Interactions of fungal pathogens and antagonistic bacteria in the rhizosphere of *Brassica napus*

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Abstract

The rhizosphere is an active interface where plants and microorganisms (pathogenic, beneficial and neutral) establish a complex and varied molecular dialogue, however knowledge of the functional mechanisms mediating interactions is still limited. Plants invest a significant proportion of their photosynthetically fixed carbon in maintaining the rhizosphere microbiome via root exudation and in return beneficial microbes provide profitable functions to the plant. The potential of naturally occurring soil microorganisms to control phytopathogens and to promote plant growth is well documented, but the functional mechanisms governing the reciprocal signaling between microbial communities and plants are not well understood. The aim of the studies described in this thesis was to gain insight into the functional basis of interactions between the fungal root pathogen *Rhizoctonia solani* and root associated antagonistic bacteria of the genus *Serratia* in the rhizosphere of *Brassica napus*.

Transcriptomic responses of the oilseed rape pathogen *R. solani*, to the plant-associated and pathogen- antagonistic bacteria *Serratia proteamaculans* S4 *and S. plymuthica* AS13, were studied using RNA-sequencing. The results demonstrate a major shift in the fungal gene expression with simultaneous alterations in primary metabolism, activation of defense and attack mechanisms and distortions in hyphal morphology.

Stable isotope probing coupled with high throughput sequencing allowed the description of the composition of bacterial and fungal communities in the rhizosphere soil and the roots of *B. napus* and the identification of active taxa capable of assimilating recently fixed plant carbon. Our results support the idea of active selection of microbial communities from the more diverse rhizosphere environment by the roots. Furthermore, the data confirm the potential of some active genera (*Streptomyces*, *Rhizobium*, *Clonostachys* and *Fusarium*) to be used as microbial inoculants for improved productivity and health of oilseed rape.

Patterns of gene expression in *B. napus* exposed to factorial combinations of *R. solani* and *S. proteamaculans* S4 were examined *in-vitro* using RNA-sequencing. Plants inoculated with *R. solani* only were almost dead at 240h post-inoculation and massive transcriptional reprogramming was observed, whereas the presence of S4 modulated the transcriptional responses and resulted in healthy plants. With *R. solani* present, we observed an interplay between stress and defense involving salicylic acid, jasmonic acid, ethylene and abscisic acid as common regulators. Induced systemic resistance when S4 present potentially depends on jasmonic acid, auxin and salicylic acid. Downregulation of stress-related and upregulation of defense-related genes were associated with transcriptional responses suggesting floral induction and plant development.

Keywords: rhizosphere, *Serratia* bacteria, plant-microbe interactions, active microbiome, *Brassica napus*, *Rhizoctonia solani,* RNA-sequencing, transcriptome, antagonism

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Interaktioner mellan svamppatogener och antagonistiska bakterier I rhizosfären av *Brassica napus*

Sammanfattning

Rhizosfären är ett aktivt gränsskikt där växter och mikroorganismer (patogena, mutualistiska och neutrala) upprättar en komplex och varierad molekylär dialog, men kunskap om de underliggande funktionella mekanismerna bakom deras samspel är fortfarande begränsad. Växter investerar en betydande del av sin energi från fotosyntesen för att upprätthålla rhizosfärsmikrobiomen med hjälp av sitt energirika rotexudat och i gengäld får de näring samt skydd mot växtpatogener av nyttiga mikrober. Potentialen hos dessa jordlevande mikrober med avseende på skydd mot växtpatogener och stimulera växternas tillväxt är väldokumenterad, men vi förstår fortfarande inte de bakomliggande funktionella mekanismerna, eller de signaler som styr samspelet mellan dessa organismer. Syftet med studier i denna avhandling är att få insikt om de funktionella verkningssätten för samspelet mellan rapspatogenen *Rhizoctonia solani*, antagonistiska bakterier av släktet *Serratia* och oljeväxten, *Brassica napus*.

RNA-sekvensering användes för att studera samspelet mellan rapspatogenen, *R. Solani*, och de växtassocierade antagonistiska bakterierna *Serratia proteamaculans* S4 och *S. plymuthica* AS13. Svampens mycelmassa genomgick drastiska strukturella förändringar vid saminokuleringen med bakterierna och omfattande förändringar observerades i svampens genuttryck avseende den primära metabolismen, samt en aktivering av svampens försvar och angreppsmekanismer.

I en separat studie, pulsmärktes unga rapsplantor med $_{13}CO^2$ i syfte att identifiera de mikroorganismer som förekommer i rhizosfären och som lever på kol i rotexudat. Det inmärkta kolet följdes i mikroorganismerna med metoden $_{13}$C-RNA-SIP. Kombinationen av $_{13}$C-RNA-SIP med massiv sekvensering av DNA- och RNA-markörer möjliggjorde identifieringen av aktiva bakterie- och svampsamhällen i rhizosfärsjorden i jämförelse med i rapsens rötter. Våra resultat stödjer idén att rötterna har förmågan till aktivt urval av mikrooganismer från den artrika rhizosfären. Flera aktiva mikroorganismer såsom *Streptomyces*, *Rhizobium*, *Clonostachys* och *Fusarium* upptäcktes också i unga rapsrötterna med potential att förbättra produktivitet och hälsa hos raps genom att säkerställa uppkomst och tidig etablering av grödan.

Genuttrycken hos *B. napus* fröplantor studerades med hjälp av RNA-sekvensering i faktoriella kombinationer med *R. solani* och *S. proteamaculans* S4 i en steril gnotobiotisk miljö. Då växtrötter inokulerades med enbart *R. solani* var de nästan döda efter 240h och en omfattande transkriptionell omprogrammering observerades. Inokulering med S4 däremot resulterade i måttliga transkriptionella förändringar av genuttrycket, samt friska växter. Vid inokulering med *R. solani* ett samspel mellan stress- och försvarsassocierade gener observerades, som involverar salicylsyra, jasmonsyra, eten och abscisinsyra. Inducerad systemisk resistens observerades vid inokulering med S4, vilket kan potentiellt förklaras med ett samspel mellan jasmonsyra, indole ättiksyra (auxin) och salicylsyra. Nedreglering av stressrelaterade gener medan uppreglering av försvarsrelaterade gener var kopplade till uttryck av gener som styr blomning och plantornas tillväxt och utveckling.

Dedication

To my beloved family and especially to my grandparents…

There is only one good, knowledge, and one evil, ignorance.
Socrates

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I **Gkarmiri, K.,** Finlay, R.D., Alström, S., Thomas, E., Cubeta, M. A., and Högberg, N., (2015). Transcriptomic changes in the plant pathogenic fungus *Rhizoctonia solani* AG-3 in response to the antagonistic bacteria *Serratia proteamaculans* and *Serratia plymuthica*. *BMC Genomics*, 16 (1), 630, https://doi.org/10.1186/s12864-015-1758-z.

II **Gkarmiri, K.,** Mahmood, S., Ekblad, A., Alström, S., Högberg, N., and Finlay, R.D., (2017). Identifying the active microbiome associated with roots and rhizosphere roil of oilseed rape. *Applied and Environmental Microbiology*, 83 (22), pii: e01938-17. doi: 10.1128/AEM.01938-17.

III **Gkarmiri, K.,** Alström, S., Finlay, R.D., and Högberg, N., Modification of the *Brassica napus* transcriptome by *Serratia proteamaculans* S4 during interaction with the plant pathogenic fungus *Rhizoctonia solani* AG2-1. (Manuscript)

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The contribution of Konstantia Gkarmiri to the papers included in this thesis was as follows:

I Participated in the experimental design, set up the assay, carried out the experiment, performed the bioinformatic analysis and wrote the paper assisted by co-authors.

II Participated in the design of the experiment, carried out the experimental part, analyzed the data. Wrote the paper in cooperation with the co-authors.

III Participated in the design of the project, conducted the experiment, analyzed the transcriptome data and wrote the manuscript with comments and suggestions from the co-authors.

Abbreviations

ABA	Abscisic acid
ACC	1-aminocyclopropane-1-carboxylatedeaminase
AM	Arbuscular mycorrhiza
BCA	Biological control agent
BRs	Brassinosteroids
CFUs	Colony forming units
CK	Cytokinin
DEG	Differentially expressed genes
ER	Endoplasmic reticulum
ET	Ethylene
ETI	Effector-triggered immunity
ETS	Effector-triggered susceptibility
GA	Gibberellin
GO	Gene ontology
GSLs	Glucosinolates
HR	Hypersensitive response
IAA	Indole acetic acid (auxin)
IPM	Integrated pest management
ISR	Induced systemic resistance
JA	Jasmonic acid
MAMP	Microbe-associated molecular pattern
MS	Murashige and Skoog basal salt mixture
PDA	Potato dextrose agar
PGPR	Plant growth promoting rhizobacteria
PRRs	Pattern recognition receptors
PTI	Pattern-triggered immunity
qRT-PCR	Quantitative real-time PCR
RN	Root nodules

ROS	Reactive oxygen species
SA	Salicylic acid
SAR	Systemic acquired resistance
SIP	Stable isotope probing
TSA	Tryptic soy agar
TSB	Tryptic soy broth

1 Introduction

1.1 Plant growth and health

In the twenty-first century, one of the major challenges of agriculture is to increase crop yields in a sustainable way. Such intensification in crop production is necessary to fulfill the food demands of an increasing human population as well as the need for the use of renewable energy and feed (Berg, 2009). At the same time plants, as sessile organisms, live in constantly changing environments, which are usually stressful and unfavorable for their development and growth. These adverse conditions include stresses that are both biotic (pathogen infection, herbivore attack) and abiotic (drought, cold, nutrient deficiency, heat and excess salinity and toxic metals) (Zhu, 2016).

Plants have developed a plethora of complex immune response pathways, enabling them to survive specific as well as combined stresses (Nejat & Mantri, 2017). There is increasing evidence suggesting that there is a significant overlap between defense genes being commonly involved in response to different biotic and abiotic stresses (Zhang *et al.*, 2016; Massa *et al.*, 2013; Mantri *et al.*, 2010). Moreover, abiotic stresses have a negative impact on biotic stress resistance and can result in an increased susceptibility of the plant to biotic stresses (Kissoudis *et al.*, 2015; Wang *et al.*, 2009a). On the other hand, another potential outcome of multiple stress exposure is that plants that are able to defend themselves against one stress can become more resistant to other stresses, a phenomenon called cross-tolerance, implying that plants have a powerful regulatory system allowing them to adapt quickly to a changing environment (Capiati *et al.*, 2006; Bowler & Fluhr, 2000).

Knowledge on the complex signaling plant immune cascades could potentially be exploited by plant biotechnology via molecular engineering. In addition, the development of several new crop varieties with greater resistance, enhanced tolerance to salt and drought and improved nutritional value has been achieved via plant breeding (Berg, 2009). However, the ability of plants to create mutualistic associations with microbiota colonizing the plant roots as well as the soil surrounding the roots (rhizosphere) is also of great significance. Mutualistic interactions can either help the plant to acquire nutrients from the soil, provide indirect pathogen protection or release phytohormones to stimulate plant growth (Lugtenberg & Kamilova, 2009) thus plant microbiota are an emerging novel trait, which extends the capacity of plants to adapt to their environment (Bulgarelli *et al.*, 2013). Very well studied endosymbioses include the establishment of arbuscular mycorrhizal (AM) fungi in most flowering plants and nitrogen-fixing rhizobia in legumes (Oldroyd *et al.*, 2009). Other mutualistic interactions between plants and microbes include those with Biological Control Agents (BCAs), which can be either bacteria known as Plant Growth Promoting Rhizobacteria (PGPRs) with biocontrol ability or biocontrol fungi. These microorganisms exist naturally in the soil surrounding the plant roots, on the root surface or can even be endophytic (Lugtenberg & Kamilova, 2009). Another ecological niche, the phyllosphere microbiome is of great interest as well, since microbial colonizers of the aboveground part of the plants can also exert beneficial effects to the plants, such as growth promotion or protection against biotic and abiotic stresses (Ritpitakphong *et al.*, 2016; Schlaeppi & Bulgarelli, 2015; Penuelas & Terradas, 2014; Vorholt, 2012).

In 2017, the Organic Materials Review Institute (OMRI) listed 174 products as ‘microbial inoculants’ and 274 products as ‘microbial products’ used as crop fertilizers or as crop management tools (Finkel *et al.*, 2017). Despite such a commercialization, there is very strict registration regulation and the process is long and costly, but ensures that the product is safe for humans as well as for the environment (Tranier *et al.*, 2014). Commercialization of microbes producing antibiotics is discouraged since there is the potential for cross-resistance with other antibiotics applied for human and animal use, whereas microbes competing (with pathogens) for nutrients and niches have better potential (Lugtenberg & Kamilova, 2009).

The most commonly used method in agriculture to control diseases is the use of chemical pesticides, however the EU has already removed a number of chemical products and further restrictions are expected (Berg, 2009). Integrated Pest Management (IPM) is the most effective and environmentally sensitive approach for pest management and relies on the use of alternative practices such as crop rotation, resistant cultivars, use of resistant varieties, mechanical controls, biological control or other cultural practices and has as a principle to responsibly use chemical pesticides (Barzman *et al.*, 2015).

1.2 The rhizosphere

The rhizosphere is the narrow zone surrounding and influenced by plant roots. It is occupied by different groups of organisms, pathogenic, beneficial and neutral and is considered as one of the most complex ecosystems on Earth (Raaijmakers *et al.*, 2009; Pierret *et al.*, 2007; Hinsinger & Marschner, 2006). It is an active interface in which plants and microorganisms establish a complex and varied molecular dialogue, which involves nutrient transfer as well as specific interactions mediated by the release of signaling molecules from plant roots (van Elsas *et al.*, 2012; Prosser *et al.*, 2006), potentially resulting in increased plant productivity (van der Heijden *et al.*, 2016). Between 20% and 50% of photoassimilated carbon is transferred to the roots and half of this is subsequently released into the soil (Kuzyakov & Domanski, 2000). More precisely, rhizosphere microbiome members are capable of utilizing a large amount of nutrients released by the roots, known as rhizodeposits (exudates, border cells, polysaccharide mucilage), which are supposed to be the primary driving force that regulates microbial diversity and activity on plant roots. These exudates affect soil microbial community structure and activity, resulting in the 'rhizosphere effect' (i.e. significantly elevated number of microorganisms) (Philippot *et al.*, 2013; Jones *et al.*, 2009). This implies that plants might be capable of adjusting the rhizosphere microbiome to their benefit, either via helping the plant to acquire nutrients, via providing indirect protection from pathogens or by improving root architecture (Pieterse *et al.*, 2016; Venturi & Keel, 2016; Mendes *et al.*, 2013; Cook *et al.*, 1995). The rhizosphere competence of PGPRs suggests they are well adapted to utilize carbon resources (Lugtenberg &

Kamilova, 2009). Rhizodeposition also includes the release of a specialized cell population known as root cap border cells into the rhizosphere, being very attractive candidates for contributing to the 'rhizosphere effect' because of their capacity to remain alive into the soil for a long time (Dennis *et al.*, 2010; Hawes *et al.*, 2000). During lateral root emergence, cellular disjunction on the root surface takes place, providing a potential entry gate for the rhizosphere microbiome into the root interior (Bulgarelli *et al.*, 2013) (Figure 1).

Another interesting phenomenon is the presence of disease-suppressive soils, soils in which little or no disease occurs under conditions that are favorable for disease development (Kinkel *et al.*, 2011). Disease suppressiveness can be either a natural characteristic of certain soils or can be induced after many years of monoculture of the same crop. Disease control in such soils is primarily attributed to the root microbiota (Berendsen *et al.*, 2012; Kinkel *et al.*, 2011). Interestingly bacteria of the genus *Pseudomonas* have been identified as key players in disease suppressive soils either via the production of nonribosomal peptide synthetases or via the bacterial production of the antibiotic 2,4-diacetylphloroglucinol (Mendes *et al.*, 2011; Raaijmakers & Weller, 1998).

Moreover, the rhizosphere microbiome is known to have at least a degree of specificity for each plant species, since the root exudates composition is determined by factors such as plant cultivar and species, plant developmental stage, soil type and pH, temperature and the presence of microorganisms (Badri & Vivanco, 2009). Recent deep sequencing studies have demonstrated that the soil type affects the bacterial rhizosphere microbiome to a greater extent rather than the plant genotype (Schlaeppi *et al.*, 2014; Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012). It has also recently been proved that both plant and microbe genotype contribute to whether or not a rhizosphere microbiome provides a beneficial or harmful effect on the plant (Haney *et al.*, 2015). However, it has been demonstrated that microbial communities in the rhizosphere of wheat, pea and oat differed significantly at the kingdom level between plant species (Turner *et al.*, 2013). Lastly, the plant developmental stage also has documented effects on fungal community structure in the rhizosphere in potato (Hannula *et al.*, 2010).

