Modulation of plant chemistry by rhizosphere bacteria

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Chapter 1

General introduction and thesis outline

General introduction

Plants produce a large number of primary and secondary metabolites with diverse functions. While primary metabolites are fundamental to growth and reproduction, secondary metabolites contribute to adaptation of plants to environmental change (Bourgaud *et al.*, 2001) and play a critical role in plant defense against herbivory and pathogen attack (Bennett & Wallsgrove, 1994; Rattan, 2010; Boulogne *et al.*, 2012). Over the past 70 years, natural product chemistry has led to the identification of more than 100,000 secondary metabolites (Wink, 2010). Many of these metabolites exhibit a vast array of pharmaceutical activities either as metabolite itself or as a scaffold for the synthesis of derivatives with enhanced or other bio-activities (Bourgaud *et al.*, 2001; Hartmann, 2007). Recent studies have shown that microorganisms colonizing plant surfaces (phyllosphere, rhizosphere) and internal plant tissue (endosphere) can induce changes in the plant metabolome, leading to alterations in the biosynthesis of known plant metabolites or of yet unknown plant metabolites (Scherling *et al.*, 2009; van de Mortel *et al.*, 2012; Huang *et al.*, 2014; Ryffel *et al.*, 2016). Hence, microbe-plant interactions have been proposed as a novel, generic means to boost the production of nutritionally and/or pharmaceutically valuable plant metabolites and to discover new plant metabolites and their corresponding biosynthetic genes and pathways. My thesis focuses on microbe-mediated modulation of plant chemistry and identification of bacterial traits involved in the induction of these plant metabolome changes. Specific emphasis is given to root-associated beneficial bacteria also referred to as Plant Growth-Promoting Rhizobacteria (PGPR).

The rhizosphere, the narrow zone (± 1-2 mm) surrounding and influenced by plant roots, is rich in small- and large-molecular weight compounds that serve as a carbon source for microbial growth (Bais *et al.*, 2006). In return, the rhizosphere microbiome provides a first

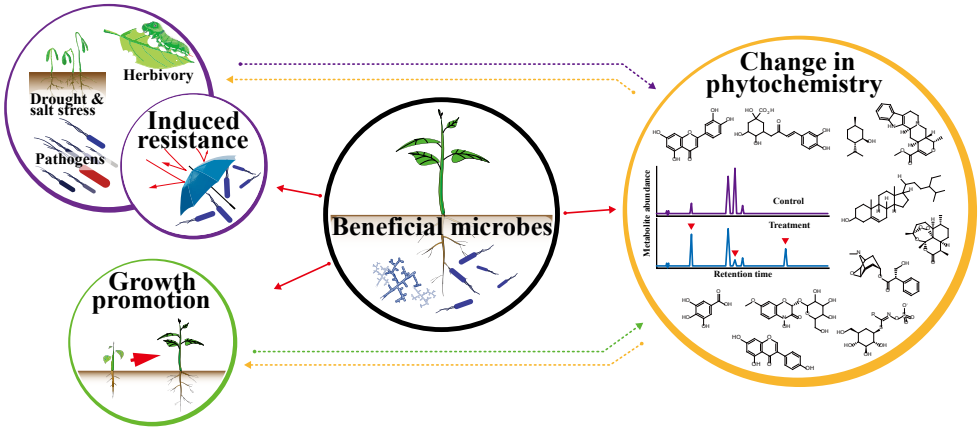

Fig 1. Direct and indirect mechanisms by which beneficial root-associated microorganisms can impact on plant growth and on plant tolerance to biotic and abiotic stress factors.

line of defense against infections by root pathogens (Raaijmakers & Mazzola, 2016) as well as other life-support functions for the plant including nutrient acquisition and growth promotion (Van Loon, 2007; Bhattacharyya & Jha, 2012), induction of systemic resistance against above-ground pathogens and herbivorous insects (Raupach *et al.*, 1996; Van Wees *et al.*, 1999; Ryu *et al.*, 2004; Haas & Défago, 2005), and enhanced plant tolerance to abiotic stress (e.g. salinity, drought) (Dimkpa *et al.*, 2009; Yang *et al.*, 2009). Several microbial traits and mechanisms involved in these interactions have been identified (Han *et al.*, 2006; Nam *et al.*, 2006; Kim *et al.*, 2007; Sumayo *et al.*, 2013; Cheng *et al.*, 2017), but how microorganisms alter plant chemistry and if/how these phytochemical changes affect plant growth and health are not well understood (**Fig 1**).

