Modulation of plant chemistry by rhizosphere bacteria

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Chapter 2

Modulation of plant chemistry by beneficial root microbiota

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Abstract

Plants are colonized by an astounding number of microorganisms that can reach cell densities much greater than the number of plant cells. Various plant-associated microorganisms can have profound beneficial effects on plant growth, development, physiology and tolerance to (a)biotic stress. In return, plants release metabolites into their direct surroundings, thereby feeding the microbial community and influencing their composition, gene expression and the production of secondary metabolites. Similarly, microbes living on and in plant tissue may induce known and yet unknown biosynthetic pathways in plants leading to diverse alterations in the plant metabolome. Here, we provide an overview of the impact of beneficial microbiota on plant chemistry, with an emphasis on bacteria living on or inside root tissues. We will also provide new perspectives on deciphering the yet untapped potential of microbe-mediated alteration of plant chemistry as an alternative platform to discover new pathways, genes and enzymes involved the biosynthesis of high value natural plant products.

Keywords: microbe-plant interactions; beneficial rhizobacteria; phytochemistry; natural products

Introduction

Plant metabolites are estimated to be more than 100,000 at present (Wink, 2010) and new analogues are still being discovered in a rapid pace. Many plant-derived compounds exhibit a diverse array of biological activities including protection against herbivores and pathogenic microorganisms. Furthermore, plant metabolites are a major source of pharmaceuticals, either as the active ingredient of a crude plant extract or as a scaffold for chemical synthesis and structural modifications(Cragg & Newman, 2013; Bauer & Brönstrup, 2014). Recent studies have shown that specific 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 to the induction of yet unknown metabolites (Scherling *et al.*, 2009; van de Mortel *et al.*, 2012; Huang *et al.*, 2014; Ryffel *et al.*, 2016). Hence, microbe-plant interactions may provide a novel and more generic means to boost the production of agriculturally and/or pharmaceutically interesting plant metabolites and to discover structurally new plant metabolites and their corresponding biosynthetic genes and pathways.

For centuries, reductionist approaches gave primary importance to the interplay between the plant, abiotic conditions (light, water, CO_2) and physico-chemical characteristics of the soil. Over the past decade, Next Generation Sequencing (NGS) technologies have demonstrated that plants are colonized by an astounding number of taxonomically diverse (micro)organisms that can reach cell densities much greater than the number of plant cells. Several members of this plant-associated microbial community can influence plant growth, development and health(Mendes *et al.*, 2013; Panke-Buisse *et al.*, 2015). Consistent with the terminology used for microorganisms colonizing the human body, the collective communities of plant-associated microorganisms, their genomes and interactions are referred to as the plant microbiome (Mendes *et al.*, 2013). Plants attract and feed their microbiome by the exudation of photosynthetically fixed carbon into their direct surroundings, i.e., spermosphere, phyllosphere, rhizosphere and endosphere (Haichar *et al.*, 2008; Rudrappa *et al.*, 2008; Shidore *et al.*, 2012; Reinhold-Hurek *et al.*, 2015). 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 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), but how microorganisms alter plant chemistry and if/how these

phytochemical changes affect plant growth, development and health are not well understood yet (**Fig 1**). Here, we provide an up-to-date overview of studies on microbe-mediated modulation of plant chemistry, with specific emphasis on endophytic and rhizosphere bacteria. The impact of other (micro)organisms, including plant pathogenic fungi, arbuscular mycorrhizal fungi, symbiotic nitrogen-fixing bacteria and parasitic weeds, on plant chemistry is not reviewed here; we refer the reader to other relevant literature (Bouwmeester *et al.*, 2003; Hahlbrock *et al.*, 2003; Furuhashi *et al.*, 2012; Okmen *et al.*, 2013; Schweiger *et al.*, 2014; Brusamarello-Santos *et al.*, 2017). In specific cases, we will address the putative mechanisms involved in the molecular interplay between beneficial bacteria and plants. Finally, we propose a conceptual framework for the potential use of plant-microbe interactions to elucidate and engineer plant metabolic pathways.

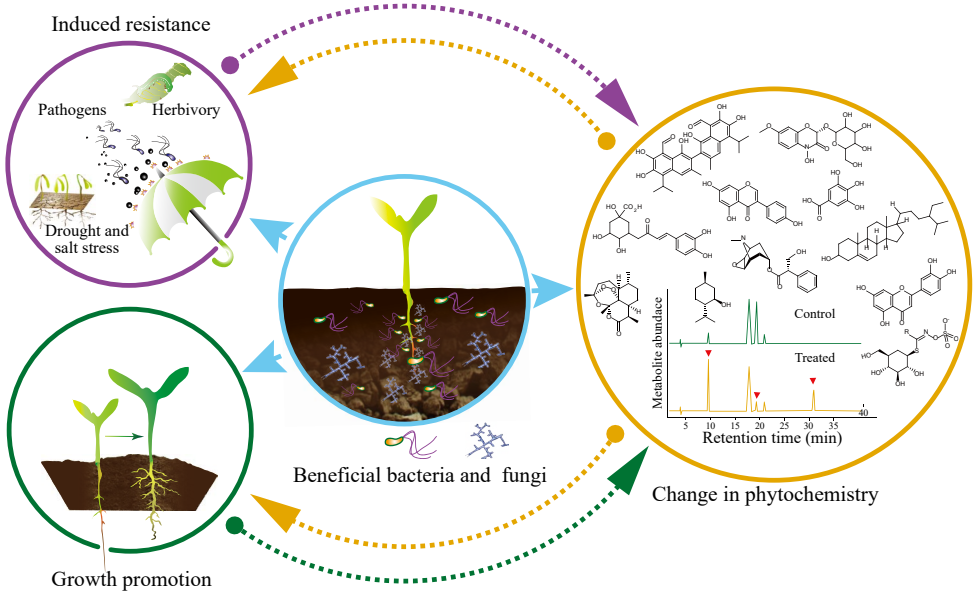

Fig 1. Influences of beneficial microbes on plant growth, health and chemistry. Beneficial microbes can enhance biomass, alter root system architecture, prime the plant defense against pathogens and phytophagous insects, enhance tolerance to drought and salt stress and alter phytochemistry. Phytochemical changes include induction, repression or biosynthesis of new metabolites in treated plants. Plants can also influence the composition and activity of root-associated beneficial microbes through their exudates. These beneficial microbes reside in the rhizosphere and endosphere of the host plant. The causal relationships between changes in phytochemistry, growth promotion and induced resistance (dotted arrows) are not well understood.

