Modulation of plant chemistry by rhizosphere bacteria

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Chapter 7

General discussion

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Plant growth-promoting rhizobacteria (PGPR) are bacteria that colonize roots of specific or several plant species and promote plant growth directly or indirectly by suppressing pests or diseases. In recent years, advances in mass spectrometry and chemo-informatics are contributing immensely to our understanding of the impact of rhizobacteria on the chemistry, providing a mechanistic understanding of growth promoting and disease suppression but also new opportunities to explore and exploit rhizobacteria as a new platform to produce high value natural plant products (HVNP) (Etalo *et al.*, 2018). To date, the majority of studies on rhizobacteria-mediated reprogramming of plant chemistry are descriptive in nature and provide limited insights into the biological relevance of the changes induced by the rhizobacteria in the plant metabolome and if or how these changes affect host phenotype. Particularly most information on rhizobacteria-induced changes in common and specific metabolic pathways that are central to plant growth and defense is either preliminary or non-existent. When it comes to the rhizobacteria side, if and how the intrinsic or altered metabolic state of the host influences rhizobacterial traits that are directly or indirectly affecting host fitness is still an undiscovered island. On the technological side, the majority of past and present studies employed targeted metabolite profiling and those that used untargeted metabolomics are often bound to either profiling of primary metabolites or focused on a specific subset of secondary metabolites. To address these knowledge gaps and to advance our understanding of the chemical continuum between rhizobacteria and their host, my thesis focused on investigating the impact of three bacteria genera comprising five bacterial species (*Pseudomonas fluorescens* SS101, *Microbacterium*, and *Paraburkholderia graminis*, *P. hospita*, and *P. terricola*) on different phenotypes and on the shoot metabolome of three plant species (*Arabidopsis thaliana*, *Artemisia annua*, *Brassica oleracea* var. *italica*).

The **aims of my thesis** were to i) identify common and specific changes in the plant metabolome induced by different rhizobacterial species ii) link rhizobacteria-induced changes in plant chemistry to other plant phenotypes, iii) identify rhizobacterial genes, pathways and traits associated with changes in plant chemistry and plant phenotypes. Although the results of the experiments conducted in my thesis do not provide all the answers to the above-mentioned questions, it does give new insights into the diverse effects of rhizobacteria on the host metabolome and the potential impact of the metabolome changes on host fitness. Furthermore, with the magnitude of data generated during the course of my PhD study more questions were generated than answered. Here, I will discuss the major findings as well as the limitations of my study and provide future directions to investigate the chemical continuum between rhizobacteria and plants.

1. Are plants the sole masters of their metabolic regulation?

Plants synthesize a vast array of secondary metabolites. Several metabolites from wild or cultivated plant have shown pharmaceutical activities either as metabolite itself or as a

scaffold to synthesize pharmaceutically improved drugs candidates (Oksman-Caldentey & Inzé, 2004; Etalo *et al.*, 2018). Because of this, a great deal of efforts has been invested in identifying factors that influence the biosynthesis, metabolic fluxes, transport and storage of plant metabolites with medicinal and nutritional value for human and animals as well as metabolites contributing to plant tolerance against (a)biotic stress factors. Earlier studies focused more on the impact of external environmental conditions such as light, drought, temperature and edaphic factors on primary and secondary plant metabolism (Gautier *et al.*, 2008; Griesser *et al.*, 2015; Yang *et al.*, 2018). Recently the impact of rhizobacteria and other plant-associated microbes, collectively referred to as the plant microbiome, on metabolism of their host is gaining significant attraction (Etalo *et al.*, 2018; Korenblum & Aharoni, 2019). Particular interest also emerged for metabolites that were initially thought to be of plant origin but now appear to be either exclusively or in part synthesized by the microbes that live on or in the host plant tissue (Kusari *et al.*, 2011; Kusari *et al.*, 2014; Ludwig-Muller, 2015). Such evidences of a microbial footprint on the host metabolome not only signifies the importance of microbes in plant metabolism but also poses questions on the long-held assumption that portrays plants as the sole masters of their metabolism.