Plant growth-promoting rhizobacteria

Since Kloepper and colleagues termed plant growth promoting rhizobacteria (PGPR) for the first time in their experiment on radish in the 1970s (Kloepper, 1978), a large number of rhizobacterial genera including root endophytic bacteria have been described for their plant growth-promoting properties; these genera include, among others, *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Micrococcus*, *Pseudomonas* and *Serratia* (Lodewyckx *et al.*, 2002; Gray & Smith, 2005; Bhattacharyya & Jha, 2012). Based on the underlying mechanisms of plant growth promotion, PGPRs are generally categorized into **biofertilizers**, **phytostimulators** and **biopesticides** (Lugtenberg & Kamilova, 2009; Bhattacharyya & Jha, 2012).

Biofertilizers refer to rhizobacteria that increase the availability and uptake of micro- and macronutrients such as iron, phosphate and nitrogen. Iron is an essential element that functions as a cofactor for many metabolic pathways including respiration and photosynthesis (Brittenham, 1994; Miller *et al.*, 1995). In soil, however, iron exists as Fe^{3+} which is unavailable to plants and microorganisms. Hence, iron is the third most limiting nutrient for the plant (Zhang *et al.*, 2009). Siderophores, high affinity iron chelators, are produced by several rhizobacterial genera (Kloepper, JW *et al.*, 1980; Sharma & Johri, 2003; Rajkumar *et al.*, 2010; Radzki *et al.*, 2013) and have been implicated in plant growth promotion via Fe-siderophore complex (Sharma & Johri, 2003). The possible mechanism implies that the microbial siderophores-Fe complex is taken up by the plant or do a ligand exchange with phytosiderophores (Masalha *et al.*, 2000; Vansuyt *et al.*, 2007; Ahmed & Holmström, 2014). Next to iron, also phosphate can be made available to the plant by microbes, albeit via other mechanisms. In addition to the 'classic' phosphorus acquisition via symbiosis with arbuscular mycorrhizal fungi, another wide-spread mechanism involves mineralization of inorganic phosphorus through acidification by organic acids produced by rhizobacteria (Rodríguez & Fraga, 1999). The hydroxyl and carboxyl groups of organic acids chelate the cation of phosphate converting the mineral phosphate into soluble forms (Kpomblekou-a

& Tabatabai, 1994). A number of reports investigated the impact of phosphate-solubilizing bacteria on plant growth promotion in various crops (Han & Lee, 2006; Zhao *et al.*, 2014; Kudoyarova *et al.*, 2017; Manzoor *et al.*, 2017). The conclusive role of P-solubilization in growth promotion is not evident in several of these studies. For example, De Freitas *et al.* (1997) reported that induced growth of canola by phosphate-solubilizing rhizobacteria was not via P-uptake but some other yet unknown mechanisms. A third essential element for plant growth is nitrogen. Apart from nitrogen fixation by symbiotic rhizobia, N_2 can also be fixed into ammonia by free-living rhizobacteria. Several studies have demonstrated that N_2-fixing *Azospirillum* can significantly increase crop yield (Baldani *et al.*, 1983; Rodrigues *et al.*, 2008), a phenotype that is also associated with an increased number of root hairs and lateral roots, thereby enhancing uptake of minerals and water (Okon *et al.*, 1998; Bashan *et al.*, 2004).