Modulations of plant chemistry by endophytes and beneficial root microbes

Endophytes are microbes that colonize living, internal tissues of plants without causing any immediate, overt negative effects on plant performance (Hardoim *et al.*, 2015). Although the number of studies on plant-endophyte interactions is increasing rapidly, the number of plant as well as microbial species investigated to date only represent the tip of the iceberg. With approximately 300,000 higher plants and their endophytic microbiomes, there is an enormous metabolic potential yet to be discovered. To date, effects of endophytes on the plant metabolome are more frequently studied and reported for fungi than for bacteria (Zhilin *et al.*, 2007; Huang *et al.*, 2008; Staniek *et al.*, 2008; Shukla *et al.*, 2014).

Endophytic fungi

Endophytic fungi are an important source of therapeutically active compounds. Some of the well-known examples include amongst others the tetracyclic diterpenoid anticancer drug paclitaxel from *Taxomyces andreanae* (Stierle *et al.*, 1993), the potent anticancer, antiviral, antioxidant, antibacterial and anti-rheumatic agent podophyllotoxin from *Sinopodophyllum hexandrum* (Yang *et al.*, 2003), the cognitive enhancer lycopodium alkaloid huperzine-A from *Shiraia* sp. (Zhu *et al.*, 2010), the antimicrobial agent enfumafungin from *Hormonema* sp. (Schwartz *et al.*, 2000), the lactone cholesterol-lowering agent lovastatin from *Aspergillus luchuensis* (El-Gendy *et al.*, 2016), a nonpeptidal antidiabetic agent from *Pseudomassaria* sp. (Zhang *et al.*, 1999) and the diterpene pyrones immunosuppressive agents subglutinol A and B from *Fusarium subglutinans* (Lee *et al.*, 1995). Furthermore, in plants, endophytic fungi are effective as biocontrol agents of plant diseases, insect pests and nematodes (Rowan, 1993; Ostlind *et al.*, 1997; Daisy *et al.*, 2002; Schwarz *et al.*, 2004; Tanaka *et al.*, 2005; Wicklow *et al.*, 2005; Li *et al.*, 2008; Wang *et al.*, 2013; Gupta *et al.*, 2016; Kim *et al.*, 2016; Schouten, 2016; Singh *et al.*, 2016). For more comprehensive information on bioactive metabolites from endophytic fungi we recommend the reader to excellent reviews of Newman and Cragg (Newman & Cragg, 2015).

In addition to having the biosynthesis machinery for the above mentioned bioactive metabolites, endophytic fungi are also know to play an important role in boosting metabolite biosynthesis of their host. Inoculation of endophyte-free *Catharanthus roseus* plants with the fungi *Curvularia* sp. and *Choanephora infundibulifera* enhanced the level of vindoline, a terpenoid indole alkaloid (TIA), in the leaves by 403% and 229%, respectively. Real-time PCR further showed that structural and regulatory genes involved in the TIA biosynthesis pathways were significantly upregulated in endophyte-inoculated plants when compared to endophyte-free plants (Pandey, Shiv S. *et al.*, 2016). Polysaccharide fraction from *Trichoderma atroviride*, an endopyte isolated from *Salvia miltiorrhiza,* significantly boosted the biosynthesis of tanshionones in hairy root cultures and also induced transcription of genes involved in tanshinone biosynthesis (Ming *et al.*, 2013). Also comparative metabolomics and

transcriptomics of ryegrass infected with *Epichloe festucae* and non-infected plants revealed that the endophyte caused 'reprogramming' of the host's metabolism, favoring secondary metabolism at the expense of primary metabolism. Particularly, flavonoids and anthocyanins showed significant accumulation in plants infected with the endophyte (Dupont *et al.*, 2015).

Endophytic and rhizosphere bacteria

Similar to endophytic fungi, endophytic and rhizospheric bacteria isolates are capable of producing a large number of bioactive metabolites and detailed reviews on this topic are provided by, among others, Gunatilaka (2006) and Singh *et al.* (2017). Several studies have shown profound effects of bacterial endophytes on their host's primary and secondary metabolism. For example, comparative primary metabolome analysis of poplar plants that were inoculated with *Paenibacillus* sp. showed significant increases in the levels of asparagine, urea and threitol, whereas a number of intermediates such as organic acids (malate, succinate, fumarate, citrate), amino acids (phenylalanine, 2-ketoglutatate, oxo-proline) and the sugar phosphate (fructose-6-phosphate) were reduced in the inoculated plants (Scherling *et al.*, 2009). Secondary metabolite profiling of grapevine treated with the endophytic bacterium *Enterobacter ludwigii* showed a significant increase in the level of vanillic acid and a decrease in the concentration catechin, esculin, arbutin, astringin, pallidol, ampellopsin, D-quadrangularin and isohopeaphenol. In roots and stems, also changes in the levels of epicatechin, procyanidin 1, taxifolin and the sum of quercetin-3- glucoside and quercetin-3-galactoside were detected (Lopez-Fernandez *et al.*, 2016).

