Modulation of plant chemistry by rhizosphere bacteria

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Plants and microbes have a history of coevolution that extends over 450 million years. The rhizosphere encompasses a few millimeters of soil layer surrounding and influenced by plant roots. This zone is rich in organic compounds released by roots and is a hot spot for microbial activity and plant-microbe interactions. Plant-microbe interactions in the rhizosphere can have a positive, negative or neutral influence on plant fitness. The rhizosphere bacteria with beneficial effects on plant growth and health are also referred to as plant growth-promoting rhizobacteria (PGPR). Recent studies also revealed that PGPR can alter plant chemistry which in turn can lead to effective or ineffective partnerships. Hence, understanding the chemical continuum between plants and microbes during their interaction could provide us with new tools to modulate plant growth, defense and the level of high value natural plant products (HVNP). The **overall aim of this thesis** was to investigate the impact of rhizobacteria on plant metabolism and how these changes are associated with plant growth and plant defense. Factorial combinations of different plant species, including *Arabidopsis thaliana* (model plant), *Brassica oleracea* var. italica (crop) and *Artemisia annua* (medicinal plant), and phylogenetically distinct rhizobacterial species, including *Pseudomonas fluorescens* SS101 (*Pf* SS101), *Microbacterium* and three *Paraburkholderia* species, were used as study model systems in this thesis. Untargeted metabolomics was used to assess the impact of these rhizobacteria on the shoot chemistry of the host plant species. Transcriptome profiling was employed to assess the impact of the host on the gene expression in the rhizobacteria.

A series of *in vitro* bioassays revealed specific phenotypic and chemotypic responses of the host plants to rhizobacterial colonization. For example, *P. fluorescens* (*Pf* SS101) established an effective partnership with Arabidopsis and Artemisia while in Broccoli it led to significant reduction in shoot biomass. Similarly, *P. graminis* (*Pbg*) exhibited an effective partnership with Artemisia and Broccoli while its partnership with Arabidopsis was characterized by stunted growth with concomitant accumulation of stress-related metabolites in the leaves. Untargeted metabolomics demonstrated that under ineffective partnerships i.e. *Pf* SS101-Broccoli and *Pbg*-Arabidopsis, secondary metabolites downstream of the phenylpropanoid pathway, such as flavonoids, anthocyanin and stilbenoids, showed a sharp increase. These classes of metabolites were either suppressed or showed no change in effective partnerships. Particularly, flavonoid accumulation was associated with retarded plant growth most likely by interfering with auxin transport, distribution and turnover. This study revealed that root treatment of different plant species with rhizobacteria altered 18-78% of the detected plant secondary metabolites in the shoot. Fueling such carbon skeleton and energy demanding or competing plant responses without compromising plant growth requires a robust metabolic regulation. The combined primary and secondary metabolite analysis revealed that rhizobacterial treatment boosted the production of soluble sugars in the plant shoot, potentially enabling the plant to accommodate the high demand for energy and carbon required for enhanced growth and secondary metabolite production. Fructose was the

central target of rhizobacteria in the plant shoot. Rhizobacteria-treated plants showed more than 280-fold increase in fructose abundance when compared to control plants. Fructose is the primary substrate for fructose-6-phosphate, a key substrate for the biosynthesis of phosphoenolpyruvate and erythros-4-phosphate. These two intermediates are the pillars of energy and secondary metabolism. Furthermore, fructose is one of the potent chemotaxis agent for bacteria. By targeting such multipurpose metabolite, rhizobacteria could potentially alter multiple processes in plants to establish an effective partnership. *Paraburkholderia* species were also able to induce a systemic resistance response (ISR) in Broccoli against the bacterial leaf pathogen *Xanthomonas campestris*. The causal relationship between the induced defense response and the induced metabolites remains to be resolved.

Beyond their impact on plant growth and defense, rhizobacteria treatment also showed their effectiveness in boosting the indigenous level of a number of metabolites with nutritional, health promoting and pharmaceutical importance. For instance, *Pbg*-Artemisia significantly upregulated the abundance of dihydroartemisinin, an antimalarial agent, whereas *Pbg* and *Pf* SS101 boosted several indolic glucosinolates, cancer chemo preventive agents in Broccoli. Considering the greater impact of rhizobacteria on the host phenotype and chemotype, we also assessed the impact of a known bacterial trait on plant phenotype and chemistry. Previous studies that employed genome-wide analysis of *Pf* SS101 followed by mutagenesis and genetic complementation revealed that sulfur assimilation attested by *cysH* gene, plays an important role in the induction of growth and defense in Arabidopsis. Our current study revealed that the *cysH* mutation in *Pf* SS101 affected the chain elongation step of aliphatic glucosinolate biosynthesis in Arabidopsis whereas in Broccoli, the *cysH* mutation led to an accumulation of indolic glucosinolates and flavonoids. These results indicated that sulfur assimilation in *Pf* SS101 modulates shoot metabolism in a plant species specific manner. In the "blind date experiment" that aimed at finding the right partnership between different rhizobacteria and host plants, *Pbg*-Broccoli exhibited an effective partnership that led to significant increase in shoot biomass and changes in shoot metabolome. To shed light on the bacterial traits that are altered by the host during their interaction, genome wide transcriptome analysis was carried out on *Pbg* grown in the presence and absence of the host. Among the differently expressed genes (DEGs) in *Pbg*, genes involved in flagellar assembly, chemotaxis, and motility together with nutrient uptake and (an)ion transporter were upregulated in the presence of the host. Future studies that examine the role of plant root exudates in the modulation of rhizobacteria gene expression will be instrumental in our quest for understanding architecture of the chemical continuum between plants and their rhizosphere microbiome.

Integrating *in vitro* assay, plant phenotyping and plant chemotyping by state-of-the-art technologies, this thesis provided new insights into the strong influence rhizobacteria and plants have on each other. The findings of this thesis can potentially feed into the theory that asserts plant and their microbiome as multipartite entities potentially co-evolving as a holobiont. Plant breeding strategies and agricultural practices that gear towards steering the

composition and function of plant microbiomes in such a way to influence plant phenotypic and chemical traits will be the next frontier in agriculture and will have a profound contribution for the next green revolution.