Phytostimulators are rhizobacteria that directly affect plant growth via the production of phytohormones such as indole-3-acetic acid (IAA), gibberellins, cytokinins, and abscisic acid (Lugtenberg & Kamilova, 2009; Cassán *et al.*, 2014). Several PGPR genera including *Rhizobium*, *Bradyrhizobium*, and *Azospirillum* can produce IAA via the indole-3-pyruvic acid (IPyA) pathway (Burdman *et al.*, 2000)) that utilizes tryptophan released by roots as the precursor. Other PGPRs such as *Azospirillum brasilense* can also produce IAA via tryptophan-independent pathways although the underlying mechanism(s) is yet not fully resolved (Jha & Saraf, 2015; Goswami *et al.*, 2016). Also other plant hormones such as giberellins, cytokinin, and abscisic acid, produced by various PGPR genera such as *Azospirillum* (Cassán *et al.*, 2014), *Bacillus* (Gutiérrez-Mañero *et al.*, 2001; Joo *et al.*, 2005) and *Pseudomonas* (García de Salamone *et al.*, 2001) can impact on plant growth and development. Another well-studied mechanism of hormone-mediated plant growth promotion by PGPRs is via 1-aminocyclopropane-1-carboxylate (ACC) deaminase, an enzyme that degrades ACC, a precursor of the plant hormone ethylene, into ammonia and α-ketobutyrate (John, 1991). ACC deaminase producing bacteria were shown not only to affect plant growth but also to provide protection against abiotic stresses such as drought, salinity, flooding, temperature, ultraviolet radiations, or heavy metals (Honma & Shimomura, 1978; Glick, 2014; Etesami *et al.*, 2015; Goswami *et al.*, 2016).

Over the past decade, substantial interest has emerged on the role of microbial volatile organic compounds (mVOCs) in plant growth promotion and induced systemic resistance (Schmidt *et al.*, 2015). mVOCs are small molecules (< 300 mw) that are highly diffusible through the air-filled spaces in the soil matrix and thereby can interact with plants and other organisms from a distance (Hiltpold & Turlings, 2008; Schulz-Bohm *et al.*, 2018; Sharifi & Ryu, 2018). Ryu *et al.* (2003; 2004) first reported 2,3-butanediol produced by *Bacillus subtilis* as an inducer of growth and systemic resistance (ISR) in Arabidopsis. Since then, various other bacterial species such as *Arthrobacter* (Velázquez-Becerra *et al.*, 2011), *Microbacterium* (Cordovez *et al.*, 2018), *Pseudomonas* (Park *et al.*, 2015; Jishma *et al.*, 2017; Rojas-Solís *et al.*, 2018),

and *Stenotrophomonas* (Rojas-Solís *et al.*, 2018) have been investigated mVOCs-mediated effects on growth and health of various plant species such as Arabidopsis, lettuce, moss, tobacco, and tomato. The classes of bioactive mVOCs include alkenes, alcohols, ketones, terpenes, benzonoids, and pyrazines (Schmidt *et al.*, 2015; Sharifi & Ryu, 2018). The study by Meldau *et al*. (2013) further showed that dimethyldisulfide (DMDS)-producing *Bacillus* sp. B55, when grown in minimal medium containing ^{35}S-labeled Na_2SO_4 as the sole S source, leads to incorporation of ^{35}S into plant proteins, suggesting that sulfurous mVOCs can feed directly into the plant's sulfur metabolism. Similarly, Arabidopsis exposed to mVOCs from *Bacillus amyloliquefaciens* (GB03) showed enhanced sulfur accumulation when traced with radioactive sulfate ($^{35}SO4^{-2}$). Subsequent microarray data analysis further indicated that mVOCs of strain GB03 induced transcription of genes responsible for sulfur assimilation and for aliphatic, indolic glucosinolate biosynthesis further evidenced by increases of 33 and 70% of the total glucosinolate content in shoots and root, respectively (Aziz *et al.*, 2016). In addition, treated plants exhibited a significant protection from herbivory by the insect *Spodoptera exigua*.