To have a broader understanding of the specific impact of rhizobacteria on the host metabolism, we performed non-targeted metabolomics in **Chapters 3**, **4**, and **5** and revealed that different rhizobacteria-plants combinations can significantly (18-74%) perturb the relative abundance or presence/absence of the host metabolome when compared to non-bacterized plants. For example, in **Chapter 3**, root tip inoculation of Arabidopsis with *Pseudomonas fluorescens* SS101, *Microbacterium* EC8, or *Paraburkholderia graminis* significantly altered 64% of secondary metabolites in the shoot in comparison with non-treated control. Interestingly, in **Chapter 4** root tip inoculation of two Broccoli cultivars Coronado and Malibu with three different *Paraburkholderia* species led to 49% and 74% alteration of their shoot metabolome, respectively. These results strongly suggest that rhizobacteria have substantial effect on their host metabolome and that the magnitude of alteration of the host metabolome is highly dependent on the interacting partners. Beyond their overarching impact on the metabolome, we also showed that rhizobacteria can modulate specific metabolic pathways of their host. In **Chapters 3** and **4,** we showed that the phenylpropanoid pathways is the prime target of rhizobacteria and its induction is associated with enhanced plant defense against bacterial pathogens. Similarly, I showed in **Chapter 4** that rhizobacteria influence both sugar generation and utilization of their host and the combined effect showed an association with plant growth. Results shown in my thesis and by other researchers magnifies the enormous impact of rhizobacteria on their host metabolism, growth and defense and force us to re-think both the mechanisms underlying the regulation of the metabolic pathways. On the other side, results in **Chapter 6** showed that plants also have big impact on rhizobacteria transcriptome landscape with significant variation between different plant cultivars. Perhaps this way of looking at the partnership between plants and rhizobacteria could help to redefine the question of who is the driver of the association. Altogether, mechanisms governing the

partnership between plants and rhizobacteria are still largely elusive as microbe-host plant interaction is the product of over 450 million years of co-evolution (Zhalnina *et al.*, 2018).

2. Metabolic signatures of plant growth, defense and their trade-off

A recent study on closely related strains within the *Pseudomonas fluorescens* species complex tied the transition between pathogenic and commensal bacterial life style in Arabidopsis to particular genetic loci such as lipopeptide/quorum island whose presence is essential to pathogenicity in the bacterium (Melnyk *et al.*, 2019). Contrary to that, both phenotype- and metabolome-based assessment of the host plants in our "blind date experiment" involving Arabidopsis, Artemisia, and Broccoli as hosts and *Pseudomonas fluorescens* SS101 (*Pf* SS101), *Microbacterium* (MB) and *Paraburkholderia graminis* (*Pbg*) as interacting rhizobacteria revealed that none of the rhizobacterial genera promoted plant growth of all plant species (**Chapter 3**). While *Pf* SS101 enhanced biomass of Arabidopsis and Artemisia, it reduced biomass of Broccoli when compared to the non-treated control. Likewise, *Pbg* root inoculation enhanced growth of Artemisia and Broccoli but showed detrimental effects on Arabidopsis growth. The shoot metabolome profiles of Broccoli treated with *Pf* SS101 and Arabidopsis treated with *Pbg* were characterized by the accumulation of defensive and stress-related phenolic metabolites such as anthocyanin. Moreover, *Paraburkholderia terricola* exhibited Broccoli cultivar dependent growth promotion (**Chapter 4**). These results strongly suggest that the impact of rhizobacteria on plant growth and health is not only linked to specific bacterial determinants but also to the genetic background of the interacting plant. During the course of my study, we used the metabolic signature of several rhizobacteria-plant interaction to understand metabolic networks governing growth, defense and the potential trade-off between these processes. Trade-off between growth and defense is a widely accepted concept and arises from resource limitation and differential allocation of resources for defense, growth and development (Herms & Mattson, 1992; Pieterse *et al.*, 2012; Lozano-Durán & Zipfel, 2015). Plant metabolites can be structurally categorized into five major groups, i.e. polyketides, isoprenoids (e.g. terpenoids), alkaloids, phenylpropanoids and flavonoids (Oksman-Caldentey & Inzé, 2004). Among others, metabolites that belong to phenylpropanoid pathways such as hydroxycinnamates and flavonoids represent the largest group of plant secondary metabolites, existing ubiquitously across the plant kingdom and playing important roles as chemical weapons against biotic stresses (Treutter, 2005; Korkina, 2007; War *et al.*, 2012). In **Chapters 3**, **4** and **5,** we showed that accumulation of metabolites belonging to the phenylpropanoid pathway, in particular hydroxycinnamates and flavonoids, was associated with ineffective partnerships between the plants and rhizobacteria tested, i.e. partnerships that had an adverse effect on specific phenotypes such as plant growth. The accumulation of defensive compounds such as hydroxycinnamates and flavonoids particularly the flavonol subclass could potentially affect plant growth through several ways. High abundance of flavonoids particularly the flavonol subclass may impact auxin transport (Besseau *et al.*, 2007), auxin biosynthesis and its conjugation/degradation

