Artificial selection of rhizosphere microbial communities associated with plant resistance to leaf pathogens

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1. The soilborne supremacy

The rhizosphere microbiome contributes to host nutrient acquisition, protection against biotic and abiotic stresses and, ultimately, changes in plant development and physiology. Due to plant domestication, these microbiomes have been severely mutilated, reducing plant fitness, especially during pathogenic attacks[1,2]. Several studies have led to the development of commercial products that uses these beneficial effects of soil microbes on plant health. However, the sustainability of these isolated strains after inoculation into natural soil needs to be optimized. In these purpose, identifying healthy and functionally diverse microbiomes and their application for enhancing crop yield represent another big and necessary challenge to venture[1].

2. Description of the project

The aim of the current project is to go from the classical dissected approach (e.g. isolating one specific strain) to a holistic one, as it exists in nature[3]. Our aim is to better understand the impact of rhizospheric microbiome in its entire complexity. In this purpose, we initiated experiments to artificially select rhizospheric microbial communities which, when co culivated with *Arabidopsis thaliana,* are associated with a change in its resistance to the foliar pathogen *Pseudomonas syringae* DC 3000 (Figure 1). Besides, we propose in parallel an original *in vitro* setup (Figure 2) in which Arabidopsis seedlings are co cultivated with soil bacteria *in vitro*. The response to the bacterial peptide Flg22 -which mimics pathogen attack- will be assessed.

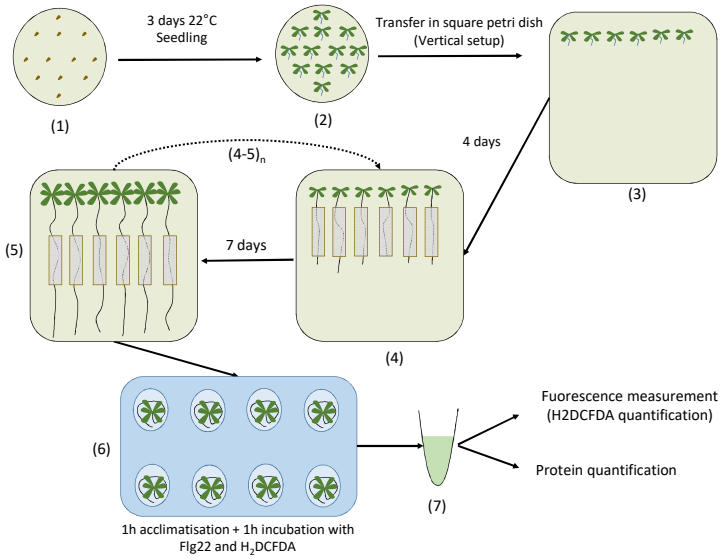

Figure 2. Original *in vitro* setup for co-cultivation of plants and soil microbes under gnotobiotic conditions. Plants were sown and grown vertically on MS ½ medium (1-2) then transferred (3) into square Petri dishes three days after sowing (d.a.s). Soil microbes are inoculated on rectangle glass fibre filter 7 d.a.s and put in contact of plants roots (4). *In vitro* assessment of plant response to flagellin is done 14 d.a.s. Briefly, plants are transferred into 8 well plates containing liquid buffer and acclimated for 1 hour. Then, bacterial peptide (Flg22) and ROS fluorescent probe (H_2DCFDA) are added (6). One hour later, plants are grinded and fluorescence emission and protein content is measured (7).

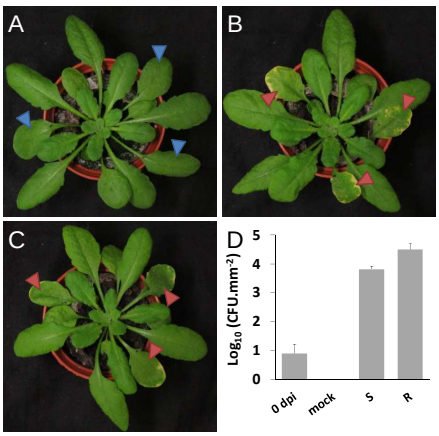

Figure 3: Visual assessment of symptoms associated to *Pst* DC3000 infection on *Arabidopsis thaliana* leaves. (A) Mock ($MgCl_2$) treated plant. (B and C) Symptoms classification on *Pst* DC3000 inoculated plants: (B) Susceptible, (C) Resistant. Blue and red arrows point out mock infiltrated leaves and *Pst* DC3000 infiltrated leaves respectively. (D) CFU measurement at 0 d.p.i and 7 d.p.i on mock treated plant (3.A) and susceptible or resistant *Pst* DC3000 inoculated plants (3.B and 3.C respectively). S and R stands for susceptible and resistant respectively. Data shows means ± S.D. on 3 different leaves.