Biopesticides refer to PGPRs that suppress disease-causing agents (e.g. fungi, bacteria, nematodes) directly via specific metabolites such as antibiotics, hydrolytic enzymes, and mVOC such as hydrogen cyanide (HCN), or indirectly via induced systemic resistance (Vessey, 2003; Pieterse *et al.*, 2014). Over the last four decades, a large number of bacterial genera, including *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Collimonas*, *Pantoea*, *Pseudomonas*, *Serratia*, *Stenotrophomonas*, and *Streptomyces* have been identified as biopesticides (Raaijmakers *et al.*, 2009; Raaijmakers & Mazzola, 2012). *Pseudomonas* and *Bacillus* are the most broadly studied genera as agronomic biocontrol agents. Despite similarities in their effects on plant growth and health, there is a large diversity of functional traits among the numerous species within these genera, with unique or shared gene clusters encoding bioactive compounds such as 2,4-diacetylphloroglucinol (2,4-DAPG), pyrrolnitrin, pyoluteorin, phenazines, 2,5-dialkylresorcinol, quinolones, rhamnolipids, and various lipopeptides (LPs) (Raaijmakers & Weller, 1998; Raaijmakers *et al.*, 2006; Gross & Loper, 2009; Raaijmakers *et al.*, 2010). LPs operate largely via membrane disruption leading to lysis of infectious propagules (e.g. zoospores) of plant pathogens (de Souza *et al.*, 2003) or trophozoites of the bacterivorous amoeba-flagellates (Mazzola *et al.*, 2009). Similarly, 2,4-DAPG produced by *Pseudomonas* spp. also exhibits broad-spectrum antimicrobial activities but, at high concentrations can also be phytotoxic (Raaijmakers & Weller, 1998; Haas & Défago, 2005; Weller *et al.*, 2007; Kwak *et al.*, 2012; Schlatter *et al.*, 2017). Similar to *Pseudomonas*, also *Bacillus* species harbor a large diversity of biosynthetic gene clusters for lipopeptides and polyketides such as surfactins, fengycins, iturins, macrolactin, difficidin, and oxidifficidin (Chen, X *et al.*, 2009; Raaijmakers *et al.*, 2010). These metabolites are active against a wide range of plant pathogenic fungi (Chen, X-H *et al.*, 2009; Yuan *et al.*, 2012) such as *Fusarium graminearum*, *Botrytis cinerea*, *Podosphaera fusca*, *Colletotrichum demiatium*, *Penicillium roqueforti*, *Aspergillus flavus*, and *Rhizoctonia solani* (Moyne *et al.*,

2001; Hiradate *et al.*, 2002; Yu *et al.*, 2002; Chitarra *et al.*, 2003; Toure *et al.*, 2004; Romero *et al.*, 2007; Wang *et al.*, 2007).

Several of these PGPRs and some of the bioactive compounds can also provide indirect plant protection by priming disease or pest resistance responses in a systemic manner. Such defense system is classified into two forms referred to as systemic acquired resistance (SAR) and induced systemic resistance (ISR). The onset of SAR is initiated when the surface-localized pattern-recognition receptors (PRRs) of the plant recognize conserved pathogen-associated molecular patterns (PAMPs) such as flagellin of avirulent or necrotrophic pathogens (Boller & Felix, 2009; Campos-Soriano *et al.*, 2012; Macho & Zipfel, 2015; Couto & Zipfel, 2016). This then increases the level of signaling molecules such as salicylic acid (SA) that in turn upregulate the expression of the antimicrobial pathogenesis-related (PR) genes, fortifying the plant against subsequent infection (Durrant & Dong, 2004; Fu & Dong, 2013). ISR is mediated by PGPR primarily through jasmonic acid (JA) and ethylene (ET), although some PGPRs can also induce resistance via the SA pathway (De Meyer & Höfte, 1997; van de Mortel *et al.*, 2012). Among the ISR-inducing genera, *Pseudomonas* and *Bacillus* are again the most well studied to date. ISR-inducing determinants of *Pseudomonas* and *Bacillus* identified to date include siderophores, SA, lipopolysaccharides (LPS), antibiotics (Meziane *et al.*, 2005; Bakker *et al.*, 2007), and also mVOCs such as 2,3-butanediol (Ryu *et al.*, 2004).