Other studies on bacteria-mediated changes in phytochemistry reported to date involve the model plant species *Arabidopsis thaliana,* medicinal plants, food crops, ornamentals and trees/shrubs. Crop and medicinal plants categories, each encompassing 25 and 16 plant species, respectively, were the major hosts used in the reviewed articles. Among the reviewed articles, a total of 53 plant species belonging to 28 families were used to investigate bacteria-mediated phytochemical alterations. Lamiaceae, representing the herb plant that encopasses 8 plant species, was the most widely used plant family, followed by Fabaceae (7) and Asteraceae (5).

When investigating bacteria-mediated alteration in phytochemistry, seudomonadales and Bacillales were the most widely used bacterial orders (on 16 plant families each), followed by Actinomycetales (on 9 plant families) (**Fig 2**). More detailed information pertinent to phytochemical alterations induced by rhizobacteria is summarized in **Supplementary Tables S1 and S2**. Furthermore, the chemical structures of several plant metabolites whose biosynthesis is altered by endophytic and rhizospheric bacteria are shown in **Fig 3**. The overall aim of the studies with medicinal plants was to investigate if rhizosphere or endophytic bacteria can affect the level of a specific medicinal compound(s). For example, endophytic actinobacterium *Pseudonocardia* sp. isolated from *Artemisia annua* induced artemisinin (an antimalarial agent) production in *Artemisia* by upregulating the expression

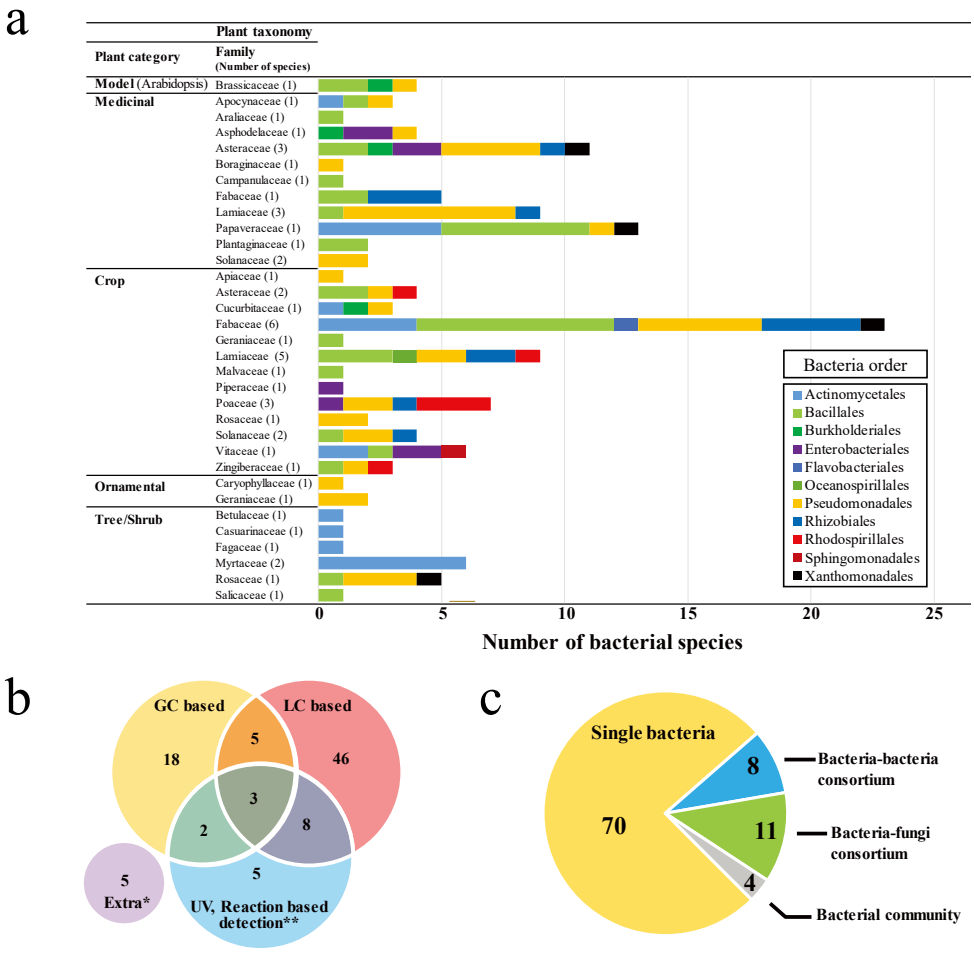

Fig 2. Plant families and bacterial orders described in the reviewed articles addressing microbe-mediated changes in phytochemistry. (**a**) taxonomy of the plants is indicated at family level along the Y-axis with the number of plant species belonging to the respective families indicated between brackets. Plant species are divided into five categories: model plant, medicinal plant, crop plant, ornamental plant and trees/shrubs. Similarly, the bacteria used in these studies are indicated at order level by colored bars and the number of bacterial species belonging to a given order is indicated along the X-axis. Details regarding the plants and bacterial species indicated in this graph are provided in the **Supplementary Tables S1 and S2**. (**b**) Analytical tools used to investigate changes in phytochemistry in response to beneficial microbes. The numbers in the Venn diagram correspond to the number of reviewed articles that used the indicated analytical tools to analyze microbe-mediated changes in phytochemistry. (**c**) Methods of application of beneficial microbes in studies involving microbe-mediated changes in phytochemistry. The numbers in the pie chart correspond to the number of reviewed articles that used one of the indicated application methods. *includes TLC and NMR, **involves reaction-based colorimetric/spectrophotometric quantitative analysis of specific groups of compounds such as total flavonoids, total phenolics, total proanthocyanidin, alkaloids and tannins.

