Identifying indicators of soil suppressiveness to fungal diseases

Katarzyna Siegel, Sébastien Aimé, Emilie Chapelle, Véronique Edel-Hermann, Jos M. Raaijmakers, Philippe P. Lemanceau, Christian Steinberg

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Subtitle : Ecofinders: Ecological Function and Biodiversity Indicators

K. Siegel[1], S. Aimé[1], E. Chapelle[2], V. Edel-Herman[1], J. Raaijmakers[2], P. Lemanceau[1*], C. Steinberg[1]

[1] INRA, UMR 1347 Agroécologie, 17 rue Sully, BP 86510, 21065 Dijon, France ; (Phone: +33 380 693 051, E-mail: katarzyna.siegel@dijon.inra.fr),
[2] Wageningen University; Bld 107, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands
* *Coordinator of the EU project Ecofinders FP7-ENV-2010-264465*

Background

Soils suppressive to soil-borne diseases are defined by a low disease incidence in spite of the presence of a virulent pathogen and a susceptible plant.
A better survival of host plant (figure 1) is generally observed in suppressive soils (red curve) compared to conducive soil (blue curve). Moreover, the suppressive effect of a suppressive soil can be transmitted to the conducive soil by mixing 10% of the former into the latter (green curve). Therefore, we assume a biotic origin of the soil suppressiveness based on the activity of the resident **soil microbiome.** Such activity may include competition, antibiosis, and mycoparasitism towards the pathogenic fungi, as well as an induction of the plant defense reactions. These interactions among fungi and bacteria in the rhizosphere of the host plant are probably regulated by the abiotic environment but **the microorganisms involved in these interactions are still not identified.**
Metagenomic approaches could be a way to evaluate and compare the microbial diversities of suppressive and conducive soils in order to depict fungal and/or bacterial taxa associated to the character.

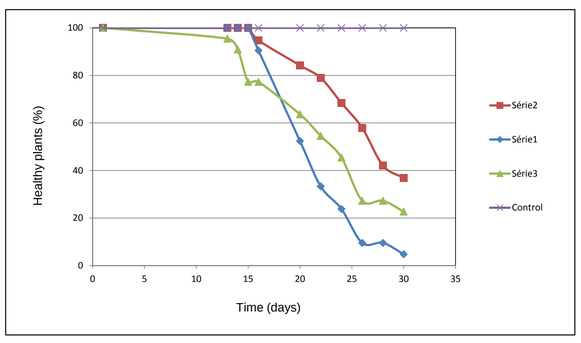


Fig1. Percentage of healthy plants remaining in the suppressive soil (SS), conducive soil (CS) and mix of both (CS+SS). Soils were inoculated with pathogen (10^3 conidia/ml of soil). Control: soil not inoculated.

Objectives

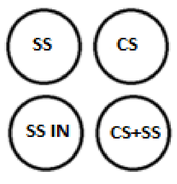
Identification of **taxonomic microbial indicators** of suppressive phenotype of soils by estimation and comparison of microbial biodiversity in two different soils suppressive to either ***Rhizoctonia solani* damping-off disease** of sugar beet and ***Fusarium* wilt disease** and check if this indicators are common to both soils**.** In the present situation, only soil suppressiveness to *Fusarium* wilt is reported.

Root necrosis of sugar beet caused by *Rhizoctonia solani*

Fusarium wilt of melon

Experimental setup

Assessing of bacterial & fungal soil diversity



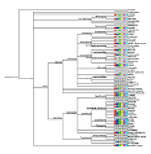
Greenhouse bio-assay

SS: Suppressive soil
CS: Conducive soil
SS IN: Suppressive soil inoculated with pathogen
CS+SS: Conducive soil amended with 10% of suppressive soil

Rhizospheric soil collection

16S (bacteria) and ITS1 (fungi) rDNA amplifications

454 Amplicon pyrosequencing



Bioinformatic analysis

Progress

Assessement of fungal diversity of *Fusarium* wilt suppressive soil

125 602 reads generated by pyrosequencing, **114 641** reads kept after filtering by bioinformatic pipeline (*J. Langellé, INRA Nancy*):

Raw sff files → Trimming & denoising → Extract ITS region (Fungal ITS Extractor) → Select ITS region>100pb → Clustering at 97% identity (Uclust) → BLAST against UNITE database → OTU clustering according to taxonomic assignments