Recent studies in our lab led to the identification of other genes and traits of *Pseudomonas fluorescens* strain SS101 (*Pf* SS101) involved in ISR and growth promotion (Cheng *et al.*, 2017). Following a screening of a genome-wide random mutant library, we identified 21 mutants out of 7,488 that was compromised in their ability to promote Arabidopsis growth and to induce systemic resistance against the bacterial leaf pathogen *Pseudomonas syringae* pv. tomato (Pst). Subsequent analysis of root colonization, site-directed mutagenesis and genetic complementation revealed the involvement of phosphogluconate dehydratase gene *edd*, the response regulator gene *colR* and the adenylsulfate reductase gene *cysH* in growth promotion and ISR by *Pf* SS101. Further comparative plant transcriptome analysis indicated that sulfur metabolism of *Pf* SS101 influenced sulfur assimilation, auxin biosynthesis and transport, steroid biosynthesis and carbohydrate metabolism in Arabidopsis (Cheng *et al.*, 2017). These results were in line with results of a non-targeted metabolomics approach that showed that *Pf* SS101 differently regulated 50 metabolites in Arabidopsis (van de Mortel *et al.*, 2012). Genome-wide transcriptomics and screening with seven Arabidopsis mutants disrupted in *myb51, cyp79B2cyp79B3, cyp81F2, pen2, cyp71A12, cyp71A13,* or *myb28myb29* revealed that camalexin and indolic glucosinolates, sulfur containing metabolites, may contribute to the induced resistance response against *Pst* and the herbivorous insect *Spodoptera exigua* (van de Mortel *et al.*, 2012).

Modulation of plant metabolism by PGPRs

While growth promotion and induced resistance by PGPRs have drawn the attention for approximately 40 years (Kloepper, 1978), relatively few studies have addressed how PGPRs modulate plant metabolism. Nevertheless, the number of studies on PGPR-mediated effects on plant secondary metabolism shows an increasing trend (**Fig 2**), exemplifying its potential for the coming decade. The increasing interest is due in part to the overwhelming attention in research for plant microbiome assembly and functioning (Cordovez *et al.*, 2019) as well as the observation that PGPRs represent a novel and promising platform for boosting or redirecting the production of high value natural products (HVNPs) in plants. Compared to conventional breeding and plant genetic engineering, steering HVNPs via PGPRs has a few distinct advantages. Such merits include simplicity and generality of application, and attested safety to environmental issue in using bacteria as biological elicitors in farmland (Tabassum *et al.*, 2017). In addition, endless yet undiscovered rhizospheric and endophytic microbial candidates make this tool a future-directed platform.

Studies on PGPR-mediated phytochemical changes have covered a broad range of plant species from Arabidopsis as a model to medicinal plants and agricultural/horticultural crops. The majority of these studies focus on economically important plant species belonging to the Lamiaceae, Fabaceae and Asteraceae. On the PGPR-side, again *Pseudomonas* and

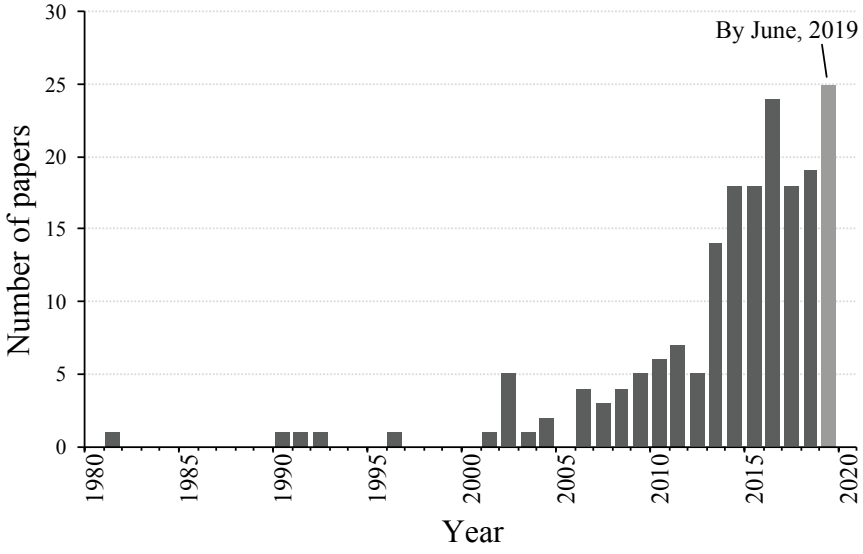

Fig 2. Number of articles available online (up to June 2019) that focus on "modulation of phytochemicals by beneficial bacteria". In the search for these articles we used the keyword combinations of "bacteria, beneficial rhizobacteria, PGPR, plant chemistry, plant secondary metabolite, plant metabolomics /metabolites, plant chemistry alteration/change, metabolite accumulation, HPLC, LC-MS/MS".