The rhizosphere microbiome has been shown to differ significantly from that of the endophytic root compartment (Gkarmiri *et al.*, 2017; Edwards *et al.*, 2015; Bulgarelli *et al.*, 2012; Lundberg

et al., 2012) as well as from that of the surrounding bulk soil (Bulgarelli *et al.*, 2015).

Furthermore, it is of great significance to identify not only the microbial taxa that are present in the rhizosphere, but also those that are capable of actively assimilating plant-derived carbon, in order to get an insight of the active microbiome. This can be achieved by the exploitation of Stable Isotope Probing (SIP) (Bressan *et al.*, 2009; Haichar *et al.*, 2008; Vandenkoornhuyse *et al.*, 2007; Dumont & Murrell, 2005). A considerable number of research studies have focused on that aspect for both bacteria and fungi (Gkarmiri *et al.*, 2017; Dias *et al.*, 2013; Haichar *et al.*, 2012; Hannula *et al.*, 2012; Gschwendtner *et al.*, 2011; Rasche *et al.*, 2009).

Understanding of the processes that determine the composition, dynamics and activity of the rhizosphere microbiome is thus important, since it has been suggested that the number as well as the diversity of microorganisms in the rhizosphere microbiome are linked to the quality and quantity of the rhizodeposits as well as to the outcome of microbial interactions taking place in the rhizosphere (Somers *et al.*, 2004).

1.2.1 Impacts of root exudates on rhizosphere microbial communities

The elucidation of the impact of rhizosphere interactions at the microbial community level is of great significance since plants are capable of selecting and attracting specific microbes, and can thus alter the composition and diversity of rhizosphere microbial communities in a plant-specific manner (Broeckling *et al.*, 2008; Houlden *et al.*, 2008). For example, it has been shown that an *Arabidopsis* ABC transporter mutant in secreting more phenolics than sugars in comparison to the wild-type, promoted significant changes in the natural microbial community, associated with PGPRs, nitrogen-fixing bacteria and metal remediation bacteria (Badri *et al.*, 2009a). Similarly, when *Arabidopsis* natural chemicals were added to soil, distinct rhizosphere communities were selected that were capable of degrading the herbicide atrazine and a greater variation of symbiotic bacteria was observed (Badri *et al.*, 2013). Moreover, it has been demonstrated that application of the root exudate *p*-coumaric acid to soil grown with cucumber seedlings, stimulated buildup of both bacterial and fungal communities and changed their organization and composition as well as increased the abundance of

the soil-borne pathogenic fungus *Fusarium oxysporum* f.sp. *cucumerinum* Owen (Zhou & Wu, 2012). More recently it has been shown that two *Arabidopsis* mutants impaired in the production of the hormone jasmonic acid (JA), which has significant roles in plant development and defense, exhibit distinct exudation patterns compared to wild-type plants and harbor distinct bacterial and archaeal rhizosphere communities implying a role of exudates in plant defense responses too (Carvalhais *et al.*, 2015). On the other hand, root exudation can also have a positive effect on plant pathogens, as it has been documented in tomato, where exudation of citrate and glucose allow the germination of spores of the tomato root pathogen *F. oxysporum* f. sp. *radicis-lycopersici* (Kamilova *et al.*, 2008).

1.2.2 Impacts of rhizosphere microbial communities on plant root exudation

The rhizosphere microbiome can also affect the exudation pattern of plant roots (Matilla *et al.*, 2010; Jones *et al.*, 2004). For instance, it has been shown that plant colonization with arbuscular mycorrhizal fungi, quantitavely changes exudation by increasing the secretion of gibberellins, phenolics and nitrogen and via reducing the secreted phosphorus, sugars and potassium ions (Jones *et al.*, 2004). Moreover, it has been demonstrated that the abundance and identity of fungi associated with the roots affects the exudation rates in pine seedlings (Meier *et al.*, 2013).

1.2.3 Complex tripartite interactions in the rhizosphere

The rhizosphere is known as a battlefield between soilborne pathogens and antagonistic microbiota and their interactions can influence the outcome of pathogen infection in the plant since the activity of pathogens can be inhibited by beneficial microbes (Raaijmakers *et al.*, 2009). Microorganisms found in the rhizosphere can be beneficial (e.g. PGPRs, nitrogen-fixing bacteria, mycorrhizal fungi, BCAs), deleterious to the plant (pathogenic bacteria and fungi, oomycetes, nematodes), or even pathogenic to humans (Mendes *et al.*, 2013). In this highly dynamic niche complex tripartite interactions occur between beneficial microbes, pathogens and plants and these complex relationships are based on reciprocal signaling

between diverse microbial consortia and plants both in the rhizosphere soil as well as the endophytic root compartment (Evangelisti *et al.*, 2014; Badri *et al.*, 2009b). Regarding biocontrol of plant pathogens, interactions that take place can be a) direct and reciprocal between the BCA and the plant pathogen, b) direct between the BCA and the plant or c) indirect between the BCA and the pathogen (i.e. responses are mediated via the plant). Until now, most studies have focused on the behavior and the mechanisms that BCAs (mainly bacteria of the genera *Bacillus*, *Burkholderia*, *Collimonas*, *Pseudomonas, Azospirillum, Serratia, Flavobacterium,* but also fungi of the genus *Trichoderma* and *Clonostachys*) utilize against fungal pathogens (Hennessy *et al.*, 2017; Martinez-Medina *et al.*, 2017; Kamou *et al.*, 2016; Lahlali, 2014; Rodriguez *et al.*, 2011; Compant *et al.*, 2005; Haas & Defago, 2005; Whipps, 2001). However, it is also of great significance to examine how fungi respond to bacteria and this aspect has received much less attention at the transcriptome level (Schmidt *et al.*, 2017; Ipcho *et al.*, 2016; Deveau *et al.*, 2015; Gkarmiri *et al.*, 2015; Mathioni *et al.*, 2013; Mela *et al.*, 2011; Schroeckh *et al.*, 2009). However, there are studies focusing on the production of fungal volatiles and on the effects that these have on bacteria (Schmidt *et al.*, 2017; Schmidt *et al.*, 2015). Moreover, analysis of the plant response to colonization with both a BCA and a pathogen with the exploitation of the new and powerful highly throughput RNA sequencing technology has started to shed greater and more comprehensive mechanistic understanding of the molecular communication between the different organisms (Laur *et al.*, 2018; Imperiali *et al.*, 2017; Vogel *et al.*, 2016; Daval *et al.*, 2011; Pozo *et al.*, 2008).

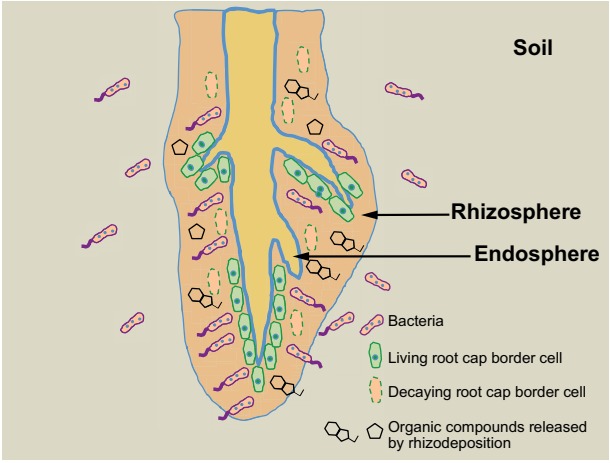


Figure 1. Niche differentiation and root exudation at the root-soil interface. Plant roots selectively secrete organic compounds and root cap border cells (rhizodeposits) that function as semiochemicals for the assembly of the root microbiome. Selected bacterial strains from the bulk soil communities specifically respond to host signals and reprogram to express traits related to root colonization. Once PGPRs are established on the root, cell wall polysaccharides from the host function as environmental cues to promote biofilm formation on the root surface. Within the biofilm matrix, individual members and/or microbial consortia integrate host and self-derived signals to activate processes in the plant that result in enhanced plant growth, induced systemic resistance (ISR) antibiotic production and competition for nutrients and niches. (Adapted from Bulgarelli *et al.* 2013, *Annu. Rev. Plant Biol.* **64**:807-838.)

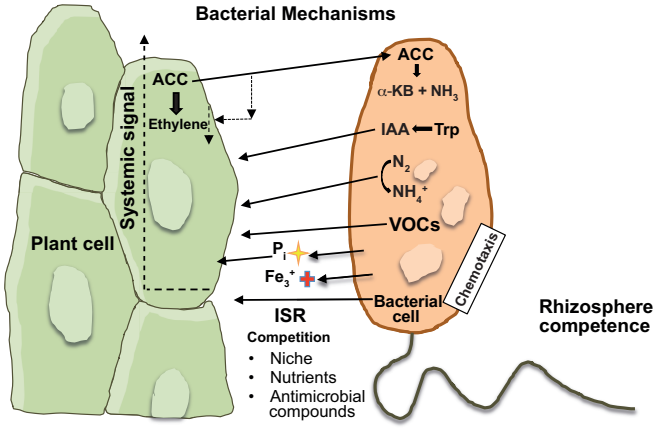


Figure 2. Mechanisms used by PGPRs to promote plant growth and health. Rhizosphere competence is illustrated by the flagellum and chemotaxis. Traits shown: (ACC:1-aminocyclopropane-1-carboxylate deaminase activity, IAA: auxin biosynthesis, biological nitrogen fixation, VOCs: volatile organic compound production, P: phosphorus solubilization, Fe_3^+: siderophore production, ISR: induced systemic resistance). (Adapted from Bulgarelli *et al.* 2013, *Annu. Rev. Plant Biol.* **64**:807-838.)

1.3 Plant growth promoting rhizobacteria

PGPRs are capable of establishing mutualistic interactions with plants and exert either direct or indirect beneficial effects on the host, related to plant growth and health (Bulgarelli *et al.*, 2013; Berg, 2009; Lugtenberg & Kamilova, 2009) (Figure 2). To date many plant-associated bacterial species have been identified as PGPRs, including bacteria of the genus *Serratia*, which are the main focus of the studies described in this thesis (Neupane, 2013; Taghavi *et al.*, 2009; Alstrom, 2001; Berg, 2000; Kalbe *et al.*, 1996).

In order to exert their beneficial effects, PGPRs need to be rhizosphere competent implying that they must be able to successfully compete with other rhizosphere microorganisms for the nutrients and carbon secreted from the roots and to occupy root niches. Competitive root colonization is thus crucial for many mechanisms of action of PGPRs (Lugtenberg & Kamilova, 2009; Lugtenberg *et al.*, 2002). Besides that, it is very crucial to consider that in the complex rhizosphere, beneficial bacteria co-exist with other bacteria and fungi where a competition for nutrient uptake and ecological niche occupation occurs. The signal transduction systems between different microbial members of the rhizosphere have been demonstrated to play different roles in fine-tuning responses towards the nearest competitor, thus placing their competitors at a competitive disadvantage (Garbeva *et al.*, 2011). On the other hand, it is also known that rhizosphere bacterial populations cooperate with each other, therefore a deep understanding of bacterial behavior and microbial cooperation could allow a more efficient and successful use of PGPRs in sustainable agriculture (Besset-Manzoni *et al.*, 2018).

1.3.1 Competitive root colonization

The steps of colonization can be divided into: recognition, adherence, invasion (for endophytes and pathogens), colonization, biofilm formation and growth followed by strategies to establish interactions (Berg, 2009).

Bacterial chemotaxis is the basic sensing mechanism by which bacteria swim towards high concentrations of root exudate chemoattractants and is activated by changes in factors such as the pH, osmolarity, temperature, viscosity and chemicals (Blair, 1995; Zhulin & Armitage, 1992).

Bacterial adhesion to plant roots is another requirement for successful establishment of PGPRs in the rhizosphere and is regulated by adhesion factors such as flagellin, pilin and haemagglutinin, and the pili play the most significant role (Kline *et al.*, 2009).

Biofilm formation is crucial too, since it provides protection from external stress, it decreases microbial competition, is capable of providing protecting effects to the host plant and allows exchange of nutrients and toxins (Kasim, 2016; Costerton, 1999). Its development is dependent on bacterial surface components and extracellular compounds (flagella, lipopolysaccharides, exopolysaccharides) combined with quorum-sensing and environmental signals, where the latter triggers the process, while flagella are crucial for the biofilm community to approach and move across the surface. Outer membrane proteins are responsible for the initial steps of adherence and after microcolonies are formed, quorum-sensing signals are required for the production of a mature biofilm (Bogino *et al.*, 2013).

1.3.2 Direct plant growth promotion

Direct plant-growth promotion is exerted in the absence of known pathogens via the utilization of mechanisms that directly influence plant growth. Some bacteria are capable of acting as biofertilizers implying that they are capable of supplying the plant with nutrients. One example is nitrogen fixation by symbiotic N_2 fixing bacteria (e.g. *Rhizobium*, *Bradyrhizobium*) and by non-symbiotic free living (e.g. *Azospirillum*) (Ferguson & Mathesius, 2014; Okon, 1998; Vanrhijn & Vanderleyden, 1995). Moreover, the solubilization of phosphorus, the second most important plant growth-limiting nutrient after nitrogen, from organic or inorganic phosphates is another biofertilization property of PGPRs (Adesemoye *et al.*, 2009; Rodriguez *et al.*, 2006; Vassilev *et al.*, 2006). Some strains of PGPRs produce siderophores, which help the plant to acquire the insoluble iron present in the soil, especially under iron-limiting conditions (Loper & Henkels, 1999). When plants are grown in stressed conditions, vitamin-producing rhizobacteria can provide vitamins of the B group (e.g. thiamine, riboflavin, biotin, niacin), as it has been documented in several studies (Marek-Kozaczuk & Skorupska, 2001; Revillas *et al.*, 2000) and there is further evidence suggesting that even root development is favored by this PGPR mechanism

(Mozafar & Oertli, 1992). Phytohormone production is another of the beneficial bacterial traits with auxin (indole-3-acetic acid, IAA) playing one of the most significant roles mainly due to the fact that IAA interferes with several plant developmental processes (Glick, 2012) but also because it acts as a reciprocal signaling molecule that affects gene expression profiles in other microorganisms (Spaepen & Vanderleyden, 2011). Additionally, IAA is capable of increasing root surface area thus providing greater access to soil nutrients by the plant as well as of loosening of plant cell walls, thereby enhancing root exudation (Glick, 2012). PGPRs can also synthesize other phytohormones such as ethylene, cytokinins, gibberellins, and glucosinolates (Neupane, 2013; Berg, 2009). PGPRs are involved not only in the production of phytohormones, but can also influence the hormonal balance of the plant, with the best-studied example being that of ethylene. This hormone promotes plant growth in *Arabidopsis* at low levels, but it normally inhibits plant growth and is involved in senescence (Pierik *et al.*, 2006). Interestingly, some PGPRs produce a precursor of ethylene (1-aminocyclopropane-1-carboxylate deaminase, ACC), which degrades ACC and reduces ethylene levels, thereby reducing negative effects on plants exerted by pathogens, salt and drought and confer resistance to stress from heavy metals (Glick *et al.*, 2007). The bacterial release of small chemically diverse organic compounds, called volatiles, such as 2.3-butanediol and acetoin has been documented for some bacteria including *Bacillus subtilis*, *B. amyloliquefaciens*, *Pseudomonas fluorescens* and *Serratia plymuthica* and has implications in triggering plant growth via the modulation of endogenous signals (Kai *et al.*, 2007; Ryu *et al.*, 2004a; Ryu *et al.*, 2003).

PGPRs have been shown to affect the root development and growth via the modulation of cell division and differentiation in the primary root, thus affecting lateral root development (Verbon & Liberman, 2016). Even systemically, PGPRs can have positive effects on the whole plant. The enhancement of nutrient uptake from the roots results in modifications of the plant primary metabolism, which contributes to enhanced growth (Vacheron *et al.*, 2013).

1.3.3 Indirect plant growth promotion

PGPRs with biocontrol potential can be applied for the control of plant diseases so apart from the direct effects on plant growth, these

bacteria can also participate in indirect growth promotion by acting as biocontrol agents (Glick, 2012). The general mechanisms of biocontrol activity involve antagonism, competition for nutrients and niches and induced systemic resistance (ISR) (Lugtenberg & Kamilova, 2009).