(Kuhn *et al.*, 2016). Additionally, the higher accumulation of phenylpropanoids and other carbon and energy-costly secondary metabolites in the ineffective partnerships could pose resource limitation to plant growth-related processes and lead to an adverse effect on plant growth. For example, the metabolic cost for pro-anthocyanidins and lignin is similar to that of biosynthesis of protein and phenylalanine, the major precursor for these metabolite classes and other phenylpropanoids and the metabolically most expensive amino acid (after tryptophan) to synthesize (Hemingway *et al.*, 1989; Seigler, 1998). Other metabolites such as indolic glucosinolates induced by rhizobacteria treatment in Broccoli also could compete for tryptophan and other intermediates of the tryptophan-dependent auxin biosynthesis and concomitantly affect plant growth (Malka & Cheng, 2017). In **Chapter 4**, plants treated with *Paraburkholderia* species (*Pbg* and *Pbh*) showed substantial accumulation of various secondary metabolites via phenylpropanoid biosynthesis and surprisingly showed also enhanced growth. The primary metabolite profile of these plants revealed high accumulation of soluble sugars such as fructose (>280-fold increase relative to untreated control). Fructose is the primary substrate for fructose-6-phospate, a key substrate for the biosynthesis of both phosphoenolpyruvate (PEP) and erythrose-4-phosphate. These two intermediates are channeled into the shikimate pathway that bridges carbohydrate metabolism to biosynthesis of aromatic primary and secondary metabolites (Herrmann & Weaver, 1999). The shikimate pathway provides all the important precursors for the biosynthesis of phenylpropanoids including hydroxycinnamates, flavonoids, stilbenoids, coumarins and lignins which were significantly accumulated in Broccoli plants treated with *Paraburkholderia* species. These results suggest that rhizobacteria enable plants to sustain enhanced growth while producing numerous defensive secondary metabolites by enhancing both sugar generation and utilization to fuel both growth and defense. To our best knowledge, this is the first comprehensive study that employed combined primary and secondary metabolomics techniques to understand rhizobacteria-mediated changes in global metabolism of plants, linking metabolic flux from primary metabolites (soluble sugar and amino acid) to specific secondary metabolites that potentially function as chemical weapon against pathogen infection.

3. Tilting plant metabolism towards high value natural plant products

Recent studies on microbe-mediate phytochemical alteration have predominantly focused on medicinal plants as they are shown to be an attractive source for pharmaceutically important bioactive secondary metabolites (**Chapter 2**). Some good examples are PGPR-mediated induction of artemisinin and dihydroartemisinin, antimalarial agents in Artemisia ((Arora *et al.*, 2016), **Chapter 3**), and of morphine in Poppy (Pandey, Shiv S *et al.*, 2016). Similar attempts were also employed to improve pharmaceutically important secondary metabolites in crops. For instance, inoculations of *Glycine max* with single or consortia of various rhizobacteria strains belonging to *Arthrobacter, Azotobacter, Bacillus, Chryseobacterium, Curtobacterium, Pseudomonas, Stenotrophomonas,* and *Streptomyces,* induced the level of isoflavonoids and phytoestrogen agents with cancer chemo-prevention ability (Birt *et al.*,