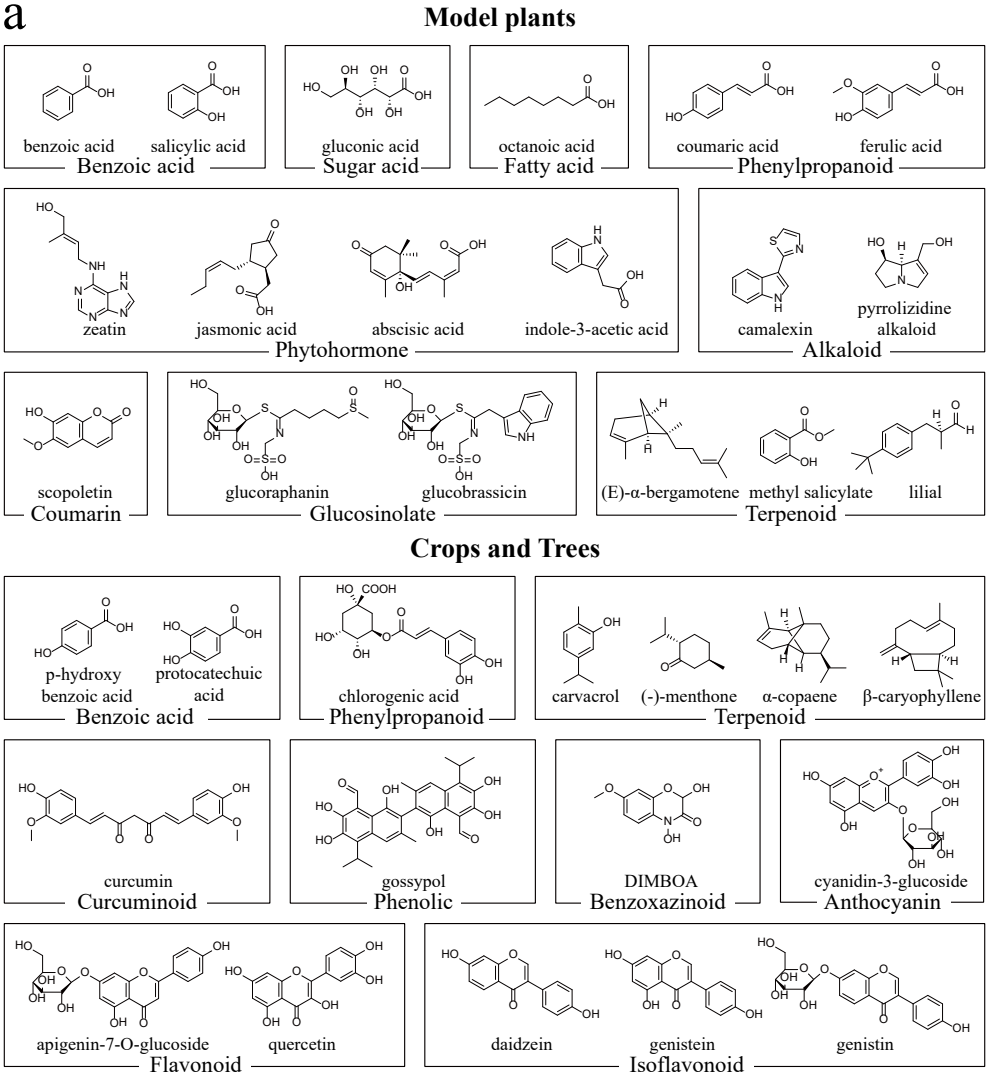

Fig 3. Chemical structures of representative plant metabolites that were changed by beneficial bacteria treatment. Detailed information on these list of metabolites is included in Supplementary **Table S1**.

of cytochrome P450 monooxygenase and cytochrome P450 oxidoreductase genes involved in artemisinin biosynthesis (Li *et al.*, 2012). Similarly, endophytic bacteria *Staphylococcus sciuri* and *Micrococcus* sp. boosted the production of therapeutically useful terpenoid indole alkaloids in *Catharanthus roseus,* including vindoline, serpentine and ajmalicine (Tiwari *et al.*, 2013). γ- Terpinene, cis-sabinene hydrate and thymol showed a significant increase when *Origanum majorana* plants were inoculated with three growth-promoting bacteria