1.3.3.1 Antagonism

Bacteria that act antagonistically synthesize and release antibiotics that kill or suppress the growth of pathogens with which they compete for the acquisition of nutrients from the roots and for occupying niches on the roots to deliver the antibiotic along the whole root system (Lugtenberg & Kamilova, 2009). It is also of great significance that the antibiotic production from the bacterium occurs in the right microniche of the root surface (Pliego *et al.*, 2008). This antagonistic capacity can be demonstrated if mutants defective in the genes involved in the biosynthetic pathways of the antibiotic are not capable of exerting biocontrol activity (Lugtenberg & Kamilova, 2009). In addition, several abiotic factors in the rhizosphere such as temperature, oxygen, carbon and nitrogen sources and microelements influence antibiotic production as well as the overall metabolic status of the cells which depends on nutrient availability (Haas & Keel, 2003; Raaijmakers *et al.*, 2002).

Different PGPRs produce a wide array of antibiotic compounds. For example, members of the genera *Pseudomonas* are known to produce phenazine, pyrrolnitrin, 2,4-DAPG, HCN, D-gluconic acid, 2-h3xyl-5-propyl resorcinol and lipopetides against different plant pathogens (de Bruijn *et al.*, 2007; Cazorla *et al.*, 2006; Kaur *et al.*, 2006; Mavrodi *et al.*, 2006; Chin-A-Woeng, 2003; Haas & Keel, 2003; Raaijmakers *et al.*, 2002; Chin-A-Woeng *et al.*, 1998; Hammer *et al.*, 1997; Defago, 1993). Bacteria of the genera *Serratia* produce a wide array of antimicrobial compounds as well, such as pyrrolnitrin, carbapenem, prodigiosin, dipeptides and bacteriocin (Neupane, 2013; Muller *et al.*, 2009; Van Houdt *et al.*, 2007; Fineran *et al.*, 2005; Ovadis *et al.*, 2004; Thomson *et al.*, 2000). Interestingly, the *Serratia* isolates used in the present study contain the gene cluster for pyrrolnitrin production as well as for bacteriocin biosynthesis and transportation (Neupane, 2013).

1.3.3.2 Competition

As mentioned earlier, for PGPRs to be able to exert their beneficial effects on the plants and their antagonistic ability towards pathogens, they must be rhizosphere competent, implying that they should be selected by the root (Kamilova *et al.*, 2005). The bacteria should be capable of competing with pathogens for iron, nutrients and niches as well as producing fungal cell-wall degrading exoenzymes.

Siderophore production is carried out by most of the rhizosphere microorganisms in order to circumvent the problem of low iron bioavailability in nature (Loper & Henkels, 1999), thus competent PGPRs can compete for iron and inhibit the growth of fungal pathogens under low concentrations of Fe^{3+} (Schippers *et al.*, 1987).

Nowadays competition for nutrients and niches has been documented as an antagonistic strategy of PGPRs and involves besides others, many inter-related processes that have already been discussed in **1.3.1**. This mechanism was firstly elucidated in a study where a crude rhizobacterial mixture of five isolates was inoculated on sterile seedlings in order to select those bacteria reaching the root tip faster. It was found out that all five isolates colonized the root tips with the same efficiency. Four isolates were able to control the tomato foot and root rot disease, one of which was using 'competition for nutrients and niches' as a biocontrol mechanism. Interestingly, despite successful root colonization of the remaining one isolate, no disease control was observed indicating that this trait alone is not efficient for biocontrol (Kamilova *et al.*, 2005). A potential explanation is related to the results of another study, where it was demonstrated that efficient disease control occurs when the exact niche in the root becomes colonized (Pliego *et al.*, 2008).

Lastly, several PGPR strains, including the *Serratia* isolates used in the present study, produce exoenzymes such as chitinases, glucanases and proteases that are known to be involved in the degradation of fungal cell wall (Neupane, 2013; Kamensky *et al.*, 2003; Frankowski *et al.*, 2001; Inbar & Chet, 1991; Tanaka & Phaff, 1965). These are a dynamic structure responsible for protection of the fungal cells from osmotic and environmental stresses and are the first barrier that needs to be overcome to achieve invasion of host cells (Bowman & Free, 2006).

1.3.3.3 Induced Systemic Resistance

Induced Systemic Resistance (ISR) is a term explaining the induced state of resistance in plants triggered by either biological or chemical inducers and which protects plant parts against future attack by pathogenic fungi, bacteria, viruses and insect herbivores (Kuc, 1982). ISR was first discovered in 1991. It was supported by the findings that resistance could be induced by a) *Pseudomonads* in beans against the Halo blight bacterial pathogen (Alstrom, 1991), b) the rhizobacterium *Pseudomonas* sp. strain WCS417r against *Fusarium* wilt of carnation (Vanpeer *et al.*, 1991) and c) by another study of selected PGPR strains against *Colletotrichum orbiculare* in cucumber (Gang *et al.*, 1991). Interestingly, extensive root colonization is not a prerequisite for ISR, in contrast to other biocontrol mechanisms (Dekkers *et al.*, 2000).

This induced state of resistance is characterized by the activation of latent defense mechanisms, which are expressed upon a subsequent challenge from pathogens (priming) and is expressed not only at the local induction site, but also in plant parts spatially separated in a systemic way and is clearly expressed at the transcriptional level (Pieterse *et al.*, 2014; Conrath *et al.*, 2006) (Figure 3). It is also generally believed that ISR is effective against a broad spectrum of attackers (Walters *et al.*, 2013). Commonly, a network of interconnected signaling pathways regulates ISR with plant hormones playing the major regulatory role (Pieterse *et al.*, 2012).

The enhanced defensive capacity of plants expressing ISR cannot be attributed to direct activation of defenses. Instead it is based on faster and stronger activation of basal mechanisms upon infection to pathogens (Frost *et al.*, 2008; Conrath *et al.*, 2006). In general, systemic resistance responses induced by beneficial microorganisms, are not associated with major changes in the expression of defense genes (Conrath *et al.*, 2002), probably because this would lead to heavy investments in resources and reduced fitness of the host (van Hulten *et al.*, 2006; Heil & Bostock, 2002).

Just like pathogens, PGPRs also possess conserved microbe-associated molecular patterns (MAMPs) raising the question of how plants distinguish between pathogens and non-pathogens at the early stages of interaction. It has been demonstrated that the MAMPs flagellin and lipopolysaccharides which are present in pathogenic *Pseudomonas* spp. (Nurnberger *et al.*, 2004) are also cell surface

components of beneficial *Pseudomonas* spp. acting as potential inducers of host immune responses, exhibiting a host recognition (Bakker *et al.*, 2007). As discussed above the activation of defense mechanisms is energetically costly and can have negative plant growth effects, however PGPRs promote plant growth, suggesting that PGPRs might have evolved strategies to reduce stimulation of local host immune responses, or to actively suppress MAMP-triggered immunity (Trda *et al.*, 2015; Zamioudis & Pieterse, 2012; Millet *et al.*, 2010; Van Wees *et al.*, 2008).

Several studies have examined the potential of root colonizing bacteria to induce systemic resistance against pathogens (van de Mortel *et al.*, 2012; Verhagen *et al.*, 2004; Cartieaux *et al.*, 2003). However, in most cases only a few transcriptional changes have been observed systemically in the leaves. More precisely, no genes were differentially expressed systemically in leaves after colonization by a *Pseudomonas* spp., however in the roots a plethora of genes were downregulated (Verhagen *et al.*, 2004). In another study, 63 genes were differentially expressed in the shoots but only a few changes in gene expression were observed in the roots of plants colonized by *Pseudomonas thivervalensis* (Cartieaux *et al.*, 2003). In both the aforementioned cases the systemic responses were found to be dependent on Jasmonic Acid- (JA) and Ethylene- (ET) signaling pathways. In contrast other studies have investigated a Salicylic Acid- (SA) dependent response of ISR either through the use of SA-producing mutants (Audenaert *et al.*, 2002b; De Meyer *et al.*, 1999) or bacteria overexpressing the SA-biosynthesis gene cluster (Maurhofer *et al.*, 1998). Even wild-type PGPR have been demonstrated to induce a SA-dependent response in *Arabidopsis* (van de Mortel *et al.*, 2012; Tjamos *et al.*, 2005). However, in those cases the production of SA by the bacteria is usually not the causal agent of the observed systemic resistance, probably because the SA produced is not released in the rhizosphere, but becomes incorporated into SA moiety-containing siderophores (Bakker *et al.*, 2014; Audenaert *et al.*, 2002b; Press *et al.*, 1997).

In most cases ISR is commonly regulated by JA- and ET-signaling pathways and the ISR-expressing plants are primed for accelerated JA- and ET-dependent gene expression that becomes evident after pathogen attack (Sarosh *et al.*, 2009; Van der Ent *et al.*, 2009; Cartieaux *et al.*, 2008; Hase *et al.*, 2008; Pozo *et al.*, 2008; Ahn *et al.*, 2007; Weller *et al.*, 2007; Ryu *et al.*, 2004b; Verhagen *et*

al., 2004; Pieterse *et al.*, 2000; Pieterse *et al.*, 1996). The NPR1 regulatory protein which is a basic component of Systemic Acquired Resistance (SAR) (will be discussed in the next section) has also been demonstrated to regulate ISR, however the downstream processes of NPR1 are divergent between ISR and SAR (Pieterse *et al.*, 1998). In ISR, NPR1 functions in the cytosol without the activation of pathogenesis-related (*PR*) genes, however the exact molecular mechanism by which it functions in the JA/ET –dependent ISR is still unknown (Pieterse *et al.*, 2012; Ramirez *et al.*, 2010). The MYB72 transcription factor is also an ISR signaling component and is specifically induced in roots under iron-limiting conditions, pointing to a direct link between iron homeostasis and the onset of ISR (Pieterse *et al.*, 2014; Van der Ent *et al.*, 2008). Additionally, the nuclear-localized transcription factor MYC2 has been identified as a potential regulator in priming for enhanced JA-dependent responses (Kazan & Manners, 2013; Pozo *et al.*, 2008). What is of great interest is that no changes are observed in the production of JA and ET in the leaves of induced plants, suggesting that ISR relies upon enhanced sensitivity to these hormones rather than an increase in their production (Pieterse *et al.*, 2000).

Evidence indicates that beneficial soil microbes have evolved decoy strategies to short-circuit hormone-regulated immune responses which are triggered in the roots upon initial recognition, thus paving the way for a prolonged association with their host (Pieterse *et al.*, 2012).

Moreover, PGPR-triggered ISR fortifies plant cell wall strength and alters host physiology and metabolic responses, resulting in enhanced synthesis of plant defense chemicals upon pathogen challenge (Nowak & Shulaev, 2003; Ramamoorthy *et al.*, 2001).

Last but not least, volatiles can also activate ISR. In *Arabidopsis* the activated signaling pathway was found to be ET-dependent and SA- and JA-independent (Ryu *et al.*, 2004a). Other studies also point to the significance of volatiles with antifungal actions produced from bacteria (Hol *et al.*, 2015; Kai *et al.*, 2007).

1.4 Plant defense against pathogens

Naturally, plants are faced with continuous biotic stress caused by diverse pathogens and pests that are capable of exploiting highly

specialized features in order to establish a parasitic relationship with their hosts (Pieterse *et al.*, 2009). Upon pathogen encounter, plants elicit an immune response to limit pathogen growth and protect themselves, whereas the pathogen needs to evade or suppress host immune responses in order to proliferate (Lu, 2013). According to their lifestyles, plant pathogens can be either necrotrophic (i.e. firstly destroy host cells via the production of phytotoxins and cell-wall degrading enzymes and then feed on the contents) or biotrophic (i.e. derive nutrients from living host tissues, primarily via specialized feeding structures) (Pieterse *et al.*, 2012; Glazebrook, 2005). Biotrophic pathogens are combatted mainly by (SA)-dependent defense responses, whereas necrotrophic pathogens are combatted by (JA)- and (ET)-dependent defense responses (Pieterse *et al.*, 2012; Jones & Dangl, 2006). The interactions between plants and pathogens can thus be explained as a dynamic interplay between host defense mechanisms and specialized pathogen factors.

1.4.1 Plant Innate Immunity

To begin with, plants have evolved an array of pre-invasive and non-specific defense layers including structural barriers and preformed antimicrobial metabolites and proteins in order to prevent a potential invasion (Pieterse *et al.*, 2009). However plants have further evolved a broad spectrum of sophisticated post-invasive strategies of defense (Jones & Dangl, 2006). In this primary immunity layer, plants are capable of recognizing MAMPs (Zipfel & Robatzek, 2010), molecules including bacterial flagellins, fungal chitin, peptides, proteins, carbohydrates, small molecules (e.g. ATP) and lipopolysaccharides as discussed earlier (Newman *et al.*, 2013; Boller & Felix, 2009; Ryan *et al.*, 2007; Nurnberger *et al.*, 2004). These molecules are recognized by pattern recognition receptors (PRRs) in the host and up to now it is known that those can be either transmembrane receptor kinases or transmembrane receptor-like proteins (Zipfel, 2008). The result is the initiation of a downstream signaling cascade resulting in pattern-triggered immunity (PTI). PTI is a basal early defense response and activates ion-flux across the plasma membrane, oxidative burst, MAP kinases (MAPK), protein phosphorylation, receptor endocytosis, protein-protein interaction, increases of Ca^{2+} concentration as well as cell wall reinforecement to a wide range of pathogens (Altenbach & Robatzek, 2007; Jones &

Dangl, 2006; Nurnberger *et al.*, 2004). Oxidative burst activation has been observed as a defense mechanism in interactions between different plants and necrotrophic fungi, including *Rhizoctonia solani* (Foley *et al.*, 2016; Pietrowska *et al.*, 2015; Foley *et al.*, 2013; Asai & Yoshioka, 2009). As direct targets of MAPK, WRKY transcription factors play broad and pivotal roles in the regulation of defenses (Eulgem & Somssich, 2007) and several studies point to the significance of MAPK signaling cascades and WRKY transcription factors in defense responses against the necrotrophic fungal pathogen *Sclerotinia sclerotiorum* to *Brassica napus* and *Arabidopsis* (Wu *et al.*, 2016; Sun *et al.*, 2014; Wang *et al.*, 2014; Chen *et al.*, 2013; Liang *et al.*, 2013; Wang *et al.*, 2009b; Yang *et al.*, 2009). It has been additionally demonstrated that there is a partial dependence of PTI-mediated gene induction on SA-signaling (Sato *et al.*, 2007) as well as an enhancement of ET biosynthesis, stomatal closure and callose deposition (Altenbach & Robatzek, 2007).

Virulent, specialized pathogens have evolved effectors capable of suppressing PTI, thus resulting in effector-triggered susceptibility (ETS), which represents the first level of molecular co-evolution between plants and pathogens. Fungal effectors are secreted through the endomembrane system and are subsequently delivered into host cells via the pathogen's Type III secretion system (Panstruga & Dodds, 2009). One common strategy of effectors to deregulate host immune responses is the manipulation of the homeostasis of plant hormones leading to deactivation of the appropriate defense response (Bari & Jones, 2009; Robert-Seilaniantz *et al.*, 2007). Intracellular recognition of effector proteins is of primary significance and is

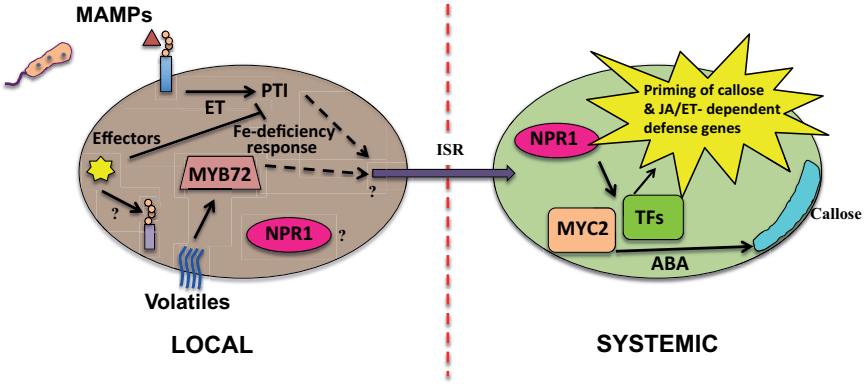


Figure 3. Schematic representation of molecular components and mechanisms involved in induced systemic resistance (ISR). Microbe associated molecular patterns (MAMPs) are recognized by pattern recognition receptors (PRRs), resulting in initiation of pattern triggered immunity (PTI). In PTI, there is an enhancement of ethylene (ET) biosynthesis, stomatal closure and callose deposition. The MYB72 transcription factor (TF) is specifically induced in the roots under iron-limiting conditions, which probably results in ISR. At the same time, since priming is transcriptionally regulated, TFs accumulate after induction of the primed state and NPR1 plays a role in activating TFs and MYC2, a master regulator of JA-dependent defenses and ISR. (Solid black lines indicate established interactions and dashed black lines indicate hypothetical interactions). (Adapted from Pieterse *et al.* 2014. *Annu. Rev. Phytopathol.* **52**:347-375.)