2001; Ramos-Solano *et al.*, 2010; Algar *et al.*, 2012; Algar *et al.*, 2013; Chamam *et al.*, 2013; Kiprovski *et al.*, 2016). Furthermore, similar studies reported modulating effects of plant-associated microbes on the metabolome of *Oryza sativa* (Mishra *et al.*, 2006; Chamam *et al.*, 2013; Chamam *et al.*, 2015), *Zea mays* (Walker *et al.*, 2011; Walker *et al.*, 2012; Couillerot *et al.*, 2013; Planchamp *et al.*, 2015; Rozier *et al.*, 2016) and *Mentha piperita* (Santoro *et al.*, 2011; del Rosario Cappellari *et al.*, 2015; Santoro *et al.*, 2015). Likewise, our result indicated that different rhizobacteria alter the host metabolome both in effective or ineffective partnerships. Both kinds of partnership can be employed to tilt the host metabolism towards nutritionally and/or pharmaceutically important high value natural plant compounds (HVPC). To empirically demonstrate the bioactivity of metabolites that were altered by rhizobacteria in plants, I subjected extracts from the shoots of plants treated on roots with (or not) rhizobacteria to a series of non-receptor-mediated CALUX bioassays to test if bacteria inoculation enhanced biological activities plant materials on various human health promoting traces. Among others, shoot extracts of *Pbg*-inoculated Broccoli cultivar Malibu demonstrated higher anti-oxidative capacity in Nrf2 basis CALUX assay when compared to the non-treated control samples (**Box**). Interestingly, in **Chapter 4** *Pbg* inoculation of cultivar Malibu led to the induction of plant metabolites belonging to different subclass of phenylpropanoid pathway such as flavonoids, hydroxycinnamates and stilbenoids that have displayed, in other studies, various bioactivities against multiple diseases and disorders (Nijveldt *et al.*, 2001; Erlund, 2004; Leopoldini *et al.*, 2011; Gülcin, 2012). Collectively these results suggest that these enhanced phenolic compounds may play a key role as anti-oxidants. Activity-guided fractionation and purification will be further strategy to shed light on these bioactive metabolites. Considering that each rhizobacteria-host plant combination differently modulates plant chemistry (**Chapters 3**, **4**, and **5**), detailed understanding of the underlying primary and secondary metabolic pathways and their interconnection will be instrumental to fine-tune the most appropriate plant and rhizobacterial species association for selected bioactivities.

4. Microbial traits affecting plant chemistry and vice-versa

While a growing number of studies is steadily reporting the effects of bacterial strains on plant chemistry (**Chapter 2**), bacterial traits that trigger specific changes in the plant metabolome remain largely unknown. In this thesis, we unraveled some bacterial traits influencing host plant chemistry changes. First, root colonization ability of rhizobacteria seemed to play an essential role, as in nature successful bacterial colonization is generally the first step to establish a beneficial interaction with the host (Lugtenberg *et al.*, 2001; Bhattacharyya & Jha, 2012). For example, in **Chapter 3**, I showed that *Microbacterium* (*MB*) did not efficiently colonize Artemisia roots and showed no significant impact on the host metabolome. Also for *Paraburkholderia* we showed that temporal changes in the rhizosphere population densities coincided with the magnitude of their impact on the host primary and secondary metabolome (**Chapter 4**). Genome-wide analysis of plant growth-promoting *Pseudomonas*