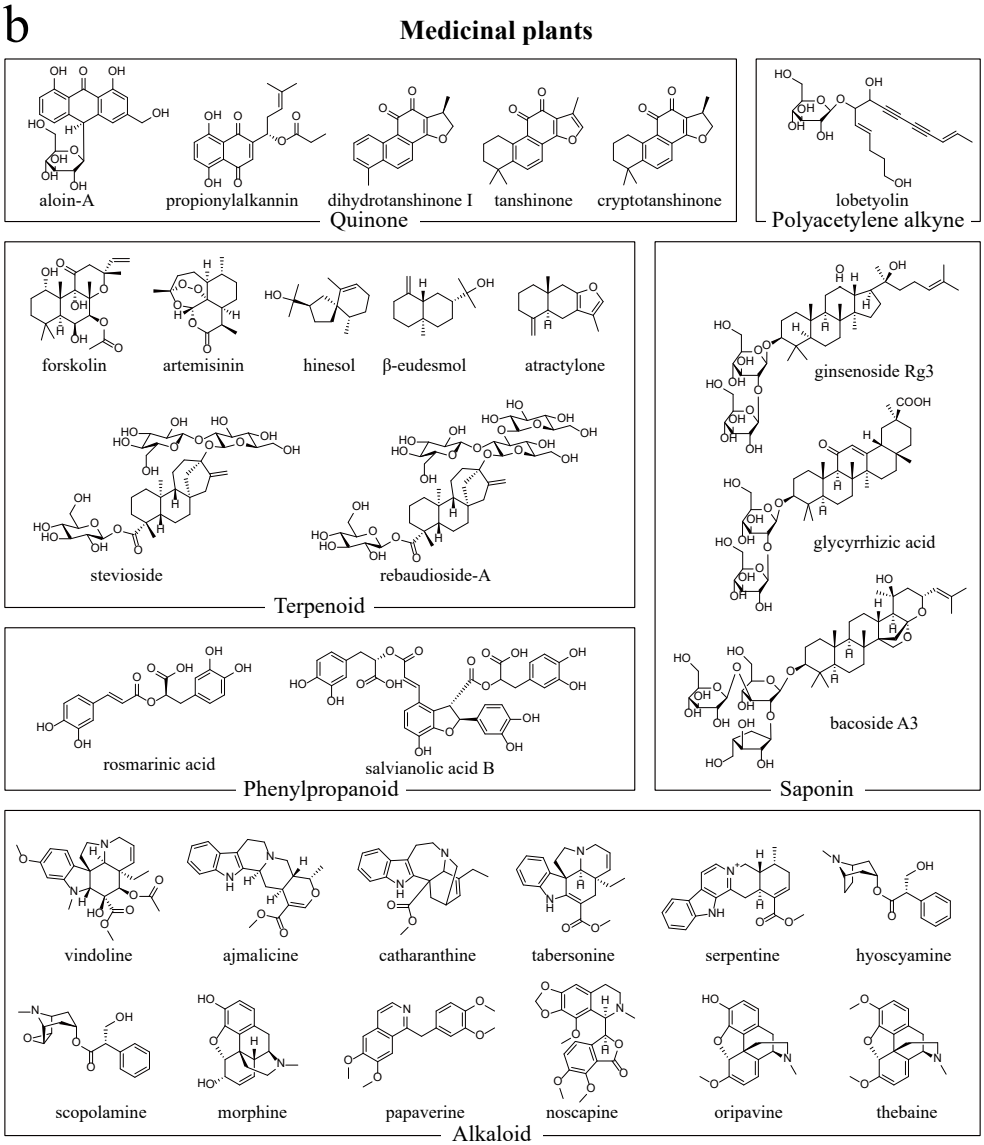

Fig 3. (Continued)

Pseudomonas fluorescens, *Bacillus subtilis* and *Azospirillum brasilense*. Inoculation of plants with *A. brasilense* only resulted in significant accumulation of carvacrol, a monoterpenoid and the major constituent of Oregano essential oils (Banchio *et al.*, 2010). Combined foliar and irrigation application of *Paenibacillus polymyxa,* an endophytic bacteria isolated from ginseng plant leaves, resulted in increased ginsenoside concentration in 1-4 year old ginseng plants by 37%, 45%, 68% and 80%, respectively (Gao *et al.*, 2015). In addition to boosting metabolite

biosynthesis of their host, root-associated bacteria are able to transform plant metabolites into different derivatives. For example, Giudice *et al.* (2015) showed the presence of root-associated bacteria in parenchymatous essential oil-producing cells. These bacteria were able to metabolize essential oil and as a consequence release large number of compounds, some of which were absent or present in very low amounts in the raw oil. Interestingly, axenic Vetiver plantlets produces *in vitro* only trace amounts of oils with strikingly different composition compared with the oils from *in vivo* Vetiver plantlets suggesting that root-associated bacteria contribute to the Vetiver oil composition. Some of the isolated root-associated bacteria were indeed able to induce the expression of the plant tepene synthase gene (Del Giudice *et al.*, 2008). Another interesting example is the conversion of the major ginsenoiside Rb1 to the potent antitumor compound ginsenoside Rg3 by *Burkholderia* sp. isolated from ginseng roots. Collectively, these studies demonstrated that endophytic bacteria can not only boost the levels of specific bioactive metabolites in their host but may also transform biologically less active forms of metabolites into active derivatives (Fu *et al.*, 2017). Other groundbreaking studies revealed that specific metabolites such as the known anti-tumor agent maytansine, a benzoansamacrolide, that was assumed to be of plant origin was actually synthesized by the endophytic bacterial community residing in the root cortex of *Putterlickia verrucosa* and *P. retrospinosa* plants (Kusari *et al.*, 2014).

Phytochemical changes and their impact on plant growth and health

In the coming sections, we will highlight several studies where plant growth promotion and plant protection against pests and diseases were investigated in relation to microbe-mediated changes in plant chemistry. Growth promotion and induced systemic resistance (ISR) are two of the most well-studied plant phenotypic responses to rhizosphere and endophytic bacteria (**Fig 1**). Similarly, a number of reports have shown global changes in the plant metabolome in response to inoculation with plant growth-promoting rhizobacteria (PGPR) (Dardanelli *et al.*, 2010; Walker *et al.*, 2011; Walker *et al.*, 2012; Algar *et al.*, 2013). Yet, the causal relationships between plant metabolome changes, plant growth promotion and/or protection (direct, indirect) conferred by these PGPRs are not well understood. In the following sections, the relationship between microbiome-mediated metabolome change and plant growth and health for model plants and crops will be discussed.

Model plants

Our earlier work showed that rhizobacterium *Pseudomonas fluorescens* strain SS101 promoted growth of *A. thaliana* and induced systemic resistance (ISR) to the bacterial leaf pathogen *Pseudomonas syringae* pv *tomato* (*Pst*) and the herbivore insect *Spodoptera exigua* (van de Mortel *et al.*, 2012). The ISR response correlated with increased levels of camalexin and glucosinolates, in particular the indole glucosinolates for insect resistance (van de

Mortel *et al.*, 2012). Mutant lines of *A. thaliana* defective in glucosinolate biosynthesis were compromised in resistance to both *Pst* and *S. exigua* (van de Mortel *et al.*, 2012), indicating an important role of these plant metabolites in the ISR response. Similar to beneficial bacteria, root colonization of *A. thaliana* by the beneficial fungus *Trichoderma asperelloides* T203 substantially altered the plant's primary metabolism with increased levels of amino acids and polyamines that are closely linked to plant growth and that act as a precursor for the biosynthesis of important defense-related secondary metabolites (Brotman *et al.*, 2012).

