

Rhizosphere interactions for disease suppression and biocontrol

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INTRODUCTION

Microbial communities play a pivotal role in the functioning of superior organisms by influencing their physiology and development (1). While many members of the rhizosphere microbiome are beneficial to plant growth, also plant pathogenic microorganisms colonize the rhizosphere striving to break through the protective microbial shield and to overcome the innate plant defense mechanisms in order to cause disease (2). To infect root tissue, pathogens have to compete with members of the rhizosphere microbiome for available nutrients and microsites. In disease-suppressive soils, pathogens are strongly restricted in growth by the activities of specific rhizosphere microorganisms (3).

Using metagenomics and metatranscriptomics studies we postulate that the invading pathogen induces, directly or via the plant, stress responses in the rhizobacterial community that lead to shifts in microbiome composition and to activation of antagonistic traits that restrict pathogen infection. Therefore, upon attack by a fungal root pathogen, plants can exploit microbial consortia from soil for protection against infections (4). In this context, we hypothesized that breeding towards resistance against soilborne pathogens influences the assembly of microbial communities and the abundance of specific functional traits associated with protection provided by the rhizosphere microbiome (5).

Here, we will discuss recent discoveries on rhizosphere interactions and soilborne pathogens aiming to elucidate the role of the rhizosphere microbiome in reducing disease impacts.

MATERIALS AND METHODS

Fungal invasion of the rhizosphere microbiome: The suppressive soil and pathosystem discussed here were previously described in Mendes et al. (2011). The soil shown to be suppressive to *Rhizoctonia solani* while infecting seedlings of sugar beet. To understand the mechanisms underlying the disease suppression, we used the suppressive soil to grow sugar beet and inoculated the pathogen *R. solani* (4). Total rhizosphere DNA was obtained and used for metagenomic and metatranscriptomics analyses to identify microbial taxa and functions related with disease suppression.

Rhizosphere microbiome assembly in a plant genotype resistant to soilborne pathogen: The methodological approach used is described in detail in Mendes et al. (2018), where four cultivars of common bean were selected with basis on their level of resistance against *Fusarium oxysporum*, including a resistant and a susceptible genotype and two moderately resistant genotypes (5). The rhizosphere microbiome was assessed in these four genotypes by 16S rRNA and shotgun sequencing and the data was used to

understand the rhizosphere microbiome assembly in genotypes with different levels of disease resistance.

RESULTS AND DISCUSSION

Changes in rhizosphere microbiome during fungal invasion: In the experiment where we evaluated the fungal invasion of the rhizosphere microbiome, we propose a model (Figure 1) in which the pathogenic fungus produces oxalic and phenylacetic acid during hyphal growth toward the plant root. These compounds feed and activate specific rhizobacterial families present in the suppressive rhizosphere microbiome, and, directly or indirectly, exert oxidative stress in specific rhizobacterial families and in the plant. This stress in turn triggers a response in these bacterial families via the ppGpp signaling pathway, leading to the activation of survival strategies such as motility, biofilm formation and the production of secondary metabolites.

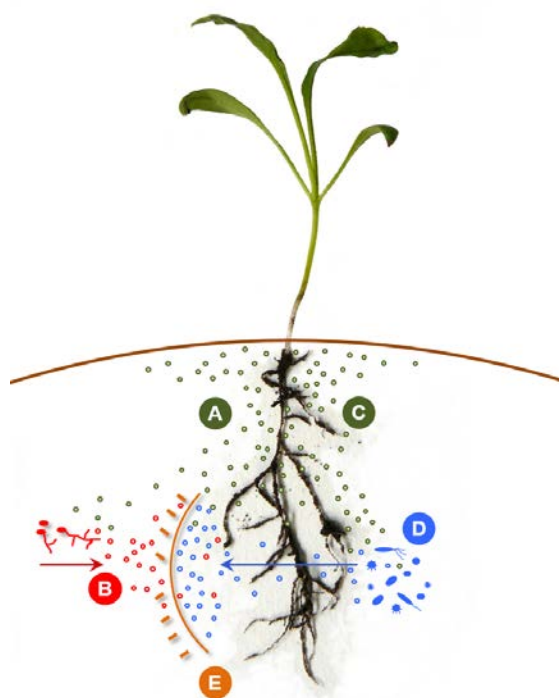


Figure 1. Sequence of events taking place in the rhizosphere of plants grown in a disease suppressive soil during fungal pathogen invasion. A) Root exudates structure and modulate the rhizosphere bacterial community and trigger fungal pathogen germination and hyphal growth, B) *Rhizoctonia solani* grows toward the root system to infect the plant and exerts oxidative and acidic stress, C) plant responds to oxalic acid, D) rhizosphere bacterial community responds to root exudates and fungal invasion by enriching and activating specific bacterial families (e.g. *Oxalobacteraceae* and *Burkholderiaceae*) and activating functions related to general bacterial stress response (e.g. HtrA/Sec, (p)ppGpp alarmone metabolism and oxidative stress), and E) Plant roots are shielded from fungal infection. (adapted from 4).