Box

CALUX bioassays

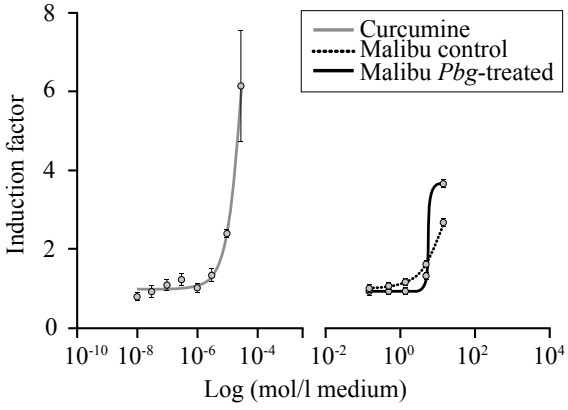

Fig 1. Results of Nrf2 CALUX assay. The dose-response curves of the tested samples are given, including curcumine, the reference compound.

Freshly frozen plant tissue materials (300 mg) were extracted with 900 ul of 97% ethanol for 30 minutes under sonication. Next, the samples were dried by speed vacuuming and were re-dissolved in 200 ul of DMSO. Serial dilution at different concentrations were prepared. Nrf1 cells were seeded in 96-wells plates and pre-incubated for 24 hours. Following pre-incubation, CALUX cells were exposed for 24 hours to the serial dilutions in triplicate (1% DMSO) and then lysed to measure luciferase activities. Thereafter, the Nrf2 CALUX activity in both control and *Pbg*-treated Broccoli cultivar Malibu samples were comparable. The Nrf2 CALUX (van der Linden *et al.*, 2014) measures activity of the Nrf2 transcription factor that trigger expression of genes involved in detoxification and the anti-oxidant stress response (Ma, 2013). As shown in **Fig 1**, *Paraburkholderia graminis* (*Pbg*) treated Broccoli shoot sample (Malibu) exhibited higher luciferase activity when compared to control, indicating *Pbg* induced anti-oxidative capacity in Broccoli sample.

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fluorescens strain SS101 (*Pf* SS101) combined with site-directed mutagenesis and genetic complementation recently revealed that sulfur assimilation plays an important role in growth promotion and induced systemic resistance of the model plant Arabidopsis (Cheng *et al.*, 2017). In **Chapter 5** of my thesis, we showed that bacterial sulfur assimilation affects both sulfur containing and non-sulfur containing metabolites in two Broccoli cultivars. Furthermore, in **Chapter 5** comparative metabolomics analysis of Arabidopsis plants treated with *Pf* SS101and its *cysH* mutant showed that sulfur assimilation in *Pf* SS101 affects side chain elongation of the aliphatic glucosinolates. Plants treated with the mutant showed an accumulation of short and medium chain aliphatic glucosinolates, whereas in plants treated

with wildtype *Pf* SS101 the long chain aliphatic glucosinolates were more abundant.

Plant-microbe interactions alter gene expression profiles of both interacting organisms. Thus, transcriptome analysis is often preferred as generic tool to unravel genes and pathways that govern the interaction. In Arabidopsis, for instance, several strains of *Pseudomonas fluorescens* were shown to alter the expression of several genes related to auxin biosynthesis and transport, sulfur assimilation and disease resistance (Wang *et al.*, 2005; Cheng *et al.*, 2017). Microbes can also alter the expression of plant genes even without direct contact through a blend of their volatile organic compounds (VOCs). Recently, exposure of Arabidopsis to *Microbacterium* EC8 VOCs was shown to alter the expression of genes involved in the assimilation and transport of sulfate and nitrate (Cordovez *et al.*, 2018). However, relatively less attention has been given on the impact of the host on the gene expression profile of the associated rhizobacteria. In **Chapter 6**, we showed that the gene expression profile of *Pbg* on synthetic media and on plant roots is significantly different. Our analysis showed that genes corresponding to flagella assembly and chemotaxis, energy metabolism, phosphate and nitrate metabolism and transport were significantly overexpressed in *Pbg*-plant interactions when compared to *Pbg* grown only on the synthetic media. These group of genes were upregulated and significantly enriched when *Pbg* interacts with both Broccoli cultivars and can be considered as a general response representing establishment of an association with the host. Our result is in line with previous study that showed maize exudate induced *Bacillus amyloliquefaciens* genes involved in nutrient utilization, bacterial chemotaxis and motility (Fan *et al.*, 2012). The induction of genes associated with energy metabolism and nutrient mobilization in *Pbg* suggests the establishment of symbiotic relationship between the plant and rhizobacteria, with the rhizobacteria contributing to nutrient acquisition and the host providing carbon sources for the energy production of the rhizobacteria. Furthermore, the results showed a number of genes expressed in *Pbg* in a Broccoli cultivar specific manner. Among those, ABC transporters were significantly enriched when *Pbg* interacted with cultivar Malibu. To further unravel the molecular interplay, site-directed mutagenesis, heterologous expression or overexpression of these specific genes is needed to identify the effects these specific mutations on plant chemistry and plant phenotypes.