Even without direct physical contact with their host, beneficial rhizobacteria can influence plant metabolism, growth and health through the production of volatile organic compounds (VOCs). *Bacillus* sp B55, a rhizobacterium naturally associated with *Nicotiana attenuata* roots, produces sulfur-containing VOCs, in particular dimethyl disulfide (DMDS), which promoted plant growth. When strain B55 was grown in minimal medum containing ^{35}S-labeled Na_2SO_4 as the sole S source, plants absorbed and incorporated ^{35}S into their proteins, suggesting that provision of reduced sulfur to the plant is a possible mechanisms underlying growth promotion (Meldau *et al.*, 2013). Similarly, Arabidopsis exposed to VOCs from *Bacillus amyloliquefaciens* (GB03) showed enhanced sulfur accumulation when traced with radioactive sulfate ($^{35}SO4^{-2}$). Interestingly, microarray data analysis further indicated that several sulfate reduction genes and transcripts encoding genes for the majority of aliphatic glucosinolates and some genes involved in the indole-glucosinolate pathway were induced by the bacterial treatment. Furthermore, treated plants exhibited a significant increase in cysteine, the precursor for methionine that acts as the main substrate for aliphatic glucosinolate biosynthesis. Interestingly, glucosinolate analysis of plants exposed to BG03 VOCs showed 33 and 70% increases in the total glucosinolate content in shoots and roots, respectively, compared to the untreated control (Aziz *et al.*, 2016). Furthermore, treated plants showed significant reduction in herbivory by the insect *Spodoptera exigua*.

Crop plants

Similar to model plants, microbe-mediated changes in phytochemistry of crop plants appear to correlate with biomass enhancement and resistance against pathogens. For example, Betelvine plants treated with *Serratia marcescens* NBRI1213 showed a significant increase in biomass and retarded infection by *Phytophthora nicotianae*, an oomycete plant pathogen with broad host range. Plants treated with *S. marcescens* showed significant accumulation of gallic acid, ferulic acid, chlorogenic acid and caffeic acid (Lavania *et al.*, 2006), phenolic acids that exhibit antifungal and anti-oomycetes activities (Sarma & Singh, 2003; Widmer & Laurent, 2006; Ockels *et al.*, 2007; Nguyen *et al.*, 2013; Li *et al.*, 2017). Similarly, chickpea treated with *Pseudomonas fluorescens* (pfs3) accumulated similar potent antifungal phenolics and the level of these metabolites coincided with seedlings survival when challenged with the fungal root pathogen *Sclerotium rolfsii* (Sarma *et al.*, 2002). Moreover, rice plants treated with *Azospirullum* sp. B510 showed enhanced resistance against the rice blast fungus

Magnaporthe oryzae and the bacterial pathogen *Xanthomonas oryzae* (Yasuda *et al.*, 2009). Interestingly, *Azospirullum* sp. B510 induced p-coumaric and ferulic acid, which are major constituents of rice phenolics (Chamam *et al.*, 2013).

Similar to what is described above for model plants, rhizobacteria-mediated changes in phytochemistry in crop plants also may have an adverse effect on phytophagous insect performance. Treatment of cotton with consortia of rhizobacteria (composed of 9 *Bacillus* spp.) resulted in accumulation of gossypol and reduced herbivory by *Spodoptera exigua* via reduced the pupation and higher larval mortality. Transcriptional analysis further revealed that the rhizobacteria significantly upregulated the expression of the (+)-δ-cadinene synthase gene family involved in the biosynthesis of gossypol (Zebelo *et al.*, 2016). Collectively, these and other examples for model, medicinal, crop plants as well as trees/shrubs (**Supplementary Table S1**), signify the magnitude of changes that can be brought about by root-associated bacteria on the plant transcriptome and metabolome and how these changes can impact plant growth, development and health. Understanding the bacterial determinants involved in the alteration of the plant metabolome will shed more light on the fundamental mechanisms underlying of the activation of specific metabolic networks and their importance for the fitness to the host plant in managed or natural ecosystems.

Microbial traits and molecular mechanisms

Most of the studies reviewed here on rhizobacteria-mediated changes in phytochemistry did not provide direct evidence or insght into the underlying molecular mechanisms. Various bacterial determinants have been identified for their role in biological nitrogen fixation (Dixon & Kahn, 2004), production and regulation of phytohormones and analogues (Cassán *et al.*, 2014), siderophore production (Neilands, 1995) and phosphate solubilization (Rodríguez & Fraga, 1999). Our recent work involving site-directed mutagenesis and genetic complementation of *Pseudomonas fluorescens* strain *Pf* SS101 pointed to three genes, i.e. phosphogluconate dehydrates gene (*edd*), the response regulator gene *coIR* and the adenylsulfate reductase gene (*cysH*), involved in plant growth promotion and ISR. Also various other bacterial traits have been identified for their role in plant growth promotion and ISR, including lipopolysaccharides (Leeman *et al.*, 1995), salicylic acid (De Meyer & Höfte, 1997), siderophores (Sayyed *et al.*, 2013), 2-aminobenzoic acid (Yang *et al.*, 2011), cyclic lipopeptides (Ongena *et al.*, 2007), exopolysaccharides (Uma Sankari J. *et al*, 2011), cell wall degrading enzymes (Kobayashi *et al.*, 2002), flagella (Meziane *et al.*, 2005), 2,4-diacetylphloroglucinol (Weller *et al.*, 2004), 2,3-butanediol (Ryu *et al.*, 2004), and N-alkylated benzylamine (Ongena, M. *et al.*, 2005). To date, however, bacterial determinants involved in the alteration of the plant metabolome remain largely elusive. Recently, phenylacetic acid (PAA) produced by beneficial bacteria *Bacillus fortis* IAGS162 was shown to be a determinant of ISR against Fusarium wilt disease in tomato. Plants exposed to PAA and challenged with the fungal pathogen *F. oxysporum* f.sp. *lycopersici* showed extensive changes in their primary metabolism. Particularly, the shikimate