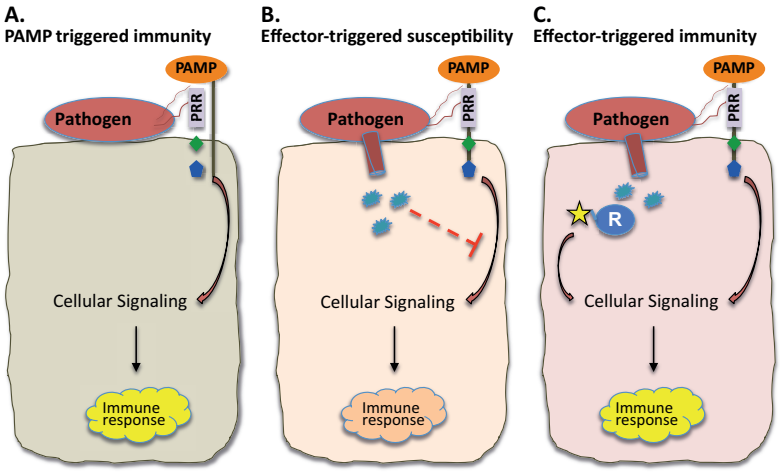


Figure 4. Schematic representation of the plant immune system. **A**. After pathogen attack, pathogen associated molecular patterns (PAMPs) activate pattern recognition receptors (PRRs) in the host plant, leading to a signaling cascade that results in PAMP-triggered immunity (PTI). **B**. Effectors of virulent pathogens are capable of suppressing PTI, leading to effector-triggered susceptibility (ETS). **C**. Plants have however evolved resistance proteins (R) that recognize pathogen effectors, leading to the secondary layer of immunity response, known as effector-triggered immunity (ETI). (Adapted from Pieterse *et al.* 2014. *Annu. Rev. Phytopathol.* **52**:347-375.)

mediated by plant NB-LRR receptor proteins which confer resistance to diverse pathogens (Dodds & Rathjen, 2010). In turn, plants have evolved resistance (R) proteins capable of recognizing either effectors or their activity, resulting in the second layer of defense, known as effector-triggered immunity (ETI) (Chisholm *et al.*, 2006; Jones & Dangl, 2006). ETI represents the second level of molecular co-evolution between plants and pathogens because effectors evolve to avoid detection, whereas R proteins evolve to maintain detection. Ultimately the final outcome of this battle determines the infection process (Chisholm *et al.*, 2006; Jones & Dangl, 2006) (Figure 4).

Despite the fact that the characteristics of PTI and ETI are different, there is a plethora of common molecular responses and an overlap in their signaling machinery has even been proposed (Cui *et al.*, 2015; Katagiri & Tsuda, 2010; Pieterse *et al.*, 2009; Abramovitch *et al.*, 2006).

Immune responses impose physiological costs due to the activation of signal cascades, production of defense metabolites and general re-organization of primary metabolism (Bolton, 2009; Berger *et al.*, 2007). So, it is necessary for plants to prioritize towards defense or growth, implying that plants should avoid unnecessary or less necessary responses (Huot *et al.*, 2014). It has been further suggested that re-modeling of primary metabolism in its own right may act as a defense component (Schwachtje & Baldwin, 2008) and it was recently demonstrated that after infection of *Arabidopsis* by *Pseudomonas syringae*, nitrogen metabolism and amino acids content were systemically reduced in leaves, probably as a priming response of the plant in order to reduce the nutritional value of the systemic tissues (Schwachtje *et al.*, 2018). This phenomenon is not that common in nature where plants have adapted and evolved sophisticated mechanisms to balance growth and defense (Baldwin, 2001). However in agricultural systems the situation is different partly due to the fact that crops have been bred for centuries with the major goal of maximizing yield-related traits which impacts genetic diversity negatively and compromises defense (Strange & Scott, 2005). PTI responses are triggered by non-specific structural microbial molecules, are known to start at the early stages of interaction and when there is continuous or enhanced MAMP signaling it increases gradually at the later stages. On the other hand, ETI responses, which are activated upon pathogen attack, are strong

and rapid even from an early stage and continue to be robust enough until the later stages (Katagiri & Tsuda, 2010).

1.4.2 Induced plant defense responses

Common defense responses between PTI and ETI include cell wall fortification via the synthesis of callose and lignin, the production of antimicrobial secondary metabolites (e.g. phytoalexins) and the accumulation of pathogenesis-related (PR) proteins (e.g. chitinases and glucanases which are common degraders of the fungal cell wall) (Pieterse *et al.*, 2009). The recognition of pathogen-specific effectors via the ETI defense system is exceptionally effective for the reason that it is followed by a burst of reactive oxygen species (ROS), which initiates a programmed hypersensitive reaction (HR) at the pathogen invasion site, assisting in keeping the pathogen isolated from the rest of the plant, thus preventing further damage (Bent & Mackey, 2007; De Wit, 1997). Since the lifestyle of necrotrophic pathogens is based on killing host cells, HR would favor such pathogens implying that it is highly effective against biotrophic or hemibiotrophic pathogens (Glazebrook, 2005).

1.4.3 Systemic Acquired Resistance

Upon activation of plant defense responses at the infection site, a systemic defense response is usually triggered in distal plant parts known as Systemic Acquired Resistance (SAR), playing significant roles in protecting undamaged plant tissues against subsequent pathogen invasion. SAR is a long-lasting and broad-spectrum induced disease resistance and its main molecular characteristic is the coordinate activation of a specific set of plant PR proteins with antimicrobial activities in both local and systemic tissues (van Loon *et al.*, 2006b; Durrant & Dong, 2004). SAR can be triggered by both PTI- and ETI-mediated pathogen recognition and is linked to elevated levels of the hormone SA, both at the local infection site but also usually in distant plant tissues (Tsuda *et al.*, 2008; Mishina & Zeier, 2007). Interestingly, it has been demonstrated that transgenic plants impaired in SA signaling are not capable of developing SAR and do not show *PR* gene activation upon pathogen infection (Durrant & Dong, 2004). A transcriptional factor, the regulatory

protein NPR1 (NONEXPRESSOR OF *PR* GENES1) has emerged as a significant transducer of the SA signal and is activated by SA. NPR1 acts as a transcriptional co-activator of *PR* gene expression (Dong, 2004) as well as a key regulator in SA-mediated suppression of JA signaling (Spoel *et al.*, 2003). This transcription factor has been shown to play crucial roles in NPR1-dependent SA-mediated signaling pathway in various plant species such as *Arabidopsis*, tobacco and rice and its function is clearly connected to the nucleus (Vlot *et al.*, 2009; Dong, 2004).

The PR proteins are inducible defense-related proteins, which are plant-specific and are classified into 17 families. Most of them are induced via the action of the signaling compounds of the hormones SA, JA or ET and are widely known for their antimicrobial properties *in-vitro*. More precisely they can exert hydrolytic activities on cell walls and presumably be involved in defense signaling. In general, PR proteins are regulated developmentally and might serve different functions in specific organs and tissues. For example, they are induced during senescence, wounding or cold stress and some of them have antifreeze activity (van Loon *et al.*, 2006b). The PR-1 family is highly conserved and is represented in almost all plant species. Despite their structural similarity and shared sequence homology, PR-1 members might differ substantially in their biological activities (van Loon *et al.*, 2006b). Glucanases and chitinases are involved in the degradation of glucan and chitin, the primary structural components of fungal cell walls. Those are constitutively present in plants consequently they can only be enhanced rather than induced upon pathogen attack and are also increased under other stress conditions. Glucanases and chitinases belong to PR-2, PR-3, PR-4 PR-8 and PR-11(van Loon *et al.*, 2006b). *Rhizoctonia solani* was the first fungus shown to be suppressed when a basic PR-3 chitinase was overexpressed in transgenic tobacco and canola plants however conflicting results were observed in transgenic cucumber plants transformed with the same chitinase type (van Loon *et al.*, 2006b). In a recent study in *Brassica napus* plants infected with *S. sclerotiorum*, genes encoding PR-2, PR-3 and PR-4 proteins were highly induced upon pathogen attack (Wu *et al.*, 2016).

A wide range of secondary metabolites with antimicrobial properties is produced in plants as a defense response. Some of them are produced via the phenylpropanoid pathway and are known to

function either as preformed or as inducible physical (e.g. lignin) and chemical (e.g. flavonoids) antimicrobial barriers, or even as signal molecules related to local and systemic signaling in plant immunity (Naoumkina *et al.*, 2010). Camalexin is a phytoalexin and in *Arabidopsis* it is a significant component of defense against necrotrophic fungal pathogens (Ahuja *et al.*, 2012; Ferrari *et al.*, 2007; Ferrari *et al.*, 2003; Thomma *et al.*, 1999). Despite the findings that the antimicrobial activity of camalexin is susceptible to fungal mechanisms of detoxification (Kliebenstein *et al.*, 2005; Pedras & Ahiahonu, 2002), almost half of the quantitative trait loci that control resistance to the ascomycete *Botrytis cinerea* are associated with camalexin accumulation (Rowe & Kliebenstein, 2008). Glucosinolates (GSLs) are the major source of phytoanticipins of plants of the Brassicaceae family (Halkier & Gershenzon, 2006). The biological activity of GSLs relies on the release of several toxic myrosinase-catalyzed hydrolytic products (Lambrix *et al.*, 2001), including the 4-methylsulfinylbutyl isothiocyanate, being an inhibitor of the growth of several pathogens in-vitro (Tierens *et al.*, 2001). According to one study (Stotz *et al.*, 2011), camalexin and glucosinolates were induced in leaves of *Arabidopsis* upon challenge with *S. sclerotiorum* and mutant lines deficient in camalexin, indole or aliphatic glucosinolate biosynthesis exhibited hypersusceptibility to *S. sclerotiorum*. Additionally, a recent study in *B. napus* challenged with *S. sclerotiorum* revealed too that the glucosinolate content was significantly enhanced in response to the fungal infection (Wu *et al.*, 2016).

1.4.4 The role of plant hormones in defense

Downstream of PTI or ETI activation, or other early molecular recognition events of microbes, different plant hormones act as central players in triggering the plant immune signaling network (Katagiri & Tsuda, 2010; Bari & Jones, 2009; Pieterse *et al.*, 2009). Crosstalk between hormonal signaling pathways provides the plant with a powerful regulatory potential that might allow it to tailor its defense response to the invaders (Kunkel & Brooks, 2002; Reymond & Farmer, 1998). The most important plant hormones in local and induced defense signaling are SA, JA and ET (Loake & Grant, 2007; van Loon *et al.*, 2006a; Pozo *et al.*, 2004). Until recently it was suggested that SA plays a crucial role in the activation of defense

responses against biotrophic or hemibiotrophic pathogens and the establishment of SAR as mentioned earlier, whereas JA and ET are associated with defense against necrotrophic pathogens and herbivorous insects (Pieterse *et al.*, 2012; Jones & Dangl, 2006; Glazebrook, 2005). However, there are studies contradicting this straightforward model.

SA is a phenolic compound involved in several plant processes including growth, development, senescence and disease resistance (Vlot *et al.*, 2009). As already mentioned, SA activates the regulatory protein NPR1 as well as its paralogs NPR3 and NPR4 (Fu *et al.*, 2012). When SA is absent, NPR1 is localized in the cytoplasm as an oligomer. Accumulation of SA in response to pathogen detection induces a redox change in the cell and triggers the release of NPR1 monomers, which subsequently translocates to the nucleus and activates defense gene expression (Tada *et al.*, 2008; Mou *et al.*, 2003). Apart from PR genes, which are activated in the presence of SA, other genes have also been identified as direct targets of NPR1, including WRKY transcription factors (Wang *et al.*, 2006). Additionally, it has recently been demonstrated that SA plays a role in the assembly of the microbiome in the interior of *Arabidopsis* roots, by analyzing the endophytic root microbiome of SA biosynthesis and signaling mutants, pinpointing plant genotypic traits related to immunity as key players in processes that structure the rhizosphere microbiome (Lebeis *et al.*, 2015).

JAs are a group of lipid-derived components, rapidly synthesized via the oxylipin biosynthesis pathway upon pathogen attack (Gfeller *et al.*, 2010). Their function apart from protection from necrotrophic pathogens is also the regulation of physiological processes such as abiotic stress responses, reproductive development and primary and secondary metabolism (Wasternack, 2007). The JA responses are regulated through the E3 ubiquitin ligase SCF^{COI1} co-receptor complex and the jasmonate ZIM-domain (JAZ) family of transcription repressors that form a complex that represses transcription of JA-responsive genes (Katsir *et al.*, 2008). When JA accumulates, the highly bioactive JA-isoleucine (JA-Ile), binds to the

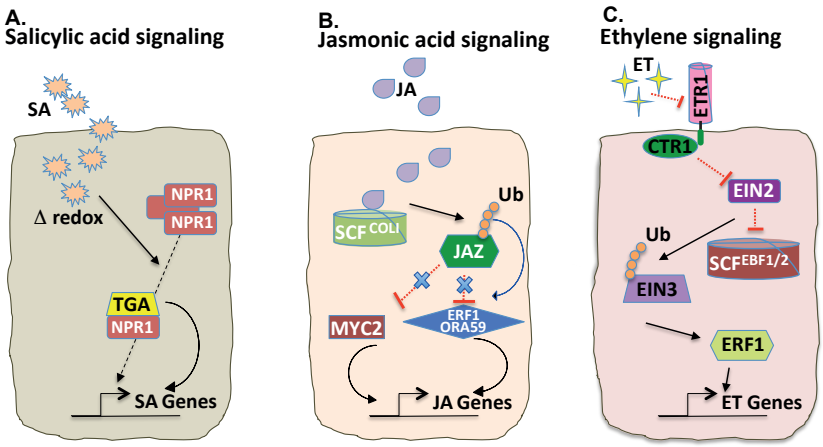


Figure 5. Schematic representation of SA, JA and ET signaling pathways. (Adapted from Pieterse *et al.* 2009. *Nature Chem. Biol.* **5**: 308-316.)

F-box protein COl1 in the SCF^{COI1} complex and then the JAZ proteins are ubiquitinated and further degraded through the 26S proteasome, resulting in the activation of JA-responsive genes via the action of the transcription factors MYC, ERF and ORA59 (Fernandez-Calvo *et al.*, 2011; Pre *et al.*, 2008). Moreover, it was recently demonstrated that PHR1, a master transcriptional regulator of phosphate starvation responses in *Arabidopsis* mutants with altered microbiomes, fine-tunes JA responses, providing new insight into the intersection of nutritional stress response, immune system and microbiome assembly (Castrillo *et al.*, 2017).

ET is a gaseous plant hormone implicated in responses to biotic and abiotic stress, but also in seed germination, leaf senescence and fruit ripening (Chen *et al.*, 2005). ET is perceived by plasma membrane receptors located in the endoplasmic reticulum (ER). From a genetic perspective, these receptors are negative regulators of the ET response because when ET is absent they maintain the negative regulatory role of CTR1, which is a repressor of the positive regulator EIN2. After ET perception, the repression by CTR1 is relieved, thus allowing further downstream signaling via the EIN2

regulator. Then EIN3, a critical positive regulator of ET-responsive gene expression becomes activated. The EIN3 activation occurs because the E3 ubiquitin ligase $SCF^{EBF1/2}$-dependent 26s proteasome degradation of EIN3 proteins becomes inhibited. EIN3-like transcription factors accumulate in the nuclei where they activate transcription factors such as ERF1, leading to the expression of downstream ET-responsive genes (Robert-Seilaniantz *et al.*, 2011; Kendrick & Chang, 2008). A simplified schematic representation of the signaling pathways of SA, JA and ET is given in (Figure 5).