5. A milestone in future breeding strategy and obstacle in field trial

In the past century, crop improvement strategies gave greater emphasis to yield, nutritional quality, disease resistance, or abiotic stress tolerance. In this context, breeding practices have emphasized more on enhancing plant intrinsic genetic characteristic to influence a desired phenotype. This approach has proven to be an effective strategy and was the corner stone of the green revolution. Currently we are facing huge challenges to meet the global food demand. Furthermore, an increasing awareness of the impact of agricultural practices on biodiversity and our environment is putting a great deal of pressure on agricultural production systems towards more sustainable practices (Wei & Jousset, 2017). Findings of my thesis

demonstrate that the use of beneficial rhizobacteria can be a novel and more generic means to complement conventional agricultural practices. Microbiome-assisted agriculture can be considered as a "taking care of microbe to take care of plant" approach. Recent studies empirically support the impact microbes can have on plant phenotype and chemistry. For example, a recent study by Carrión (Carrión *et al.*, 2019) demonstrated that invasion of the root system by the soil-borne fungus *Rhizoctonia solani* enriched for Chitinophagaceae and Flavobacteriaceae inside the roots which in turn suppressed the disease development via enhancing enzymatic activities associated with fungal cell-wall degradation as well as other secondary metabolites encoded by a novel NRPS-PKS gene cluster. Similarly, *Pseudomonas simiae* WCS417 induced root exudation of scopoletin, an iron-mobilizing coumarin with selective antimicrobial activity, suppressing the growth of fungal root pathogens but supporting root colonization by beneficial *Pseudomonas* strains (Stringlis, Ioannis A *et al.*, 2018). Thus, in future plant breeding programs, identifying plant characteristics that promote the recruitment and activities of beneficial microbes can be highly instrumental for developing sustainable agricultural practices. Furthermore, the "microbial-Gene Positioning System (m-GPS)" concept that we proposed in **Chapter 2** also can have an important contribution. The first principle resides on the identification of plant metabolic pathways that are altered by rhizobacteria and linked to plant phenotypes such as yield, quality or stress tolerance. For example, in my thesis, soluble sugars such as fructose, phenylpropanoids and glucosinolates were highly influenced by rhizobacteria treatment in plants and they are crucial for plant productivity, quality (nutritional, medicinal and flavor) and stress tolerance. The next step is to identify which part of the pathway is highly impacted by the rhizobacteria treatment. This will provide us with essential information on the rate-limiting steps in the biosynthesis of a given metabolite. For example, if we consider the glucosinolate pathway, the subclass aliphatic glucosinolate was impacted greatly by rhizobacteria treatment. Previous studies on the transcriptome profile of Arabidopsis treated with *Pf* SS101 and its *CysH* mutant showed that sulfur assimilation by the bacteria affects the chain elongation steps in aliphatic glucosinolate biosynthesis and key genes in the chain elongation steps such as MAM1 and MAM3 were significantly upregulated in *Pf* SS101 treated plants. Interestingly, in **Chapter 5** comparative metabolome analysis of Arabidopsis plant treated with *Pf* SS101 and its *cysH* mutant confirmed that long chain glucosinolate indeed accumulated in plants treated with *Pf* SS101 while short and medium chain glucosinolate were more abundant in plants treated with the mutant. Based on the expression profile of genes and the corresponding metabolites, the most influential genes in the biosynthesis pathway such as MAM1 and MAM3 can be targeted for improvement by conventional breeding or the current gene editing technologies. Furthermore, this conceptual framework allows screening for bacterial traits involved in the phenotypic and metabolome alteration in plants by using integrated ~omic technologies i.e. metagenomics, transcriptomics, and metabolomics combined with site-directed mutagenesis for functional analysis of potential bacterial traits identified by the omics analyses. The *cysH* gene from *Pf* SS101 is a good example and screening of allelic variants/orthologues across

