



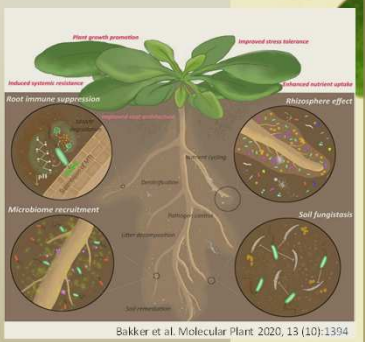
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Core bacteriome and keystone genera of the rhizosphere

INTRODUCTION

A large part of the microbes inhabiting soils live in association with plants constituting the plant microbiome. The interaction between plant and microbes is particularly close and important at the root level. This interaction has been defined through millennia of co-evolution and it plays a crucial role in plant health, nutrient uptake and stress tolerance. Microbes inhabiting the root plant compartment can be a significant ally for the plant in controlling colonization/infection by plant pathogens. NGS (Next Generation Sequencing) technologies allow the analysis of the total microbial population of a certain niche (microbiome). The current knowledge regarding the microbial community of the coffee plant is rather limited and the practical use of the microbiome data for a more sustainable agriculture is currently at large unexplored.



AIM OF THE PROJECT

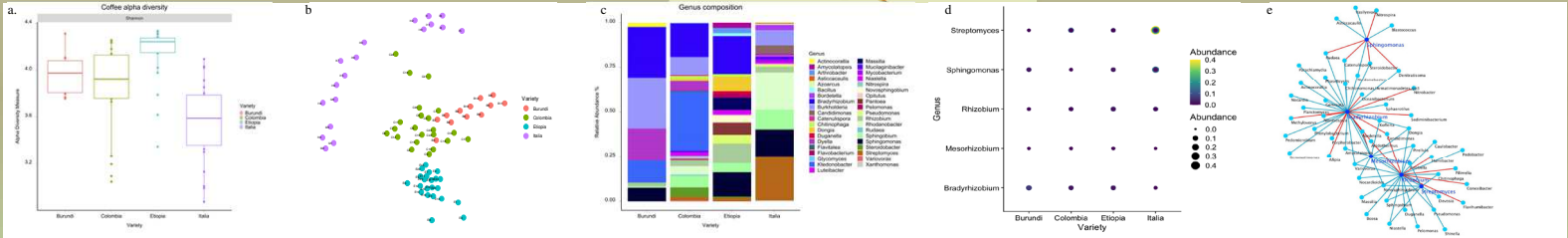
The study has been organized on 84 coffee root plant samples from 4 different geographical locations with the aim to provide information on the coffee "core bacteriome" (bacteria present across all environments) and the "variable bacteriome" (either environment- or genotype-specific bacteria). Understanding the core bacteriome in different geographical areas is the first important step to devise microbial biofertilizers and/or biopesticides products for sustainable coffee agriculture.

METHODOLOGY

The bacteriome analysis was performed on rhizospheric DNA extracted with the Power Soil DNA isolation kit (Qiagen). The 16S metagenomic library was prepared with the Illumina 16S Ribosomal RNA Gene Amplicons for the Illumina MiSeq System using Nextera XT Indexes. Libraries sequencing was performed using 2x250 bp MiSeq. Sequence reads generated were processed using scripts from Qiime2 and DADA2 v1.1.5 [1] and analysed in R using the package named phyloseq [2]. After the pre-processing, the sequences were grouped into OTUs (Operational Taxonomic Units) with a 97% of identity and each OTU was annotated with RDP reference database, giving a final OTU table.

Burundi, Colombia and Ethiopia (20 samples each from open fields);
Italy (24 samples from greenhouse growing plants);
Vietnam (in progress)

RESULTS



a. The alpha diversity estimation (Shannon index) of the different OTUs inhabiting the rhizosphere of plants growing in different geographical area, is quite similar; only the plants grown in Italy (in greenhouse and pot) show a lower a-diversity value.

b. Bacterial community composition shifts between open field grown plants and indoor grown plants. Samples from Burundi, Colombia and Ethiopia are partially overlapping suggesting that their bacteriomes are similar.

c. The bacteriomes at genus level of the samples from Burundi and Colombia are dominated, by similar genera (*Bradyrhizobium*, *Burkholderia*, *Dreella* and *Ktedonobacter*). The bacteriomes from Ethiopia and Italy are more biodiverse and characterized by genera with similar abundance. Only predominant genera (relative abundance >1%) were considered.

d. Keystone genera are characterized by their presence in the 99% of the samples independently from location, plant age and variety, agricultural practices etc. The 5 keystone taxa (abundance > 0.1%) are: *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, *Sphingomonas* and *Streptomyces*.

e. Co-occurrence network of interaction based on SparCC correlation values (>0.5) between the 5 keystone genera (labelled in yellow). The positive interaction are marked in blue, the negative ones are marked in red. *Bradyrhizobium* results as a central connection hub.

CONCLUSION/PROSPECTIVE

Data analysis is still ongoing and will be completed with the analysis of the Vietnamese samples. The results obtained suggests that Rhizobiales group plays an important role in the microbiome of coffee plants since 3 of the 5 keystone genera belong to this Order whose importance in the cycle and fixation of N₂ is fundamental. The wet work will now focus on isolating different strains belonging to these groups to test their plant biofertilization features and their potential development as bioinoculants for sustainable coffee plantations.

REFERENCE
 [1] Callahan *et al.*, 2016;
 [2] Mc Murdie and Holmes, 2013