and phenylpropanoid pathways (l-phenylalanine, cinnamic acid, benzoic acid, caffeic acid, and salicyclic acid) were upregulated (Akram *et al.*, 2016). Similarly, 3-hydroxy-5-methoxy benzene methanol (HMB), isolated from extracellular metabolites of *Pseudomonas aeruginosa* PM12, was shown to have significant impact on the plant metabolome and to be a potent determinant of ISR. Tomato plants treated with HMB showed enhanced levels of sugars (fructose, glucose, sucrose), phosphorylated sugars (fructose-6- phosphate, glucose-6-phosphate, myo-inositol-phosphate), organic acids (α-ketoglutarate, malate, oxaloacetate, citrate, succinate), polyamines (picolinic acid, pipecolic acid, putrescine, spermidine), amino acids (phenylalanine, arginine, glutamine and proline) and salicylic acid. The levels of glycerol-3-phosphate, fumarate, cis- aconitate, leucine, tyrosine and threonine were reduced in plants treated with HMB when compared to untreated control plants (Fatima & Anjum, 2017). As indicated above, beneficial rhizobacteria can also influence the host metabolome via VOCs, that may influence the expression of various plant genes including those involved in flavonoid (Zhang *et al.*, 2007) or sulfur metabolism (Meldau *et al.*, 2013; Aziz *et al.*, 2016).

Microbial-Gene Positioning System (m-GPS)

The above-mentioned examples highlight the paramount importance of plant-associated microbes in shaping the metabolome landscape of their host. Microbe-mediated alteration or reprograming of the plant metabolome is dependent on the combination of plant species/cultivar and microbial species. For example, when two rice cultivars (Cigaron and Nipponbare) were inoculated with *Azospirillum lipoferum*, *the* impact on the root metabolome was greater for Cigaron. More specifically, treated Cigaron cultivars exhibited a reduction in the level of a number of flavonoids and an increase in the level of hydroxycinnamic acid derivatives and alkylresorcinol (Chamam *et al.*, 2013). In another study, treatment of these two rice cultivars with either *A. lipoferum* 4B (rhizospheric) or *Azospirillum sp*. B510 (endophytic) resulted in *Azospirillum* strain x cultivar specific changes in the metabolome. Strain 4B (rhizospheric bacteria isolated from Cigalon) induced major changes in secondary metabolism only in Cigalon roots, while strain B510 (endospheric bacteria isolated from Nipponbare) induced metabolic changes in shoots and roots of both rice cultivars (Chamam *et al.*, 2013). Whether these differences are truly strain specific or more related to their lifestyle, i.e. rhizosphere versus endosphere, remains to be resolved. Also treatment of seeds of two maize cultivars (PR37Y15 and DK315) with three beneficial bacteria *A. brasilense* CFN-535, *A lipoferum* CRT1 and *A. brasilense* UAP-154, revealed major *Azospirillum* strain-cultivar specific qualitative and quantitative changes in secondary metabolism, primarily benzoxazinoids (Walker *et al.*, 2011). Based on these observations, we postulate that microbes, due to their long standing co-evolutionary relationship with their host, serve as specialized engineers of the plant's primary and secondary metabolism. We predict that such specific alteration of the plant metabolome when interrogated by integration of different approaches involving transcriptomics, proteomics and metabolomics, can reveal new genes, enzymes, pathways

and metabolic networks in plants associated with growth, development and tolerance to (a) biotic stresses (**Fig 4**). In this context, microbe-plant interactions may serve as a valuable tool to predict and identify rate limiting/critical steps in plant metabolic pathways. To further frame the more fundamental concept behind this approach, we propose the term "microbial-Gene Positioning System (m-GPS)", which involves identification of metabolic pathways that are perturbed by the microbial treatment followed by mapping of the changes in the expression of genes and the activity of enzymes belonging to the corresponding metabolic pathways. Based on these analyses, pragmatic decisions can be made to pick the most influential gene(s) that is/are targeted by the beneficial microbes to impact the flux of a given metabolite(s). The role of these candidate genes can be validated using mutant lines or reverse genetics tools. If indeed these genes show an influential role in the biosynthesis of high value natural plant products (HVNPs), they can be used in conventional breeding or synthetic biology programs for trait improvement. Screening for allelic variants/orthologues of the candidate genes across plant species may further help to identify an elite allelic variant and the associated sequence information can be used for protein engineering intended to create ultra-enzymes in biotechnology and synthetic biology programs (**Fig 4a**). Similarly, bacterial traits involved in the modulation of the plant metabolome can be explored as an innovative strategy to improve the collective genome of the rhizosphere microbiome. Once prominent bacterial traits are identified, further screening of allelic variants/orthologues can be performed across several rhizobacterial species and strains of a given species. Using such strategy, best performing allelic variant(s) of bacterial traits in altering the plant metabolome can be used to enhance the content of HVNPs in plants (**Fig 4b**). The current targeted genome editing techniques such as the CRISPR/Cas system (Selle & Barrangou, 2015) will have a profound contribution to our quest to understand signal perception and downstream signalling and to ultimately engineer bacterial traits to impact plant chemistry.