several rhizobacteria species and strains of a given species can be performed to identify the best performing allelic variants(s) involved in the alteration of sulfur-containing metabolites of the host. Similar approaches can be followed for other groups of plant metabolites that are altered by rhizobacteria and have influence on yield, quality or stress tolerance of crops.

While *in vitro* screening allows to identify specific rhizobacteria that enhance plant performance, getting reproducible and consistent results under field settings is the major hurdle. This phenomenon is mainly due to unsuccessful root colonization of introduced strains (Kloepper, J *et al.*, 1980; Bhattacharyya & Jha, 2012). To facilitate efficient root colonization of selected rhizobacteria in field experiments, several applications such as drenching seed and seedlings, or continuous treatments have been carried out. However, such methods are not always successful. In our soil experiment, continuous application of *Pbg* broth (50 ml per application, 3 times in three weeks) displayed induction of shoot biomass of Broccoli and Arabidopsis in early development stage when compared to untreated control but the effect faded in time (data not shown). This might be due to the 'buffering' effects of the existing root and soil-associated microbes on the proliferation and activity of the introduced rhizobacteria. Therefore, exploration of proper field application and most importantly understanding of the interaction of introduced microbes with the existing soil and root associated microbial community is vital for the success of introduced rhizobacteria. Another approach could be steering of the existing microbial community in agricultural system towards desired groups of microbes that provide specific functions to the plant using probiotic approach or by 'pre-biotics' that alter the physico-chemical properties of the soil to favor the growth or activity of desired groups within the plant microbiome. Another innovative approach is to introduce beneficial bacteria into seeds. The introduction of endophyte bacteria *Paraburkholderia phytofirmans* PsJN into maize progeny seeds by spraying flower with this bacteria is one of the success stories and was shown to significantly increase yield (Mitter *et al.*, 2017).

Concluding remarks

The research presented in my thesis demonstrated that, apart from visible phenotypic changes, rhizobacteria also modulate the levels of a substantial number of plant secondary metabolites in a rhizobacteria-specific and even cultivar-specific manner. Our findings further demonstrated that rhizobacteria-mediated changes in the plant metabolome arise from both effective and ineffective partnership between plants and rhizobacteria. Changes in metabolism of the plant by rhizobacteria showed an association with plant growth and defense. Although it is widely accepted that plant defense has an adverse effect on growth, some rhizobacteria can induce plant defense and induces growth at the same time. Integrating the data from primary and secondary metabolome analyses of plants treated with rhizobacteria showed that rhizobacteria enhance the generation and utilization of soluble sugars in the host to fuel both defense and growth. Therefore, effective partnerships can enhance the production of nutritionally and pharmaceutically important plant metabolites and at the same time