Additionally, other hormones are implicated in plant defense, such as auxin, abscisic acid, cytokinins, gibberellins and brassinosteroids. Auxin is involved in all aspects of plant development (e.g. lateral root development, vascular differentiation, cell division and elongation, flower development), but also in plant-microbe interactions (Dharmasiri & Estelle, 2004). It has been demonstrated that repression of auxin signaling could contribute to enhanced resistance in plants to bacterial pathogens (Navarro *et al.*, 2006; Thilmony *et al.*, 2006). In contrast, the repression of auxin signaling has been shown to compromise resistance of *Arabidopsis* to the necrotrophic fungi *Botrytis cinerea* and *Plectosphaerella cucumerina*, without affecting JA- or SA-mediated defense pathways (Llorente *et al.*, 2008).

Abscisic acid (ABA) regulates as well several plant developmental processes, adaptation to environmental stresses and plant defense responses (Wasilewska *et al.*, 2008; de Torres-Zabala *et al.*, 2007; Mohr & Cahill, 2007; Mauch-Mani & Mauch, 2005), however this regulation occurs in a complex and variable manner, being dependent on the different types of plant-pathogen interactions (Bari & Jones, 2009). In general ABA increases susceptibility to biotrophic and necrotrophic pathogens (Anderson *et al.*, 2004; Thaler & Bostock, 2004; Audenaert *et al.*, 2002a). It has also been demonstrated that ABA treatment suppresses SAR induction, indicating an antagonistic interaction between SAR and ABA in *Arabidopsis* (Yasuda *et al.*, 2008). However, ABA has also been reported as a positive defense regulator, either via activation of stomatal closure acting as a barrier against bacterial infections (Mauch-Mani & Mauch, 2005), or by acting as a positive signal for defense against the necrotroph *Alternaria brassicicola* in *Arabidopsis* via priming for callose accumulation and in *B. napus* against *S. sclerotiorum* (Novakova *et al.*, 2014; Ton & Mauch-Mani,

2004). It has additionally been demonstrated that in the pathosystem *Arabidopsis* and *Pythium irregulare*, ABA enhances the defense mechanism via callose priming and activation of JA biosynthesis, resulting in regulation of defense genes (Adie *et al.*, 2007). Last but not least, there is evidence that ABA might be directly involved in the control of *Arabidopsis* resistance to *Ralstonia solanacearum*, probably through a mechanism of modulating call walls (Hernandez-Blanco *et al.*, 2007).

Gibberellins (GA) promote plant growth by stimulating the degradation of the negative growth regulators known as DELLA proteins. Despite the fact that GA have received little attention regarding their roles in plant defense, there is increasing evidence that they play major roles (Bari & Jones, 2009). It is thought that DELLA proteins are capable of promoting the expression of genes encoding ROS detoxification enzymes, thus regulating the levels of ROS upon exposure to biotic or abiotic stress (Achard *et al.*, 2008). Moreover, it has been shown that DELLA proteins promote resistance to necrotrophs by activating JA/ET-dependent defense responses, but repress SA-dependent responses thereby making biotrophs more susceptible indicating that GA act as a virulence factor for necrotrophic pathogens (Navarro *et al.*, 2008; Achard *et al.*, 2006).

Cytokinins (CK) are a group of plant hormones that promote cell division and play major roles in the regulation of several biological processes associated with active growth, metabolism and development (Sakakibara, 2006), as well as with chlorophyll maintenance and chloroplast development, delaying thus leaf senescence (Gan & Amasino, 1995). These molecules play pivotal roles in the profound reconfiguration of the plant primary and secondary metabolism related to plant-microbe interactions, since an increase in CK is usually observed upon pathogen and insect attack (Giron *et al.*, 2013). Pathogens and insects potentially influence the levels of phytohormones by inducing plant genes involved in CKs biosynthesis, degradation and response, however they can also produce and secrete relevant phytohormones themselves (Robert-Seilaniantz *et al.*, 2007). Until now there is no evidence for production of CKs from necrotrophic fungal pathogens (Giron *et al.*, 2013).

Brassinosteroids (BRs) are a unique class of plant hormones and emerging evidence indicates their involvement in the regulation of

plant defense responses but in a SA-independent way (Nakashita *et al.*, 2003). An important component of BR signaling is the involvement of the BRI1-associated kinase 1 (BAK1) in the regulation of basal defense and programmed cell death in plants (Heese *et al.*, 2007). Interestingly, *bak1* mutants have shown enhanced susceptibility to necrotrophic pathogens such as *Alternaria brassicola* and *B. cinerea* (Kemmerling *et al.*, 2007).

1.4.5 Hormonal crosstalk

The hormonal pathways are known to interact with each other in a complex cross-communicating signaling network with highly interconnected components (Denance *et al.*, 2013). The balance of the hormonal crosstalk is known to significantly influence the outcome of plant-pathogen interactions (Pieterse *et al.*, 2009).

In general, SA and JA interact in an antagonistic way and different models have been proposed for this mode of action. It has been demonstrated that in *Arabidopsis*, the ability of SA to suppress JA-responsive genes is related to a SA-mediated modulation of the cellular redox state (Koornneef *et al.*, 2008). Moreover, NPR1 has been shown to modulate the antagonistic effect of SA to JA when located in the cytosol, whereas when located in the nucleus it plays a role in the activation of SA-responsive genes (Leon-Reyes *et al.*, 2009; Spoel *et al.*, 2003). On the other hand there have been reports where SA and JA seem to interact synergistically in *Arabidopsis* against the foliar pathogen *P. syringae* and the necrotroph *Alternaria brassicola* (Schenk *et al.*, 2000; van Wees *et al.*, 2000) and in *B. napus* against the necrotroph *S. sclerotiorum* (Wang *et al.*, 2012) and sometimes in a concentration-dependent manner (Mur *et al.*, 2006). The interaction between JA and ET is synergistic in most cases and it has been demonstrated that the expression of the transcription factors ERF1 and ORA59 is of great significance for the convergence between JA and ET (Pre *et al.*, 2008; Lorenzo *et al.*, 2003; Penninckx *et al.*, 1998). Furthermore. It has been shown that the key component of the JA signaling pathway, MYC2, differentially regulates JA- and JA/ET-responsive genes, being a positive regulator of the JA response but only in the absence of ET (Lorenzo *et al.*, 2004). ET is also thought to act in a synergistic manner with SA by enhancing the expression of SA-responsive genes in *Arabidopsis* (De Vos *et al.*, 2006; Glazebrook *et al.*, 2003; Lawton *et al.*, 1994). ET

has additionally been proposed to act as a key component during SA and JA interactions by enhancing the NPR1-dependent SA-responsive expression of *PR* genes (Leon-Reyes *et al.*, 2009). In the *B. napus*-*S. sclerotiorum* pathosystem a highly interconnected signaling cascade was recently demonstrated, where enhanced levels of ET, SA, JA and ABA induced a SA-dependent defense against the necrotroph (Novakova *et al.*, 2014). Despite the huge amount of studies dealing with plant hormone signaling in defense, only a limited number of pathosystems have been used. This represents a bottleneck in development of new knowledge since there does not appear to be a single model explaining all kinds of interactions taking place.

1.5 *Brassica napus*

Oilseed rape (*Brassica napus* L.) is a globally important oil crop, cultivated for edible oil, biofuel production and phytoextraction of heavy metals. However, it is susceptible to several pathogens including *Rhizoctonia solani*, *Verticillium longisporum*, *Sclerotinia sclerotiorum*, and *Alternaria brassicae* (Turan & Bringu, 2007). Under abiotic stress conditions, poor emergence and early seed development have been observed (Blake, 2004), however similar symptoms were evident upon infection with *R. solani* (Neupane *et al.*, 2013a). Studies on the rhizospheric environment of this crop have documented the presence of biocontrol bacteria (e.g. *Serratia proteamaculans*, *S. plymuthica*, *P. chlororaphis*, *P. acidovorans* and *P. putida*) as well as of fungal genera related to biocontrol (*Clonostachys*) (Gkarmiri *et al.*, 2017; Abuamsha *et al.*, 2011; Alstrom, 2001).

1.6 *Serratia* spp.

The genus *Serratia* belongs to the bacterial family Enterobacteriaceae and the class Gammaproteobacteria and its members are broadly distributed in nature and commonly found in soil, water, plants, insects and humans (Alstrom & Gerhardson, 1987; Grimont & Grimont, 1978). The genus includes diverse species from a biological and an ecological perspective, ranging from those being beneficial to economically significant crops, to others that are pathogenic or harmful to pathogens. Those establishing

mutualistic interactions with plants can be either endophytes or free-living (e.g. *S. proteamaculans* and *S. plymuthica*) and some have been screened for antagonism against plant pathogenic fungi (e.g. *R. solani* and *V. longisporum*) (Alstrom, 2001; Berg, 2000; Kalbe *et al.*, 1996). The genomes of four *Serratia* strains (AS9, AS12, AS13 and S4) have recently been sequenced (Neupane *et al.*, 2013b; Neupane *et al.*, 2012a; Neupane *et al.*, 2012b; Neupane *et al.*, 2012c). S4 and AS13 exhibit diverse colonization patterns, plant growth promoting activities and antagonistic interactions against the pathogenic fungus *R. solani*. S4 has been found to be more antagonistic to the pathogen compared to AS13 based on *in-vitro* studies and transcriptome experiments revealed different gene expression patterns compared to non-inoculated control treatments (Neupane *et al.*, 2015; Neupane, 2013).

1.6 *Rhizoctonia solani*

The soil-borne basidiomycete fungus *R. solani*, (teleomorph *Thanatephorus cucumeris*) is a damaging necrotroph causing billions of dollars of losses to agriculture worldwide (Okubara *et al.*, 2014). The pathogen has a broad host range and infects major crops such as barley, oilseed rape, legumes, potato, rice and wheat, with large variations in morphology, ecology and pathology (Vilgalys & Cubeta, 1994). This species is categorized into 14 anastomosis groups (AG-groups) (Hane *et al.*, 2014). Some anastomosis groups are capable of attacking a wide range of plant species, while others exhibit greater host specificity (Ogoshi, 1987). AG2-1 is highly pathogenic on oilseed rape (Khangura *et al.*, 1999), causing both pre- and post-emergence damping off independent of the cultivar type (Neupane *et al.*, 2013a), whereas AG-3 affects mostly potato (Anderson, 1982).

R. solani causes damping-off and root rot in oilseed rape and black scurf in potato. When the pathogen is established in the soil, it is difficult to control because of its broad host range, saprophytic life style and persistence of sclerotia and mycelium in soil and plant material (Anderson, 1982). Current control strategies based on the use of chemicals and crop cultivation practices have limited efficacy or are not feasible from an economical point of view, implying that there is a great need for alternative strategies such as biocontrol in order to improve sustainability (Paulitz, 2006).

2 Objectives

The rhizosphere is an environment in which complex interactions take place between the plant roots, fungal pathogens and antagonistic bacteria. Despite the economical importance of *B. napus*, there is lack of knowledge about the underlying mechanisms involved in the protection of this crop from fungal pathogens as well as its interactions with naturally occurring antagonistic bacteria. The overall aim of the studies described in this thesis was thus to increase the knowledge on the interactions taking place in the rhizosphere of this crop. More specifically, the objectives were to:

- Identify the differentially expressed genes of the necrotrophic fungal pathogen *R. solani* when challenged with the antagonistic bacteria *S. proteamaculans* (S4) and *S. plymuthica* (AS13) and to elucidate the underlying molecular mechanisms that the fungus uses to respond to the antagonists (Paper I).

- Characterize and compare the structure and composition of the bacterial and fungal communities colonizing the roots and the rhizosphere of *B. napus* and to identify active taxa capable of competing for recently fixed plant-derived carbon (Paper II).

- Examine differential transcriptomic responses in the roots and leaves of *B. napus* during interactions with *R. solani* alone, or interactions with *R. solani* and *S. proteamaculans* in order to elucidate potential mechanisms that trigger

differential plant responses and to investigate whether S4 are capable of inducing a systemic response (Paper III).

3 Materials and Methods

3.1 Bacterial strains and growth conditions

The bacterial strains used in the studies described in this thesis were *S. plymuthica* AS13 and *S. proteamaculans* S4. AS13 was isolated from the rhizosphere of oilseed rape in 1998, in Uppsala, Sweden and was selected on the basis of its ability to inhibit the fungal pathogen of oilseed rape *Verticillium longisporum* in both controlled and non-sterile growth conditions (Alstrom, 2001). S4 was isolated from the rhizosphere of *Equisetum* sp. in 1980, in Uppsala, Sweden and exhibited similar patterns of inhibition of fungal growth (Alström and Andersson, unpublished). Both bacterial strains were previously shown to inhibit the growth of the fungal pathogen *R. solani* under *in-vitro* conditions. They exhibited different levels of antagonism and also promoted the growth of oilseed rape (Neupane *et al.*, 2015; Neupane, 2013).

Lyophilized bacterial cells were taken from glycerol stocks and cultured on half-strength Tryptic Soy Agar (TSA) for 48h. After confirmation of the purity of the cultures, a single colony was inoculated onto half-strength TSA and incubated for 24h at 20°C.

For the experiment described in Paper I, a loop containing 30µl of this bacterial culture was further inoculated onto half-strength Potato Dextrose Agar (PDA) for 24h.

For the experiment described in Paper III, a loop containing 30µl of this bacterial culture was incubated in half-strength Tryptic Soy Broth (TSB) for 24h on a rotary shaker. Serial dilution was carried out to estimate the number of Colony Forming Units (CFUs/ml) before seed inoculation.

3.2 Fungal isolates and growth conditions

The *R. solani* AG3 isolate, strain Rhs1AP used in Paper I, was isolated from an infected potato stem in 1988, in Maine, USA. The fungus was taken from glycerol stock and was cultured on half-strength PDA at 20°C for 8 days, followed by sub-culturing of a 5-mm diameter plug from the edge of the actively grown colony of *R. solani* onto half-strength PDA at 20°C for 4 days. The genome of this strain became publicly available in 2014 under the accession number (GenBank: JATN00000000) (Cubeta *et al.*, 2014).

The *R. solani* AG2-1 isolate used in Paper III, was isolated from diseased oilseed rape seedlings and was cultured on half-strength PDA at 20°C for 4 days, followed by sub-culture on half-strength Potato Dextrose Broth (PDB) and further incubation for 6 days at 20°C, until the diameter of the fungal colony was about 4 cm. The mycelium was washed twice in sterile distilled water before blending in Phosphate Buffer Saline (PBS) solution. The resulting mycelial suspension was plated on half-strength PDA to confirm viability, and serially diluted to estimate the number of CFUs/ml before seed inoculation. This strain was selected because it had previously been shown to have negative effects on pre- and post-emergence of oilseed rape in a greenhouse experiment (Neupane *et al.*, 2013a).

3.3 Plant material

Oilseed rape was selected for the experiments described in this thesis, firstly because studies examining the microbiome of this crop are not many and secondly because it is an economically important crop worldwide, often exhibiting poor and failed establishment. Moreover, the genome of this crop has been publicly released (Chalhoub *et al.*, 2014) and is available at the European Nucleotide Archive (ENA) under the accession numbers (CCCW010000001-CCCW010044187).

3.3.1 Greenhouse experiment in Paper II

The *B. napus* winter cultivar ‘Libraska’ was used. Surface sterilization of the seeds was performed by suspending the seeds in 95% ethanol for 2 minutes, followed by rinsing in a 15% sodium hypochlorite solution with 0.1% Tween-20 for 15 minutes and finally

rinsing in sterile distilled water for 10 minutes. The seeds were subsequently sown on half-strength PDA for 4 days in order to confirm that sterility had been achieved and to select seedlings of uniform size for the experiment described below.

The greenhouse experiment was performed with soil collected from an organically managed field in Ultuna. Following collection, the soil was homogenized, sieved and transferred to pots, where two seedlings of uniform size were planted in each and thinned to one seedling after four days. Five pots containing only soil ('bulk soil') were also included and served as controls in order to confirm that ^{13}C enrichment was achieved because the maximum level of carbon was allocated to soil through rhizodeposition. The plants were incubated for four weeks before $^{13}CO_2$ pulse labeling and subsequently rhizosphere soil and roots were destructively harvested on days 0, 1, 3, 7 and 14 post-labeling. Further details are described in Paper II.

3.3.2 *In-vitro* gnotobiotic experiment in Paper III

The *B. napus* winter cultivar 'Banjo' was used. Surface sterilization of the seeds was performed as described for Paper II. Following surface sterilization, the seeds were sown on half-strength PDA and were incubated in a controlled chamber for 2 days. Seedlings of uniform size were then inoculated aseptically on Murashige and Skoog basal salt mixture (MS medium) (Sigma-Aldrich), in sterile multi-well tissue culture plates (Thermo Fisher Scientific) and further incubated for 24h. Details of the incubation parameters are given in Paper III.