Bacillus were the most extensively studied rhizobacteria but also other genera are being tested for their effects on the plant metabolome (Etalo *et al.*, 2018). The overall objective in this research field is to investigate if PGPRs can increase the level of medicinal compounds in herbs and plant species. One of the best examples to date is the boost of artemisinin, the antimalarial bioactive chemical, in *Artemisia annua* by endophytic *Pseudonocardia* sp. via upregulation of the artemisinin biosynthesis genes *cyp71av1* and *cpr* (Li *et al.*, 2012). Similarly, an endophyte consortium, consisting of *Acinetobacter* and *Marmoricola* spp. induced the biosynthesis of benzylisoquinoline alkaloids (BIAs), bringing about a substantial increase of morphine production in *Papaver somniferum* (Ray *et al.*, 2019). Another recent study investigated the impact of various endophytic bacteria in *Panax ginseng* on the levels of ginsenosides, which are antitumor bioactive glycosylated triterpenes (Ji *et al.*, 2019).

Next to these medicinal plant compounds, PGPRs can also induce metabolome changes that affect (a)biotic stress tolerance. For instance, soybean (*Glycine max*) treated with *Stenotrophomonas maltophilia* N5.18 accumulated more isoflavonoids, phytoestrogen (Algar *et al.*, 2014). This study further demonstrated that another PGPR *Curtobacterium* sp. strain M84 also induced isoflavonoids levels in soybean after infestations by the bacterial leaf pathogen *Xanthomonas axonopodis* pv. *glycines*, suggesting a potential correlation between isoflavonoids accumulation and systemic resistance. In the same manner, *Bacillus velezensis* YC7010 significantly induced tricin, a flavonone glycoside, as well as contents of lignin and cellulose in rice (*Oryza sativa*), thereby triggering defense mechanism against the brown planthopper *Nilaparvata lugens* (Rashid *et al.*, 2018). Similarly, treatment of cotton with consortia of PGPR (9 *Bacillus* spp.) resulted in significant expression of (+)-δ-cadinene synthase gene family, gossypol accumulation and anti-herbivory against *Spodoptera exigua* (Zebelo *et al.*, 2016).

Notwithstanding the growing efforts to investigate PGPR-plant interactions, the bacterial traits involved in rhizobacteria-mediated plant metabolome changes remain largely unknown. Recently, the production of phenylacetic acid (PAA) by *Bacillus fortis* IAGS162 ameliorated *Fusarium* wilt disease in tomato (Akram *et al.*, 2016). Exposure of PAA to the media supporting tomato seedling growth led to changes in defense-related pathways together with up-regulation of various phenylpropanoid precursors. For *Pseudomonas aeruginosa* PM12, methoxybenzene methanol (HMB) was identified as a bacterial determinant that triggered systemic resistance in tomato against *Fusarium* wilt disease. An additional chemical analysis by GC-MS revealed the impact of HMB on primary and secondary metabolism, signaling and defense pathways in tomato (Fatima & Anjum, 2017). Moreover, several other studies revealed that mVOCs from various PGPR strains also impact on primary metabolism (Wenke *et al.*, 2019), flavonoid biosynthesis (Zhang *et al.*, 2007), or sulfur metabolism (Aziz *et al.*, 2016) but a comprehensive analysis of the specific mVOCs that trigger these responses and the signal transduction pathways leading to these plant metabolome changes has not been conducted yet for most of these PGPRs.