Concluding remarks and future perspective

In this review, we provided an overview of microbe-mediated changes in plant chemistry and other plant phenotypes (enhanced growth, ISR). Establishing a causal relationship requires comprehensive understanding of the metabolic pathways involved in plant growth and defense, and of the microbial traits and mechanisms underlying the alteration of plant phenotypic and metabolic traits. Unravelling the chemical interplay between microbial metabolites, enzymes and elicitors and how these affect plant signal perception, signal transduction and the downstream metabolic networks requires systems-based approaches to connect host- and microbial traits with the observed changes in the metabolome, biomass and resistance to biotic stresses. The number of publications in the area of bacteria-mediated phytochemical alterations is increasing due to exciting developments in next generation sequencing and state-of-the-art LC/GC hyphenated mass spectrometry technologies **(Fig 2b)**. Future studies that focus on the beneficial microbe-mediated plant metabolome reprograming

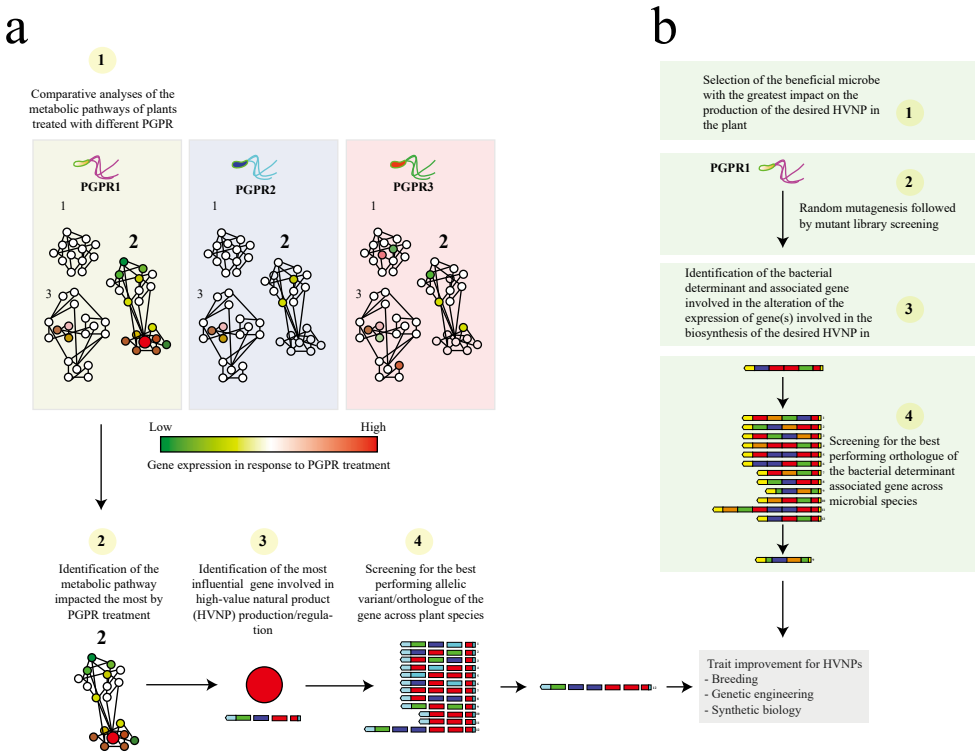

Fig 4. Illustration of the microbial Gene Positioning System (mGPS), a conceptual framework that proposes to use beneficial microbe-mediated alteration of plant metabolic pathways as a means to identify influential genes involved in the biosynthesis and regulation of High Value Natural Products (HVNPs). (**a**) The first strategy initially involves comparing the influence of different beneficial microbes on the plant metabolic pathways involved in the production of HVNPs (1), after which follows the identification of the metabolic pathway that is impacted the most by the treatment of beneficial microbes (2). Subsequently, the most influential gene in the biosynthetic pathway of HVNPs is identified (3); following this step, the performance of allelic variants/orthologues of the identified gene from different plant species is evaluated and the best performing allelic variant/orthologous gene is then used for improvement of HVNPs production in plants (4). (**b**) A second potential strategy to improve the production of HVNPs in plants is to screen a number of beneficial microbes for their impact on the production of targeted compounds (1). The best performing microbe is then subjected to random mutagenesis (2) to identify the microbial determinant involved in the alteration of HVNPs (3). Then, orthologous genes of the microbial determinant are evaluated for their ability to elicit maximum production of the targeted HVNPs (4).

will expand our horizon beyond the use of microbes as a biocontrol agent or as plant growth promotor only. Endophytes are receiving particular attention in this research field as they are assumed to have a more intimate association with their host plant than microorganisms that live in the 'spheres' (rhizosphere, phyllosphere). Interestingly, metabolites detected in crude extracts from plants could originate from the plant, the endophyte (Kusari *et al.*, 2014), from the combined effort of both (Kusari *et al.*, 2011; Heinig *et al.*, 2013), from modification

of the plant metabolites by endophytes (Tian *et al.*, 2014; Fu *et al.*, 2017), or even from modification of endophyte metabolites by the host plant (Strobel & Hess, 1997). Moreover, endosymbiotic bacteria residing inside the fungal hyphae (Hoffman & Arnold, 2010) as well as endophyte-endophyte interactions may even increase the complexity of the overall 'plant' metabolome. These examples underscore how the genetics and chemistry between plants and their microbiomes (i.e. the holobiont (Rohwer *et al.*, 2002; Rosenberg & Zilber-Rosenberg, 2016)) are intertwined to each other. Although most studies to date have focused on the impact of one microbial strain on the plant metabolome (**Fig 2c**), future studies that interrogate the relationships between the microbiome composition, its functions and plant metabolome dynamics will greatly help to understand the ecological significance of microbe-mediated alteration of the plant metabolome.

Supplementary materials

Table S1. Overview of studies on alterations in the chemistry of model, medicinal, crop and ornamental plant species induced by beneficial bacteria

Table S2. Overview of different bacterial genera studied for their impact on the chemistry of different plant species

Above supplementary tables are available at: https://doi.org/10.1039/C7NP00057J