Deciphering the cross-talk between growth and microbiota recognition at the root-soil interface

Plants are in a situation of constant negotiation of resources between increased growth or defense against pathogens. At the molecular level, this negotiation is mirrored by the intertwining of genetic components underlying growth and defense responses. While studies conducted on aboveground tissues (e.g. leaves) contributed to elucidating these processes, little is known of the mechanisms mediating the growth-defense trade-off in the rhizosphere. The rhizosphere is defined as the layer of soil that is firmly attached to plant roots. It has been shown that the rhizosphere is a distinct microbial environment compared to unplanted soil, and that both the plant genotype and the abiotic environment can play a role in shaping the rhizosphere microbiota. Due to the complex interactions in the rhizosphere, the plant is exposed to the confounding dilemma of distinguishing between pathogens and non-pathogens in this very confined space. Typical plant-responses to pathogen attack such as MAMP (microbe associated molecular patterns) triggered immunity (MTI) could also harm beneficial bacteria. Elucidating the complex interactions in the rhizosphere microbiota could lead to a more comprehensive understanding of the growth-defense dilemma in the context of host-microbe interactions. To gain further insights into these phenomena, I performed two experiments during my internship.

1) Application of the MTI elicitors flg22 from *Pseudomonas* (flg22*Psy*) and from *Agrobacterium* (flg22*Agro*) directly to planted and unplanted soil. This experiment aimed at exploring the effect of elicitors of plant immune response on the composition of the microbiota. Barley seeds (cv. Bowman) were germinated in petri dishes, transplanted in soil and grown under controlled environmental conditions. Two and nine days after transplantation I applied a solution containing either flg22*Psy* or flg22*Agro* directly to soil to elicit plant immune responses at the root-soil interface. In parallel, unplanted soil controls as well as mock inoculated controls were analysed. 14 days after transplantation I excavated the plants from the soil, collected the rhizosphere from the individual samples, and extracted DNA from the rhizosphere of these specimens. I used barcoded PCR primers to selectively amplify the 16S rRNA gene from the DNA preparation and generated a sequencing library. This library has been sequenced using Illumina technology and the data have been processed using R. Data analysis revealed that the exogenous application of flg22*Psy*, but not flg22*Agro,* interfere with the recruitment of a very limited number of members of the barley microbiota. Although these preliminary data need to be further validated, this observation may implicate elicitors of plant immune response in the recruitment and modification of the rhizosphere microbiota.

2) Growing of barley-mutants deficient in brassinosteroid signalling aimed at exploring the effect of the plant genotype and cross-talk between growth and immune responses on the composition of the microbiota. A number of barley genotypes with mutations in the brassinosteroid pathways were grown under controlled conditions for 14 days without exogenous application of an immune response elicitor. This experiment was conducted using two different soil types and including bulk soil and *wildtype* controls. The DNA has been extracted and processed. A sequencing library is currently under construction. I will have the opportunity to continue this investigation further during an MSci that follows on from this project. My ultimate goal will be to gain more detailed insights into the significance of immune responses in the establishment of the plant microbiota.

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Left panel: Barley seedlings growing in two contrasting soil types.

Right panel: Root of barley plant grown for 14 days before extraction of the rhizosphere.

I would like to thank the Bulgarelli lab for hosting me for this internship and for providing me with the opportunity to extending my knowledge and skillset in this exciting research area. I would also like to thank the BSPP for funding this internship.

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