sphingomonas from Autumn to Spring and the high abundance of Anoxybacillus in Córdoba. In fungal communities, we observed a clear presence of Aureobasidium in the aerial part of the plant in Malaga; the remarkable abundance of Alternaria and Mortierella in Jaén; and the greater abundance of Filobasidium in the roots, rhizosphere and soil under 'Arbequina'. In bacteria, DESeq2 analysis showed a significant differential abundance in Jaén when compared with the other 2 locations. Moreover, the main significant differences between ecological niches were found among rhizosphere and root (165 genus) with root and stem (92). The genotype reported only 1 significant genus between 'Arbequina' and 'Picual'. Similarly, fungal communities displayed the same significant differences according to ecological niche and field locations, but here the influence of the genotype was more noticeable between 'Arbequina' and 'Frantoio' (15 genera) and between 'Frantoio' and 'Picual' (10 genera). Alpha diversity showed the higher heterogeneity found in rhizosphere and soil niches from Jaén and ranked the diversity in decreasing order within the plant compartment from root, fruit, leaf, stem and xylem sap. Beta diversity determined that the main differences between bacteria and fungi communities (measured as phylogenetic distances) were due to the ecological niche of the olive tree (distinguishing especially the plant compartment from the soil compartment) followed by the environment in which the plants grow (environmental conditions) while the season (autumn or spring) and the genotype ('Picual', 'Arbequina', 'Frantoio') have a minor effect on the microbial community structure. Constrained analysis based on R2 higher than 1% selected 8 and 20 environmental variables in bacterial and fungal communities, respectively. The influence of the environmental temperature together with the internal temperature of the olive tree and the texture and availability of sodium (Na) in soil had a strong effect in both microbial communities differentiation. Co-occurrence network inference analysis in plant compartment presented a positive interaction between Actinobacteria and Proteobacteria, while the negative interaction was displayed by fungal communities mainly from phylum Ascomycota and Basidiomycota. In soil compartment the negative interaction of fungal communities was more visible, and Firmicutes plays a different role in soil than in plant compartment. Moreover, 15 keystone species were predicted in each compartment as important members to maintain the ecological interaction within the network.

FUTURE COLLABORATIONS (if applicable)

The present work is part of the first objective of my PhD focus on the characterization the structure and diversity of the olive microbiome under natural conditions based on the olive genotype, plant niche, environmental factors and their interaction. Hence, this research stay allowed me to gain experience and expand my knowledge of microbiome analysis, as well as being able to collaborate and exchange my background in the different bioinformatics processes with the host research group. In this way, we made an active collaboration between the both research groups that will be reflected thanks to the publication of a manuscript together. Moreover, both groups are open to participate jointly in future calls for projects and for student exchanges in order to strengthen this collaboration and advance together in the plant microbiome research field.