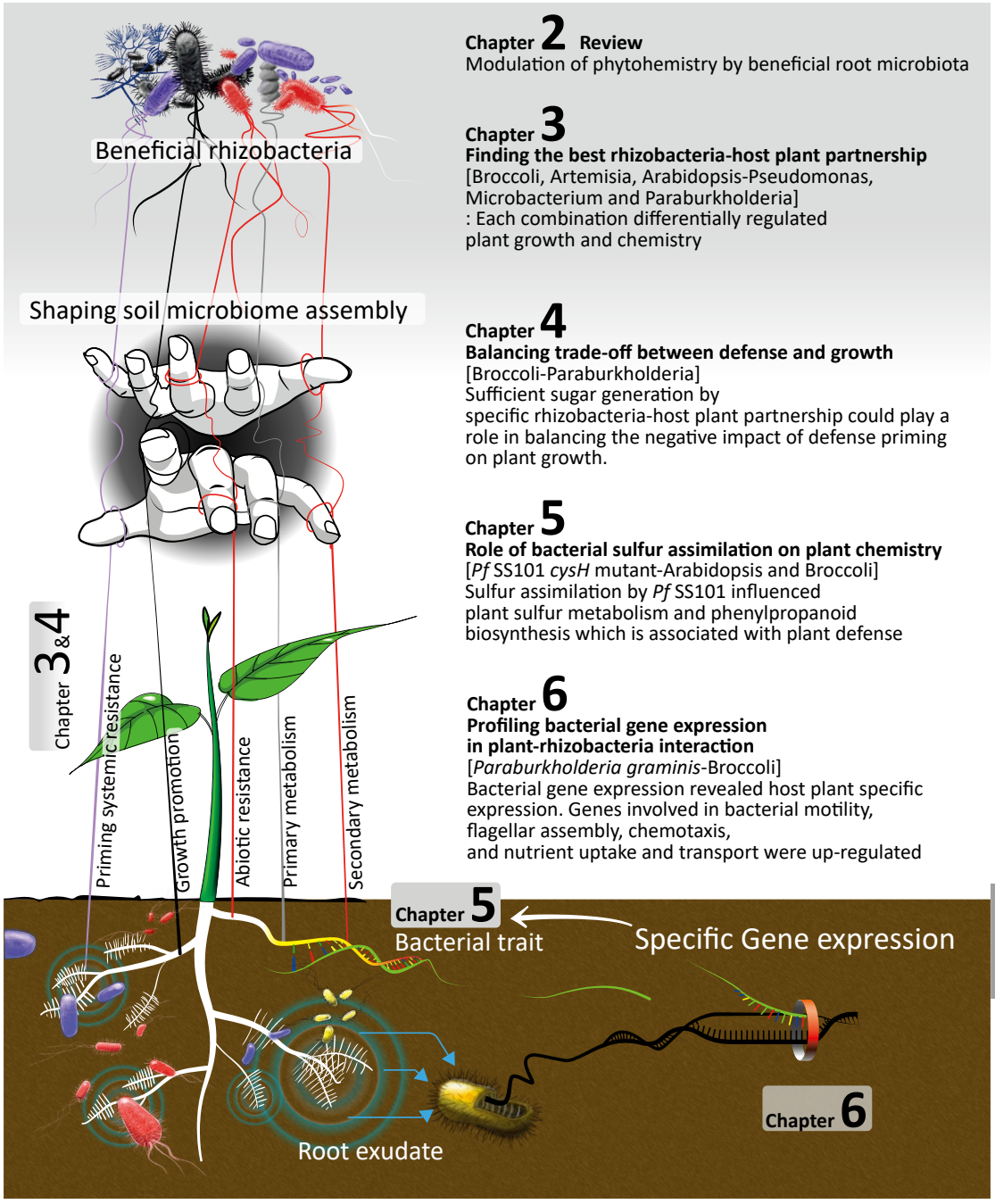

Fig 2. Summary of the main finding of the thesis.

enhance plant biomass. The combined effect could maximize the amount of plant metabolite recovered per unit of plant biomass and will make rhizobacteria-mediated host metabolome reprogramming an attractive approach to enhance the production of high value natural plant products. In conclusion, the results of this thesis exemplify that the fundamental basis of many if not all biological interactions are chemical in nature. The contribution of my work to the advancement of the field is not only tied to a number of answers that my thesis provides about rhizobacteria-host interaction but also to a number of testable hypotheses it has generated during the course of this study. So far, understood only a minute portion of the interaction between plant and rhizobacteria was resolved and the larger portion of the interaction is still a “black box” that awaits to be unlocked.