3.4 Inoculation methods

3.4.1 Paper I

For the purpose of Paper I, *in-vitro* dual-culture assays were established in 9 cm Petri dishes containing half-strength PDA. The assays were set-up in a way identical to that used earlier in order to identify the differential gene expression of S4 and AS13 bacteria in response to *R. solani* (Neupane, 2013). Briefly, a 5 mm diameter plug was taken from an actively growing colony of *R. solani* and was

inoculated in the centre of the Petri dish, whereas fresh cells of S4 and AS13 were streaked in a 3cm length parallel line on each side of the fungal plug. Control treatments inoculated only with *R. solani* were also set-up. In addition, fungal hyphae from control and non-control treatments were stained with the vital stain phenosaffranin in order to examine abnormalities in the mycelial growth, if any, due to the presence of the antagonistic bacteria.

3.4.2 Paper III

Pre-germinated seedlings incubated in sterile MS medium in multi-well plates were aseptically inoculated with 20µl of S4 bacterial suspension of 10^6 CFU/ml /seedling and incubated for 24h. The seedlings were then inoculated with 20µl of 10^5 CFU/ml AG2-1 fungal suspension and further incubated. Incubation parameters are given in Paper III. For the purpose of the experiment, four treatments were included: 1). Control (inoculated with PBS buffer), 2). +S4, 3). +AG2-1 and 4). +S4 +AG2-1.

3.5 Nucleic acid manipulations and gene expression studies in Paper I and Paper III

In both papers, the fungal or the plant materials respectively were frozen in liquid nitrogen and ground with sterile pestles and mortars prior the manipulations.

In the first study (Paper I), the fungal material used was harvested at 72 h post-inoculation. For the treatments where the fungus was challenged with the bacteria, fungal mycelia were harvested from the zone of interaction, whereas for the control treatments the peripheral fungal zone was harvested.

In the second study (Paper III), sampling was destructive, separating the root system from the aboveground part (referred as 'leaves') and was performed 6h, 12h and 24h after bacterial or fungal inoculation. Harvesting was subsequently repeated at 48h, 72h, 120h and 240h post-inoculation. For further analyses, samples harvested at 120h and 240h post-inoculation were used.

In both of the experiments, total RNA was extracted from the harvested material using the RNeasy Plant Mini Kit (Qiagen). Traces

of DNA were removed by DNase I treatment (Fermentas, St. Leon-Rot, Germany). DNase treated RNA was then analyzed for RNA integrity by electrophoresis on an Agilent Bioanalyzer using the 6000 Nano kit (Agilent Technologies, Santa Clara, CA). For Paper I, 500 ng - 1 µg of total RNA were subjected to Illumina®TruSeq, while for Paper III 1 – 5 µg of total RNA were subjected to Illumina®HiSeq, both at the SciLife Lab, Uppsala.

In the first study, verification of the expression profiles obtained from the RNA sequencing was carried out using Quantitative Real-Time PCR (qRT-PCR). For cDNA synthesis, 180ng of total DNase-treated RNA were reverse transcribed with the iScript cDNA Synthesis Kit (BioRad, Hercules, CA). Transcript levels were assessed by RT-qPCR in an iQ5 qPCR system (BioRad, Hercules, CA). Data normalization was conducted with the expression level of the reference gene Histone-3 (*H3*) and relative quantification was carried out using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001). Analysis of variance (ANOVA) was conducted using a General Linear Model implemented in SPSS ver. 21 (IBM, Armonk, NY). Pairwise comparisons were made using Fisher's test at the 95% significance level.

3.6 Data analyses in Paper I and Paper III

3.6.1 Bioinformatic analyses

For the analyses of RNA sequencing in both studies (Paper I and Paper III), the same pipeline was used. After removal of Illumina adaptor sequences and low quality bases from reads using the software Nesoni (http://www.vicbioinformatics.com/software-.nesoni.shtml) bioinformatics analyses of trimmed reads was performed using the Tuxedo Suite (Trapnell *et al.*, 2012). This software has in-built functions for mapping of the reads, abundance quantification of transcripts in terms of fragments per kilobase of exon per million mapped fragments (FPKM) and differential expression analysis of transcripts between each treatment to the corresponding control. For both experiments, differentially expressed genes (DEGs) were identified using two criteria: a) $\log_2$ fold-change > 3 and b) q-value (false discovery rate (FDR)) < 0.05. Details of the parameters used are given in Paper I and Paper III.

3.6.2 Functional classification and annotation of differentially expressed genes

For both experiments, sequence similarity was calculated using the BLASTx algorithm at the statistical significance threshold 1.0E-6. Gene Ontology (GO) annotations were used to assign functional categories to the DEGs in Blast2GO, enabling the integrated Interproscan and ANNEX functions for improved annotations (Conesa *et al.*, 2005).

For Paper I, enrichment of GO terms was evaluated by Fisher's exact test with an FDR threshold of 5%, but revealed no statistically significant differences between the two treatments (S4 and AS13). Therefore investigation of functional category assignment for DEGs was conducted using the WEGO online server (Ye *et al.*, 2006). In addition, KEGG orthology (KO) and enzyme commission (EC) numbers were obtained in KAAS (V. 1.69x) online tool (Moriya *et al.*, 2007). Principal component analysis (PCA) of the $\log_2$-transformed FPKM values was done in CummeRbund (Trapnell *et al.*, 2012).

For Paper III, functional category assignment for DEGs was obtained from Blast2GO (Conesa *et al.*, 2005). Hierarchical clustering of the genes was performed in the built-in function of Tuxedo Suite, CummeRbund (Trapnell *et al.*, 2012) with the Jensen-Shannon distances. Volcano plots were obtained in R. Venny tool (Oliveros, 2007) was used to generate the Venn diagrams. KEGG orthology (KO) and enzyme commission (EC) numbers were obtained in KAAS (V. 1.69x) online tool (Moriya *et al.*, 2007).

3.7 Stable Isotope Probing (Paper II)

Stable Isotope Probing (SIP) is a cultivation-independent technique used to identify microorganisms in environmental samples that use a particular growth substrate and helps to answer the question 'who is doing what'. In the case of plant microbiome studies, SIP can help in the identification of microorganisms that consume recently fixed plant carbon (Haichar *et al.*, 2008; Vandenkoornhuyse *et al.*, 2007; Dumont & Murrell, 2005). More precisely, the method relies on the incorporation of a stable isotope (^{13}C or ^{15}N) into nucleic acids from a labeled substrate, so microbes that incorporate plant carbon into their biomass become enriched. SIP was first applied in the analysis

of phospholipid fatty acids (PLFA), but knowledge is lacking on the PLFA patterns of non-cultivated microorganisms, rendering thus the use of DNA or RNA more sensitive. RNA-SIP is a more recently developed technique and has some advantages over DNA-SIP. DNA-SIP requires long incubation times for DNA replication and incorporation of the labeled substrate into the newly synthesized DNA, which probably leads to non-specific labeling. Since RNA is synthesized faster than DNA, it is possible to obtain ^{13}C-RNA more quickly implying that primary consumers are targeted before the label can reach secondary consumers (Whiteley *et al.*, 2007; Manefield *et al.*, 2002a; Manefield *et al.*, 2002b) and labeling times should be carefully reduced (Vandenkoornhuyse *et al.*, 2007). However that could result in incomplete labeling of microorganisms with slow growth rate (Radajewski *et al.*, 2003). Another limitation of the SIP technique in general, is the necessity of adding ^{13}C-labeled substrate in large amounts leading to an increased *in situ* availability of carbon, which potentially generates a large divergence between experimental and natural conditions (Vandenkoornhuyse *et al.*, 2007). In RNA-SIP the fractionation of SIP gradients obtained by Cesium trifluoroacetate ultracentrifugation allows access to the full range of buoyant densities resolved in gradients and that combined with quantitative analyses of the fractions can shed light on the comparative distribution of specific RNA-populations across the gradient fractions (Lueders *et al.*, 2004). Most recent studies target rRNA (for bacteria) or ITS (for fungi) to generate taxonomic information on the microbes involved in label assimilation.

The experimental procedure of SIP used in this study is schematically summarized in Figure 6 and further details are given in Paper II.

3.7.1 Nucleic acid manipulations and PCR amplifications

Rhizosphere soil and roots were frozen in liquid nitrogen and freeze-dried. Rhizosphere soil was then milled to fine powder and roots were homogenized using a Precellys 24 tissue homogenizer (Bertin Technologies, France). The material used was harvested at 3 days post-labeling.

Total DNA and RNA from rhizosphere and bulk soil were extracted using the RNA power soil isolation kit (MOBIO Laboratories, CA, USA). For the roots, DNA and RNA were

extracted using the RNeasy Plant Mini Kit (Qiagen) but without adding RNase in order to extract both nucleic acids at the same time. Traces of DNA from the extracted RNA rhizosphere soil and root material were removed by using the RTS DNase kit (MOBIO Laboratories, CA, USA).

The pooled ^{13}C-labeled RNA (heavy) and the ^{12}C-unlabeled RNA (light) fractions obtained from the cesium trifluoroacetate ultracentrifugation were reverse transcribed using the iScript reverse transcription Supermix (Bio-Rad, CA, USA).

The PCR amplifications were conducted using the Phusion high-fidelity DNA polymerase (Thermo Fisher Scientific, Germany) in triplicates, including negative controls. The bacterial primers 515F and 806R were used to target the variable bacterial region V4 (Bates *et al.*, 2011; Caporaso *et al.*, 2011), while the fungal primers fITS7

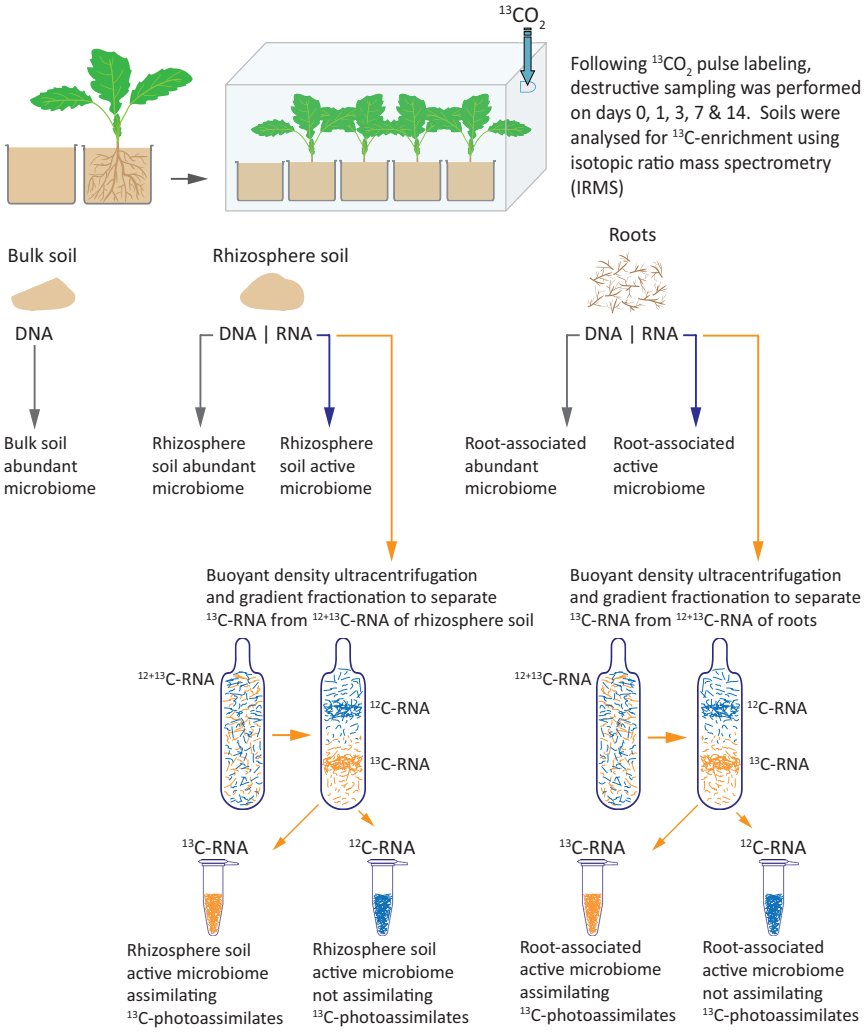


Figure 6. Schematic representation of the Stable Isotope Probing (SIP) experimental approach. *Brassica napus* seedlings were grown in pots containing organically managed soil and were subjected to $^{13}CO_2$ pulse labeling after 4 weeks growth. Roots and rhizosphere soil were harvested destructively on days 0, 1, 3, 7 and 14 and soil were analyzed for ^{13}C enrichment to determine the stage at which maximum enrichment had occurred. Then, rhizosphere soil and root samples from day 3 were used for coextraction of DNA and RNA to analyze abundant and active bacterial and fungal microbiomes using high-throughput sequencing. ^{12}C- $+^{13}C$-RNA was subjected to density gradient ultracentrifugation to separate ^{13}C-RNA and ^{12}C-RNA fractions that were used to characterize the active bacterial and fungal microbiomes assimilating recent ^{13}C-labeled photoassimilates of plants.

and ITS4 were used to target the ITS region (Ihrmark *et al.*, 2012). The primers 806R and ITS4 were uniquely barcoded for each sample. Amplification of the cDNA samples was performed using 1µl undiluted cDNA, whereas for the DNA samples the templates were diluted 10x. The triplicate PCR products were then pooled, purified using the Agencourt AMPure kit (Beckman Coulter, USA) and quantified in a Qubit fluorometer (Invitrogen, USA). Bacterial and fungal PCR products were subsequently pooled in equimolar concentrations, freeze-dried for 24 h and sent for pyrosequenving on a 2x one-quarter of a GS FLX titanium Pico titer plate (Macrogen, Seoul, Korea) according to the manufacturer's recommendations (Roche, Branford, CT, USA).

3.7.2 Data analyses in Paper II

The sequences obtained were analyzed using QIIME (Caporaso *et al.*, 2010b) (MacQIIME v. 1.9.0). Both bacterial and fungal reads were demultiplexed based on the barcode sequences and forward and reverse reads were combined. Bacterial data was denoised and sequences from both bacteria and fungi were clustered into OTUs by UCLUST (Edgar, 2010) based on 97% similarity (Caporaso *et al.*, 2010a). Details on how the read alignments and taxonomic classifications were done are given in Paper II.

Multivariate analysis of OTUs was conducted using the Paleontological Statistics package (PAST v. 2-17) (Hammer, 2001). Beta diversity community dissimilarity calculations were visualized using nonmetric multidimensional scaling (NMDS) with the Bray-Curtis dissimilarity measure. Nonparametric multivariate analysis of variance (NPMANOVA) was used to estimate the significance of the differences in microbial communities. The Venny tool (Oliveros, 2007) was used to generate the Venn diagrams.

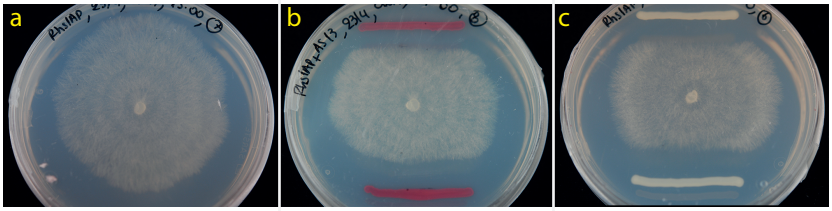
4 Results and Discussion

4.1 Paper I: Transcriptomic changes in the plant pathogenic fungus *Rhizoctonia solani* AG-3 in response to the antagonistic bacteria *Serratia proteamaculans* and *Serratia plymuthica*

We investigated the functional response of the necrotrophic plant pathogen *R. solani* AG-3 when it was confronted with two antagonistic, plant-associated bacteria, *S. proteamaculans* S4 and *S. plymuthica* AS13, with S4 being a stronger antagonist than AS13 as was earlier demonstrated (Neupane *et al.*, 2015; Neupane, 2013). Establishing an *in-vitro* dual culture assay followed by RNA sequencing of the fungus allowed us to identify the molecular mechanisms that *R. solani* exploits to respond to the bacterial challenge. We hypothesized a greater rearrangement of the fungal transcriptome in response to the stronger antagonist, as well as both a general and a more bacterial-specific differential regulation of genes involved in metabolism. Limited information is available about the responses of fungal pathogens to antagonistic bacteria. Thus, this study provides a global view of genes and potential mechanisms being differentially regulated and being required for survival and defense of the fungus.