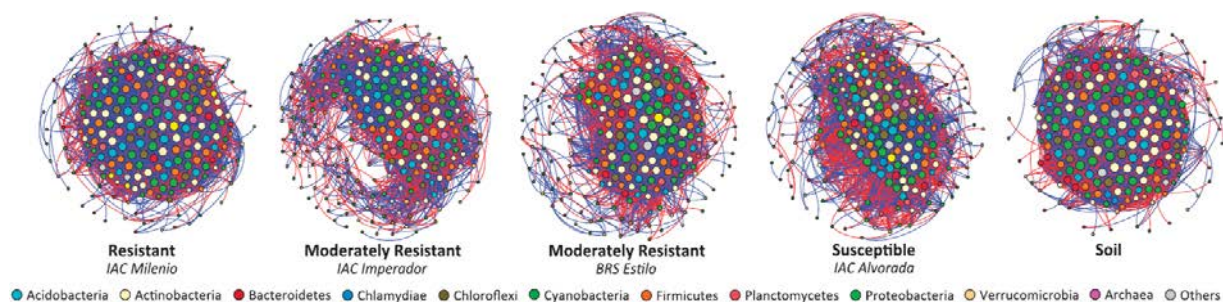


Figure 2. Network co-occurrence analysis of microbial communities of rhizosphere and soil samples. Each node represents taxa affiliated at genus level (based on 16S rRNA), and the size of node is proportional to the number of connections. (from 5).

Metatranscriptomics data pointed that *Oxalobacteraceae*, *Burkholderiaceae*, *Sphingobacteriaceae* and *Sphingomonadaceae* were significantly more abundant in the rhizosphere upon fungal invasion and metatranscriptomics revealed that stress-related genes (ppGpp metabolism and oxidative stress) were upregulated in these bacterial families.

The results suggest that in suppressive soils the rhizosphere microbiomes acts as a first line of defense against root infections by soilborne pathogens. The invasion by a pathogen causes changes in the rhizosphere microbiome composition and functions negatively affecting fungal growth, inducing a plant resistance response, and/or co-activating other microorganisms in the rhizosphere microbiome to ward off the fungal invader.

Changes in rhizosphere microbiome assembly on plant genotype resistant to soilborne pathogen

The comparison of four contrasting common bean cultivars with different levels of resistance against *F. oxysporum* showed differences not only in composition and functions of the rhizosphere microbiome, but also in the assembly of the microbiome as revealed by network analysis (5).

Interestingly, rhizobacterial abundance was positively correlated with *F. oxysporum* resistance; *Pseudomonadaceae*, *Bacillaceae*, *Solibacteraceae* and *Cytophagaceae* were more abundant in the rhizosphere of the *F. oxysporum* resistant cultivar. Metagenome analyses further revealed that specific functional traits such as protein secretion systems and biosynthesis genes of antifungal phenazines and rhamnolipids were also more abundant in the rhizobacterial community of the resistant cultivar.

The common bean genotype resistant to the soilborne pathogen showed the highest level of complexity and modular structure as revealed by network analysis, when compared with the susceptible genotype (Figure 2). This fact suggests that the underlying interaction network architecture may explain the invasion resistance observed in the genotype able to recruit and support a more complex rhizosphere microbiome.

SUMMARY

- Invading soilborne pathogen induces, directly or via the plant, stress responses in the rhizobacterial community that lead to shifts in microbiome composition and to activation of antagonistic traits that restrict pathogen infection.
- Breeding for soilborne diseases may have co-selected for other unknown plant traits that support a higher abundance of specific beneficial bacterial families in the rhizosphere with functional traits that reinforce the first line of defense.

ACKNOWLEDGEMENTS

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REFERENCES

1. Mendes R, Raaijmakers JM (2015) Cross-kingdom similarities in microbiome functions. *The ISME Journal* 9:1905-1907.
2. Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiology Reviews* 37:634-663.
3. Mendes R, Kruijt M, De Bruijn I, Dekkers E, Van Der Voort M, Schneider JHM, Piceno YM, Desantis TZ, Andersen GL, Bakker PAHM, Raaijmakers JM (2011) Deciphering the Rhizosphere Microbiome for Disease-Suppressive Bacteria. *Science* 332:1097-1100.
4. Chapelle E, Mendes R, Bakker PAHM, Raaijmakers JM (2016) Fungal invasion of the rhizosphere microbiome. *The ISME Journal* 10:265-268.
5. Mendes LW, Raaijmakers JM, De Hollander M, Mendes R, Tsai SM (2018) Influence of resistance breeding in common bean on rhizosphere microbiome composition and function. *ISME Journal*, 12:212-224.