OTUs richness

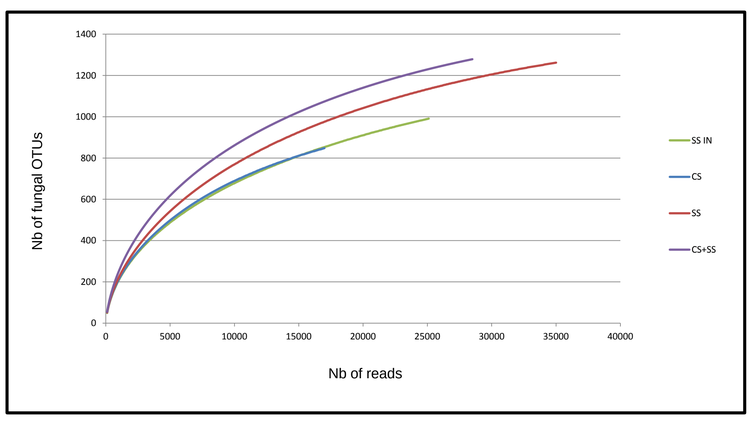


Fig2. Rarefaction curves : effect of internal transcribed spacer (ITS) sequence number on the number of operational taxonomic units (OTUs) identified from the four soil modalities. OTUs were generated at 3% sequence dissimilarity. Single-singletons (OTUs supported by only one read) were excluded from analysis.
X, number of sequences; Y, number of observed OTUs.

The number of OTUs increased with the number of reads but the plot of OTUs vs ITS1 sequences resulted in rarefaction curves that did not reached a plateau in spite of the large number of reads, what is expected in the case of soil metagenomics analysis.

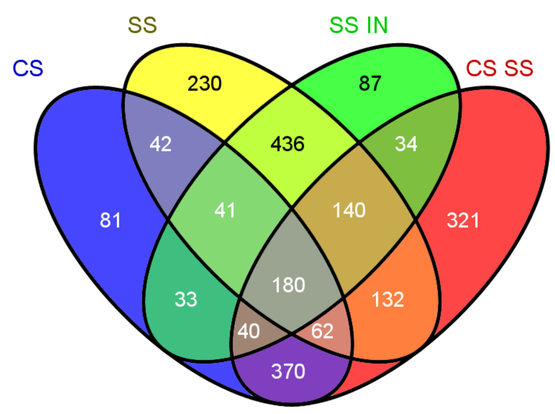


Fig3. Venn diagram indicating shared and unique observed operational taxonomic units (OTUs) between disease conducive soil (CS), disease suppressive soil (SS), suppressive soil inoculated with *F. oxysporum f.sp. lini* (SS IN) and conducive soil with 10% of suppressive soil (CS+SS).
SS and CS include respectively 1264 and 849 OTUs among which 325 are shared by both soils.

Taxonomic analysis

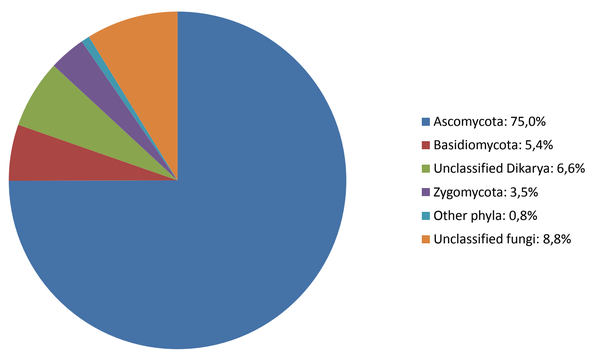


Fig4. Taxonomical distribution of different phyla and fungal groups in the rhizosphere microbiome of flax plants grown in soils with different levels of disease suppressiveness (sum of the fungal abundance of the four modalities).

Ascomycota: 75,0%, Basidiomycota: 5,4%, Unclassified Dikarya: 6,6%, Zygomycota: 3,5%, other phyla (Chytridiomycota, Glomeromycota, Blastocladiomycota): 0,8%, Unclassified fungi: 8,8%.

Conclusions & Perspectives

Although, bioinformatic and statistical analyses are not finished yet, **we can already notice a significant difference in the taxonomic diversity composition between suppressive and conducive soils which could be associated with the suppressive/conducive character of given soil**.
The next step after achieving the bacterial communities in *Fusarium* wilt suppressive/conducive soils is to assess the microbial diversity of *Rhizoctonia solani* suppressive soil.

Once the analyses of sequencing data are finished and the taxonomic assignments done, the comparison of microbial diversity of all studied soils will be performed in order to find out the similarities or/and differences in these soils which should provide the suppressiveness indicators.