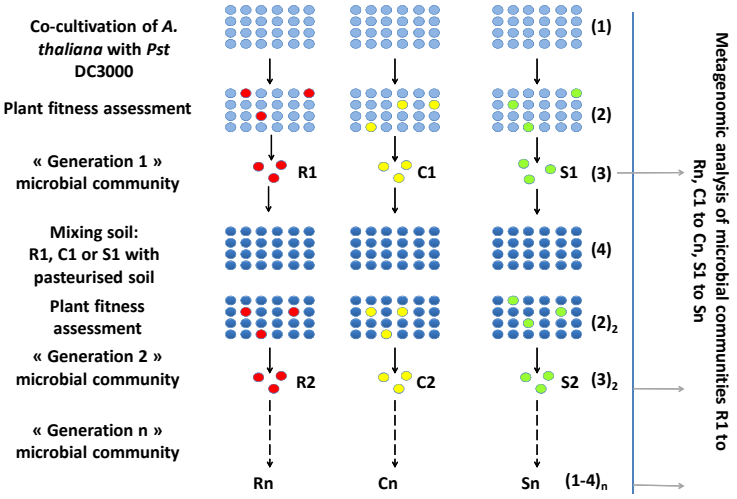

Figure 1. Experimental procedure for iterative selection of soil organisms associated to modifications of *A. thaliana* fitness in response to *Pst* DC3000. Briefly, plants grown in natural soil will be inoculated with *Pst* DC3000 (1). Seven days post-inoculation (d.p.i), rhizospheric bacterial community (2-3) associated with a higher resistance (R), lesser susceptibility (S) or selected randomly (control, C) will be retrieved and inoculated into the new "generation" (i.e. newly batch of soil in which plants shall be cultivated) (4). Steps 1 to 4 will be repeated "n" times (5). At each "generation" one aliquot of soil will be kept for future metagenomics analysis.

3. Expected results:

After few generations, we can expect to have a divergence between the different groups (Figure 4) with the plants grown with the suppressive soil microbial communities (e.g. reducing disease symptoms) having a better development in opposition with the conductive ones (e.g. enhancing disease symptoms). For the control group we expect no changes in plant responses to the pathogen since experimental setups should not influence directly soil microbial community dynamics. Metagenomics analysis will allow to unravel if these differences could be attributed to changes in soil microbial communities.

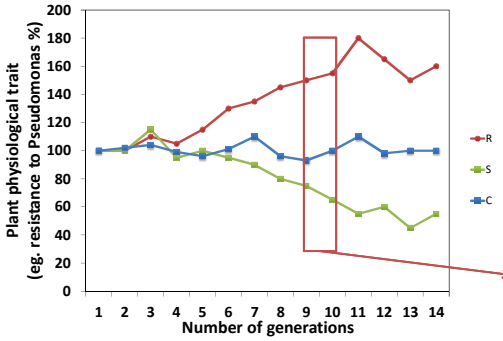

Figure 4. Expected evolution of plant physiological trait following iterative artificial selection of the 3 groups of interest (resistant (R), susceptible (S) and control (C)).

4. Main outcome:

As microbial populations indigenous to the rhizosphere can changes plant development and physiology, active manipulation of this community may be an effective means to suppress plant pathogens[1-3]. This approach should be associated to genetic studies (use of different cultivars and mutants) as plant genetic traits contribute to microbiome composition[6]. Ultimately, this will led to developing new plant breeding strategies to optimize plant-beneficial microbe interactions in crops. Root microbiota therefore represent an untapped trait in current plant breeding strategies. Through the selection of host genotypes that capture an optimal microbiota from a given soil type this will allow to reduce synthetic fertilizer and pest control inputs[1,3].

5. References:

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