Table 1. Examples of PGP R strains and traits associated with changes in the plant metabolome

PGPR strain	PGPR trait	Plant	plant metabolome changes	Reference
Bacillus fortis IAGS162	phenylacetic acid (PAA)	Tomato	induction of shikimate and phenylpropanoid pathways	(Akram *et al.*, 2016)
Pseudomonas aeruginosa PM12	methoxybenzene methanol (HMB)	Tomato	increase of sugars, organic acids, polyamines, amino acids and salicylic acid	(Fatima & Anjum, 2017)
Bacillus amyloliquefaciens GB03	Sulfurous volatiles, dimethyldisulfide (DMDS)	Arabidopsis	increase of aliphatic and indolic glucosinolates	(Aziz *et al.*, 2016)
Pseudomonas fluorescens SS101	Sulfur metabolism (*cysH* gene)	Arabidopsis, Broccoli	increase of IAA, camalexin, hydroxycinnamates, and aliphatic glucosinolates in Arabidopsis; increase of flavonoids, hydroxycinnamates, indolic glucosinolates in Broccoli	(van de Mortel *et al.*, 2012; Cheng *et al.*, 2017), Chapter **5**

Thesis outline

Numerous beneficial rhizobacteria (PGPRs) can promote plant growth and trigger systemic resistance, thereby enhancing crop yield and improving plant quality traits. The recent technological developments in LC/GC hyphenated mass spectrometry have opened opportunities to study PGPR-mediated changes in phytochemistry as a novel platform that can be integrated in microbiome-mediated plant breeding. To date, however, the underlying mechanisms, bacterial traits and specificity in plant metabolome responses to single PGPRs or consortia of PGPRs, also referred to as synthetic communities (syncoms), remain largely elusive. Hence, the **overall aim of my thesis** is to study PGPR-induced changes in the metabolome of different plant species and to identify the bacterial traits involved in the induction of these plant metabolome changes. In **Chapter 2**, I provide an up-to-date overview of the existing literature on microbe-mediated effects on the plant metabolome. It provides an overview of phytochemical changes triggered by soil and plant-associated bacteria in diverse plant species, ranging from Arabidopsis as a model plant to crop, herbal, and medicinal plant species. Furthermore, this chapter also proposes a novel concept termed "Microbial-Gene Positioning System (m-GPS)" as a comprehensive tool to investigate the underlying mechanisms and genes associated with microbe-mediated modulation of plant chemistry.

To investigate the specificity of plant metabolome changes induced by rhizobacteria, I conducted a so-called 'blind date' experiment in **Chapter 3**, combining three strains of distinct rhizobacterial genera (*Pseudomonas*, *Microbacterium*, *Paraburkholderia*) and three different plant species, representing the model plant Arabidopsis, the medicinal plant Artemisia, and the crop plant Broccoli. Bacterial and host-specific effects were investigated via untargeted plant metabolomics, aided by root and shoot phenotyping and bacterial root colonization. Also the association between altered plant metabolism and plant growth is investigated based on the resource allocation theory and pathway analysis evidenced by chemical analyses. In **Chapter 4**, I looked into the diversity of phytochemical changes upon exposure of two Broccoli cultivars to different *Paraburkholderia* species. In this chapter, I not only looked into changes in plant secondary metabolism but also into changes in primary metabolism induced by these rhizobacterial species. In the following two experimental **Chapters 5** and **6**, I focus on the identification of bacterial traits and genes associated with the changes observed in plant phenotypes and metabolome described in **Chapters 3** and **4**. More specifically, for *Pseudomonas fluorescens* SS101, I investigated if *cysH*, a gene involved in sulfur metabolism, is associated with growth promotion and ISR, and if these phenotypic changes in two Brassica plant species (Arabidopsis and Broccoli) can be explained by the observed metabolome changes. In **Chapter 6**, I conducted a genome-wide transcriptome analysis on *Paraburkholderia graminis* (*Pbg*) colonizing the roots of Broccoli to identify potential gene candidates and pathways associated with the phenotypic and metabolic changes induced in two Broccoli cultivars.

Final **Chapter 7** integrates the findings of this thesis and addresses the potential use of rhizobacteria for sustainable agriculture and as a novel technological platform for the production of pharmaceutical products such as HVNPs.