The challenge of *R. solani* with S4 and AS13 led to clear inhibition of the growth of the fungal mycelium compared to the control treatment at 72 h and this pattern persisted for at least four weeks (data not shown (Figure 7A.). In contrast exposure of

A.



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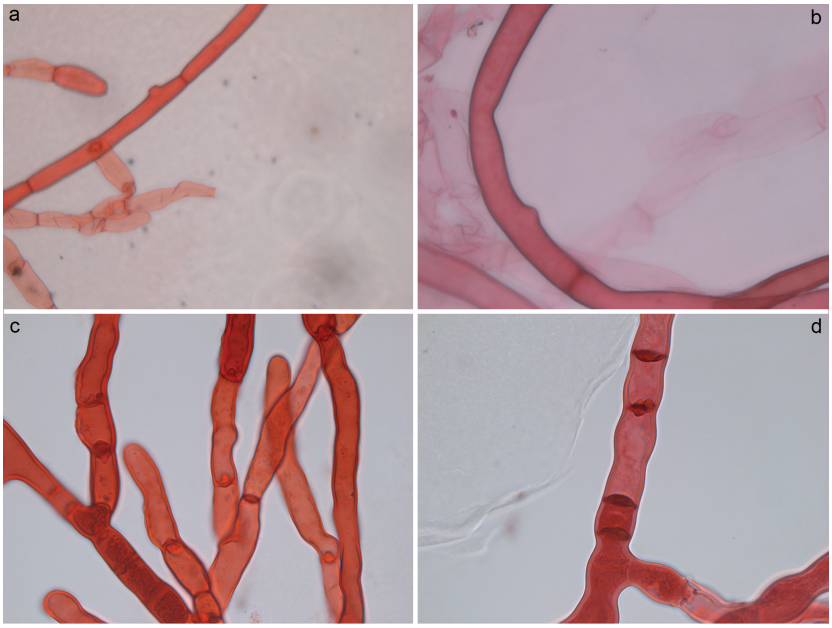

Figure 7. **A**. Dual-culture *in-vitro* bacterial assays. **a)** Control *Rhizoctonia solani* monoculture, **b)** *R. solani* challenged with *Serratia proteamaculans* S4, **c)** *R. solani* challenged with *Serratia plymuthica* AS13. **B.** Microscopic observations of *R. solani* hyphae. **a, b)** *R. solani* from control monocultures; straight mycelium, normal branching, normal septation **c, d)** *R. solani* when challenged with AS13; increased frequency of septa and branching, swollen mycelium, and dolipore septa, cell wall thickening.

Fusarium graminearum to bacterial MAMPs did not cause any observable morphological effects on the fungus (Ipcho *et al.*, 2016). Moreover, microscopic observations of the challenged fungal hyphae revealed a complete disruption of the hyphal morphology with swollen mycelium, increased septation and branching and thickened cell walls compared to straight mycelium with normal branching and septation in the control treatment (Figure 7B.). This observation is in accordance with hyphal abnormalities observed in *Fu*sarium *verticillioides* when challenged with *Bacillus mojavensis* (Blacutt *et al.*, 2016), in *R. solani* upon treatment with *Pseudomonas fluorescens* (Thrane *et al.*, 1999) and in *Aspergillus niger* during confrontation with *Collimonas fungivorans* (Mela *et al.*, 2011).

In total, almost 10% of the whole fungal transcriptome was differentially expressed. Fungal genes that were statistically differently regulated compared to the corresponding control samples (q-value < 0.05) were 1901 and 1327 in response to S4 and AS13 respectively. Among these genes, 1035 were common between both the treatments, while 866 and 292 were S4- and AS13-specific respectively. A total of 460 and 242 genes respectively had fold values exceeding +/-8x and were used for all downstream analyses.

KEGG pathway analysis revealed the presence of some common enzymes for genes being up- and downregulated in both treatments (Figure 8A. and 8B.). Among the upregulated genes, some were related to glycerophospholipid metabolism, drug metabolism by cytochrome P450, sucrose and ascorbate metabolism, pyruvate and vitamin B6 metabolism and biosynthesis of unsaturated fatty acids. Since the challenge with S4 resulted in greater restructuring of the fungal transcriptome compared to the treatment with AS13, it was expected that some genes would be prominent in the presence of S4 (e.g. metabolism of pyruvate, propanoate, methane, glycerophospholipid and glyoxylate, xenobiotics metabolism by cytochrome P450, glycolysis, fatty acid and chloroalkane degradation).

Enrichment analysis between the Gene Ontology (GO) terms revealed no statistically significant differences between the two treatments (S4 and AS13). The most functionally important common GO terms identified were associated with oxidation-reduction process (GO: 0055114), pathogenesis (GO: 0009405), threonine-type endopeptidase activity (GO: 0004298) and cellular proteolysis (GO: 0051603). We interpreted those categories as being involved in the

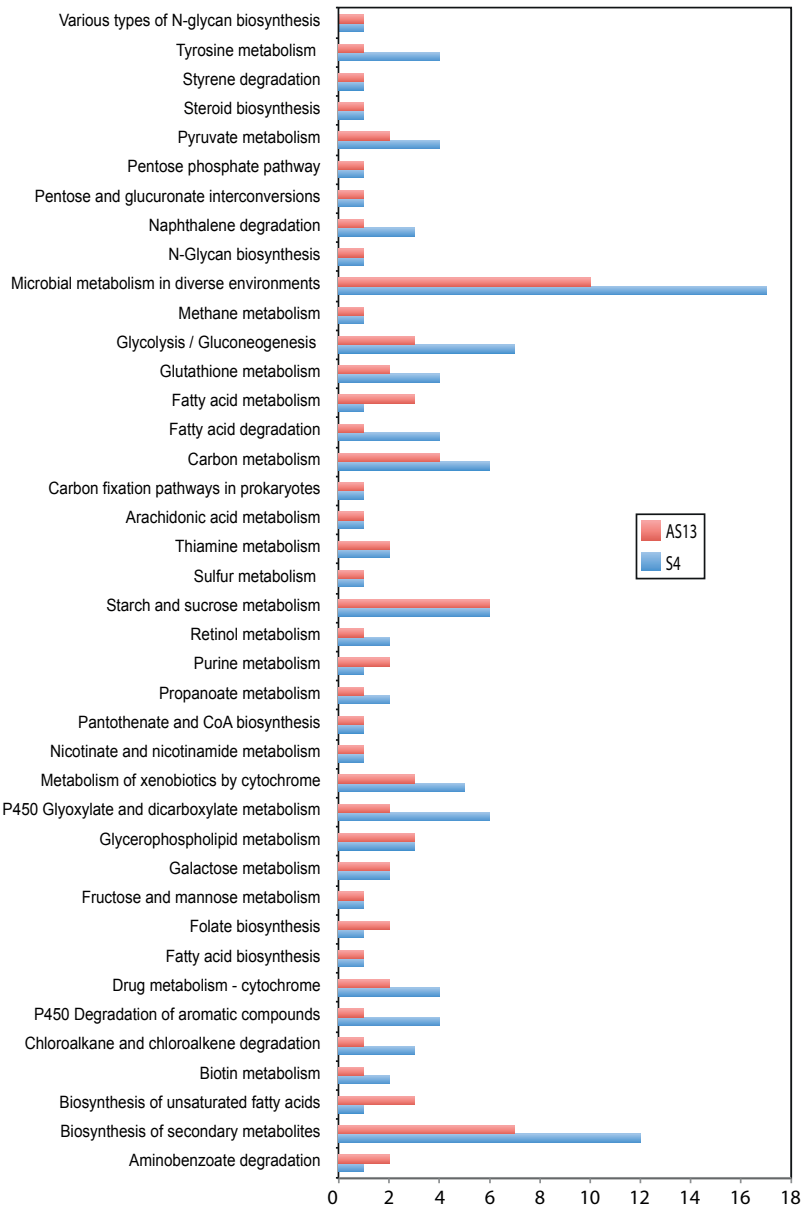

Figure 8. KEGG pathway annotations found to be common between the treatments with S4 *Serratia proteamaculans* and AS13 *Serratia plymuthica* for differentially expressed genes with fold values exceeding $\log_2(3)$. **A.** Upregulated genes.

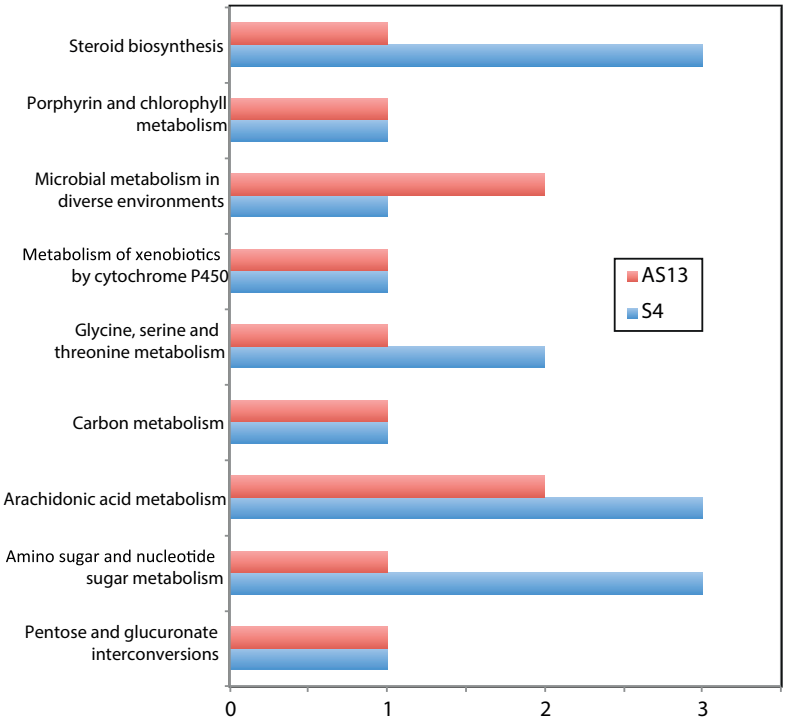

Figure 8. KEGG pathway annotations found to be common between the treatments with S4 *Serratia proteamaculans* and AS13 *Serratia plymuthica* for differentially expressed genes with fold values exceeding $\log_2(3)$. **B.** Downregulated genes.

following processes: a) arrested growth of the fungus and changes in hyphal morphology, b) defense against bacterial stress and c) attack. Similar stress response categories were identified when the fungal pathogen *F. graminearum* was confronted with bacterial MAMPs (Ipcho *et al.*, 2016). In addition, analysis of the 'cellular component' GO terms for both treatments revealed that the highest number of upregulated genes were 'integral to membrane' and 'extracellular region', suggesting that upregulated proteins are being secreted from the cell to interact directly with the bacteria or counteract exogenous antagonistic compounds.

a) Arrested growth of the fungus and changes in hyphal morphology: The dynamic fungal cell wall protects the cell from changes in osmotic and environmental stresses and is the first barrier that needs to be overcome to achieve invasion of host cells. We identified cell wall-degrading enzymes to be significantly downregulated in accordance with earlier findings where similar enzymes were repressed during challenge with live bacteria (Mathioni *et al.*, 2013; Mela *et al.*, 2011) but not during challenge with bacterial MAMPs (Ipcho *et al.*, 2016). Recently it has been demonstrated that genes related to ergosterol biosynthesis were up-regulated in *A. niger* probably as a mechanism to regulate membrane fluidity, or confer resistance to the antifungal agent amphotericin (Mela *et al.*, 2011). In contrast, in our study ERG2 to ERG6 were significantly down-regulated during challenge with S4, suggesting a strong potential of the bacteria to disrupt the fungal membrane and the fungal growth in general (Sheehan *et al.*, 1999). It is known that AS13 bacteria produce the antimicrobial compound pyrrolnitrin (Neupane, 2013) and interestingly we found that when *R. solani* was challenged with AS13 a gene encoding an ABC transporter, being involved in the active export of toxins out of the cell, was highly up-regulated suggesting its potential role in protection against bacterial metabolites.

Increased mitochondrial activity is often related to innate immunity in animals (Walker *et al.*, 2014) and there is evidence in our study that this response is conserved among fungi as well. We found that genes involved in fatty-acid degradation, the glyoxylate cycle, pyruvate and fatty acid metabolism were highly upregulated, suggesting an increased energetic demand of the fungus, as was

shown in another study (Ipcho *et al.*, 2016). This implies that under stress conditions, these compounds could be used as carbon sources through gluconeogenesis and highlights the importance of the glyoxylate cycle in growth, stress tolerance and antagonism (Dubey *et al.*, 2013). Nitrogen metabolism related genes were highly induced in the presence of S4 but not in AS13, probably because such genes were up-regulated in the transcriptome of S4 as well (Neupane, 2013), suggesting efficient nitrogen metabolism. Interestingly, genes related to nitrogen metabolism were also highly induced in the study by Ipcho *et al.,* 2016.

b) Defense against bacterial stress through antioxidant production, xenobiotics degradation and environmental alterations: In terms of defense, we found that *R. solani* can protect itself either via the production of antioxidants that remove free radical intermediates and inhibit other active oxidants, via degradation of xenobiotics, or via alterations of the environment. Antioxidant production in stressed *F. graminearum* has also been found in relation to induced thioredoxin production (Ipcho *et al.*, 2016) and is potentially involved in defense against Reactive Oxygen Species (ROS) (Powis & Montfort, 2001). Almost 20 transcripts were upregulated and related to oxidoreductase activity in our study. Examples of such identified genes are: glutathione-S-transferases, transaminases and pyridoxal-5-phosphatases implicated in Vitamin B6 biosynthesis and pyridoxal reductase. The latter has been previously shown to be an antioxidant and alleviator of ROS in fungi under stress (Bilski *et al.*, 2000) and it was also reported to be induced in *R. solani* when challenged with *Stachybotrys elegans* (Chamoun & Jabaji, 2011).

Acetoin and 2,3-butanediol are bacterial volatiles mediating growth promotion and ISR (Han *et al.*, 2006; Ryu *et al.*, 2004a; Ryu *et al.*, 2003) and it is known that the genome of AS13 contains genes for acetoin reductase, involved in conversion of acetoin to 2,3-butanediol as well as 2,3-butanediol reductase, involved in the catabolism of 2,3-butanediol (Neupane, 2013), which can be dehydrated to 1,3-butadiene (Syu, 2001). Epoxide hydrolases were highly induced in our study and their corresponding enzymes have the ability to detoxify 1,3-butadiene oxide among others (Arand *et al.*, 2003), suggesting a defense mechanism of *R. solani* to the production of these bacterial volatiles.

In the presence of both S4 and AS13, we found that aliphatic nitrilase was very highly upregulated and similar patterns have also been observed in the transcriptome of *A. niger* (Mela *et al.*, 2011). Interestingly, nitrilases have been found to be able to convert IAA precursors to IAA (Park *et al.*, 2003) and among other plant pathogenic fungi, *R. solani* is also known to produce IAA (Furukawa, 1996), and to act as a potential virulence factor during disease development (Fu *et al.*, 2015). Degradation of the antibiotic pyrrolnitrin produced by S4 and AS13 (Neupane *et al.*, 2015; Neupane, 2013) was also one of the defense mechanisms we identified in *R. solani*, since the gene haloacid dehalogenase was highly upregulated. We additionally identified four genes encoding laccase multicopper benzenediol: oxygen oxidoreductase to be highly induced in the presence of both bacteria and similar results were obtained in a study where *R. solani* was confronted with different strains of *P. fluorescens* suggesting that laccases could play a determining role in the efficacy of the bacterial biocontrol and they could also serve as a virulence factor in the host-fungus interactions (Crowe & Olsson, 2001). Another important aspect is that some fungi are capable of gaining an ecological advantage over competitors by acidifying their environment. During challenge with S4, 2 genes encoding oxalate decarboxylase were overexpressed and the role of such fungal enzymes is related to the prevention of high intracellular levels of oxalic acid as well as to the decomposition of extracellular oxalic acid (Makela *et al.*, 2002; Micales, 1997). Oxalate has direct inhibitory effects on the growth of competitors (Dutton & Evans, 1996), but it can also reduce the pH to create a less favorable environment for bacterial growth (Ownley *et al.*, 1992). Increased oxalate production by *R. solani* has been reported in response to *P. fluorescens* (Nagarajkumara *et al.*, 2005) and by *A. niger* in response to *Collimonas* (Mela *et al.*, 2011).

c) Attack via toxin productions and oxidative stress: We found the upregulation of genes related to toxin production such as volvatoxin (in treatment with S4) and delta-endotoxin (in treatment with AS13), both being members of the Endotoxin CytB protein family. Similar proteins have been found in other pathogenic fungi and bacteria with implicated roles in their virulence (Soberon *et al.*, 2013). In contrast to our results, a gene encoding a delta-endotoxin CytB was downregulated in *R. solani* when challenged with *B. subtilis* and *Stachybotrys elegans* (Chamoun *et al.*, 2015). Moreover,

a gene containing the ricin b-like lectin domain was upregulated almost 16 times in both treatments. Furthermore, upregulation of proteases in both treatments and induction of six genes encoding the metalloprotease deuterolysin in the treatment with S4 were observed in our study and this finding links to the fact that proteolytic enzymes are potential pathogenicity factors of pathogenic fungi.

Taken together, these results assisted in the identification of a large number of genes in the phytopathogenic fungus *R. solani* that are required for survival and defense in the presence of the plant-associated bacteria S4 and AS13. In general, a major shift in gene expression was evident in the presence of both bacterial strains, with a simultaneous alteration of primary metabolism, hyphal rearrangements and activation of defense and attack mechanisms. Our findings expand the knowledge on the functional responses of a fungal pathogen to antagonistic bacteria, but further *in-situ* studies are required to provide a more detailed understanding of the complex interactions taking place in the rhizosphere.

4.2 Paper II: Identifying the active microbiome associated with roots and rhizosphere soil of oilseed rape

The central aim of this study was the characterization of the active microbiomes of bacteria and fungi colonizing the rhizosphere soil and the roots of *B. napus*, the identification of taxa capable of assimilating recently fixed plant carbon (referred as ^{13}C-RNA) and their comparison with other less active groups (referred as ^{12}C-RNA). This was achieved by labeling oilseed rape plants grown in a greenhouse experiment with $^{13}CO_2$, followed by RNA Stable Isotope Probing (SIP) and high-throughput 454 pyrosequencing.

Rhizosphere soil and roots were destructively harvested on days 0, 1, 3, 7 and 14 post-labeling. Analysis of the overall isotopic signatures of δ^{13}C revealed significant enrichment of the rhizosphere soil (P<0.05) from day 1, but maximum enrichment was observed on days 3 and 7, so in order to focus on the primary consumers, and avoid secondary redistribution of label, we chose to analyze samples harvested on day 3.

In total, 325,992 bacterial and 350,798 fungal reads were obtained from pyrosequencing. After denoising and removal of chimeric

sequences, 139,074 bacterial sequences remained whereas following demultiplexing 123,804 fungal sequences remained.

Nonmetric multidimensional scaling (NMDS) ordinations and nonparametric multivariate analysis of variance (NPMANOVA) are shown in (Figure 9A.) for bacteria and in (Figure 9B.) for fungi. The analysis revealed significant differences between the three DNA-based communities (bulk soil, rhizosphere soil, roots) for both bacteria (Figure 9Ai.) and fungi (Figure 9Bi.), in accordance with previous results where such communities were found to be structurally distinct from each other (Hartman *et al.*, 2018; Edwards *et al.*, 2015; Nallanchakravarthula *et al.*, 2014; Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012). Moreover, DNA- (abundant) and RNA- (active) based communities of bacteria and fungi were also significantly different from each other, both in the rhizosphere soil and in the roots (Figure 9Aiii., 9Aiv., 9Biii., 9Biv.) and similar results have been demonstrated in other studies comparing DNA- and RNA-based bacterial profiles (Stibal *et al.*, 2015; Lillis *et al.*, 2009). The active communities colonizing rhizosphere soil and roots were significantly different as well (Figure 9Aii., 9Bii.). Interestingly comparison between the bacterial ^{13}C-RNA and ^{12}C-RNA fractions from rhizosphere soil and roots revealed similar diversity patterns, in contrast to same comparison for the fungal fractions, where there was unexpectedly greater diversity (data not shown). This could probably be due to the fact that fungi are important organotrophic organisms that receive considerable amounts of plant-derived carbon (Wu *et al.*, 2009), implying that there was probably enhanced competition.

Interestingly, similar numbers of bacterial OTUs were retrieved from all soil samples (rhizosphere soil DNA, rhizosphere soil RNA, bulk soil), whereas in the roots the number of bacterial OTUs from RNA was almost double that retrieved from DNA (Figure 10A.), however without differences among the major taxa (Figure 10B.). In total, 29 bacterial and two archaeal phyla were identified. We observed a general predominance of *Proteobacteria*, *Bacteroidetes*, *Acidobacteria*, *Actinobacteria* and *Chloroflexi* and these results were expected since these groups have been identified as common rhizosphere inhabitants in other crops (Edwards *et al.*, 2015; Peiffer

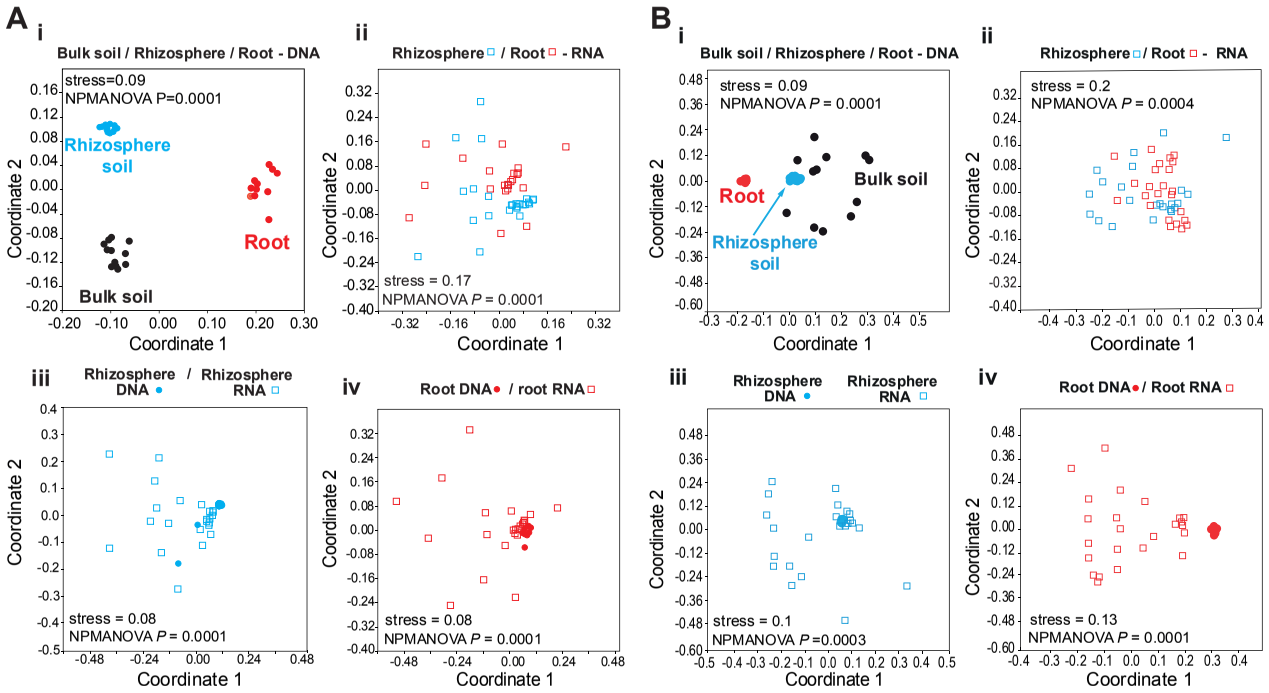

Figure 9. Nonmetric multidimensional scaling (NMDS) ordinations of changes in **A**. bacterial and **B.** fungal community structures associated with (i) bulk soil DNA, rhizosphere soil DNA and root DNA, (ii) rhizosphere soil RNA and root RNA, (iii) rhizosphere soil RNA and rhizosphere soil DNA and (iv) root RNA and root DNA.

et al., 2013; Inceoglu *et al.*, 2011), as well as highly abundant in the rhizosphere soil and in the roots of *Arabidopsis* (Schlaeppi *et al.*, 2014; Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012). We found *Proteobacteria* and *Actinobacteria* abundances almost equally high in all communities. However, *Proteobacteria* were not such abundant in rhizosphere DNA suggesting that they are proportionally more strongly represented among active bacteria in the rhizosphere, whereas *Actinobacteria* had greater relative activity in the roots than in the rhizosphere. Moreover *Bacteroidetes* were more abundant in the root compartment, where they were also proportionally more active than in the soil. *Acidobacteria*, were more abundant in bulk soil and in the two rhizosphere soil samples, but they were much more infrequent in the roots (Figure 10B.). Interestingly, it has been previously suggested that *Proteobacteria* and *Actinobacteria* are potentially associated with disease suppression in the rhizosphere of sugar beet (Mendes *et al.*, 2011).

At the genus level in all soil samples the most abundant bacterial genera were *Rhodoplanes*, *Kaistobacter* and *Candidatus* Nitrososphaera (Figure 10C.). *Rhodoplanes* and *Kaistobacter* were highly active in both the ^{13}C- and ^{12}C-RNA rhizosphere fractions, whereas *Candidatus* Nitrososphaera was most abundant in the ^{13}C-RNA fraction (Figure 10D.). *Rhodoplanes* were identified in a ^{15}N-DNA SIP study as potential nitrogen fixers (Buckley *et al.*, 2007), whereas members of the genus *Kaistobacter* have been suggested to be involved in aromatic compounds degradation (Kersters, 2006). *Candidatus* Nitrososphaera, is an ammonia-oxidizing archaeon with central roles in global nitrogen cycling (Schleper & Nicol, 2010).

In the root-derived bacterial communities, the dominant genera were *Streptomyces*, *Rhizobium*, *Flavobacterium* and *Agrobacterium* (Figure 10C.), which exhibited also high activity (Figure 10D.). Members of the genus *Streptomyces* are very well known PGPR candidates (Cordovez *et al.*, 2015; Kanini *et al.*, 2013; Lehr *et al.*, 2008) and a *Flavobacterium* sp. isolated from the rhizosphere of bell pepper was found to be associated with plant growth promotion and antagonistic potential against pathogens (Kolton *et al.*, 2012). Previous studies were either unable to identify *Streptomyces* in either rhizosphere soil or roots of *B. napus* (Haichar *et al.*, 2008), or found corresponding OTUs only in the rhizosphere soil of strawberry (Costa *et al.*, 2006). In accordance with our results, bacteria belonging to the *Flavobacteriaceae* family were a significant

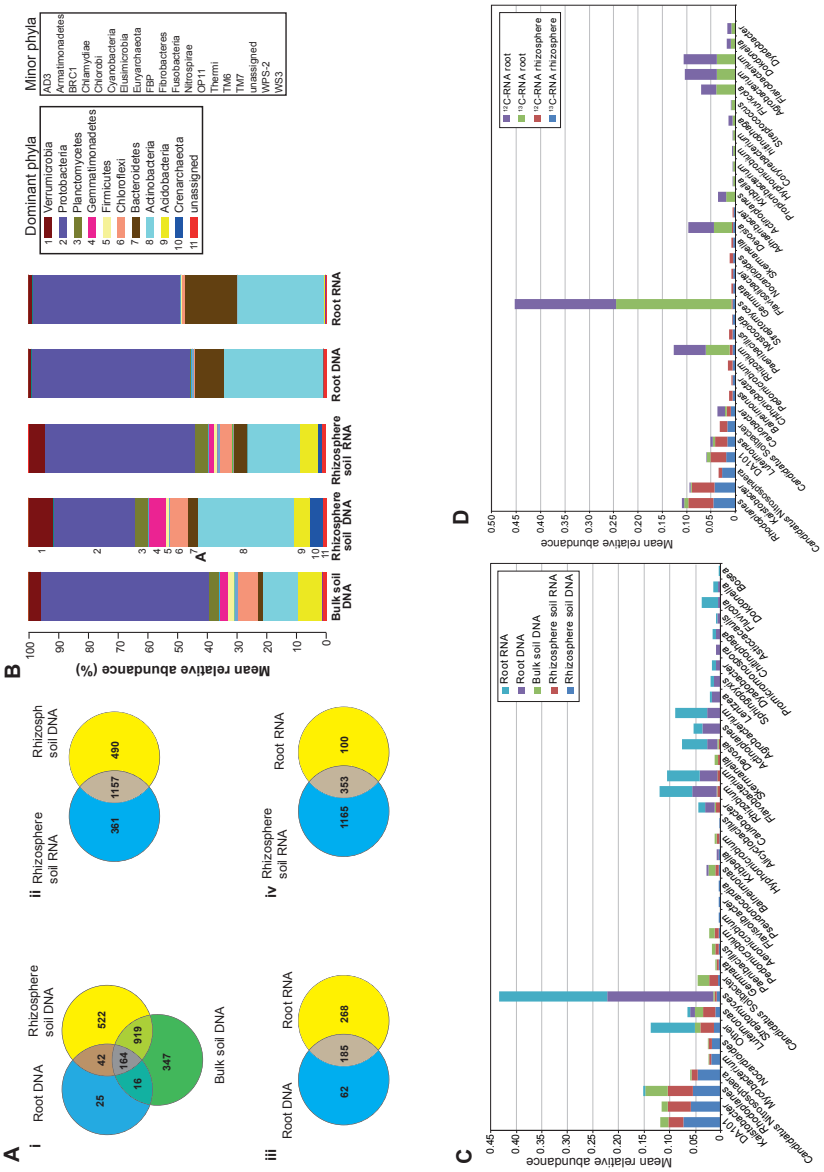

Figure 10. **A.** Venn diagrams showing unique & shared numbers of bacterial OTUs. **B.** Mean relative abundances of different bacterial phyla in bulk soil DNA, rhizosphere soil DNA and RNA, and root DNA and RNA. Dominant phyla are shown in a separate legend, supplemented with a numerical key. Minor phyla are simply listed. **C.** Mean relative abundances of the 20 most abundant bacterial genera in bulk soil DNA, rhizosphere soil DNA and RNA, root DNA and RNA. **D.** Mean relative abundances of the top 20 bacterial genera found in the ^{13}C-RNA and ^{12}C-RNA in the rhizosphere soil and in the root fractions. (Taxonomic classifications of 16S rRNA gene sequences were performed in QIIME using the Greengenes 16S rRNA reference taxonomy).

component of the *Arabidopsis* root microbiome (Schlaeppi *et al.*, 2014; Bulgarelli *et al.*, 2012). It has been demonstrated that *Rhizobium* sp. have strong potential to colonize roots of nonlegumes such as canola, lettuce and *Arabidopsis* and promote plant growth (Haichar *et al.*, 2012), suggesting that even in nonlegumes, the presence of nitrogen-fixing bacteria has the potential to reduce the use of synthetic fertilizers. In an earlier study using DNA-SIP, *Rhizobium* were ^{13}C incorporators in the rhizosphere soil of *B. napus* and wheat and they were present in the DNA-based communities of these crops (Haichar *et al.*, 2008).

The total numbers of fungal OTUs are shown in (Figure 11A.). The number of fungal OTUs retrieved from DNA was double that retrieved from RNA for the soil samples, while the opposite trend was observed for the root-derived OTUs. In total, 5 fungal phyla were identified (Figure 11B). The relative abundance values of *Basidiomycota* suggest that they are more strongly represented among the active fungi in the rhizosphere, while in the roots they appear to be only active, since corresponding OTUs were absent from the root DNA samples. *Ascomycota* formed a relatively large proportion of the active fungi in both the rhizosphere soil and the roots, but they contributed to a much smaller proportion of the total root fungal community. *Chytridiomycota* were most abundant in root-DNA followed by root-RNA-derived samples, whereas *Zygomycota* exhibited higher abundance in all soil-derived samples and a small proportion appeared in the root-RNA as well (Figure 11B).

At the genus level in all soil samples the most abundant fungal genera were *Cryptococcus* and *Mortierella*, whereas in rhizosphere DNA- and RNA- communities *Pseudaleuria*, *Clonostachys*, *Exophiala* and *Fusarium* were among the top 20 most abundant/active genera as well (Figure 11C). The aforementioned genera were thus consisting the ^{13}C- and ^{12}C-RNA rhizosphere fractions, however their relative activities were higher in the ^{12}C-RNA fraction, with the exception of *Clonostachys* whose activity was much higher in ^{13}C-RNA based community (Figure 11D). In the roots, the most active fungal genera were *Olpidium*, which is a soilborne obligate parasite, followed by the pathogen *Dendryphion*, *Clonostachys* and *Cryptococcus* (Figure 11C), whereas *Olpidium* and *Dendryphion* were more active in the ^{12}C-RNA-based root community, suggesting that they are either slow growing fungi, or

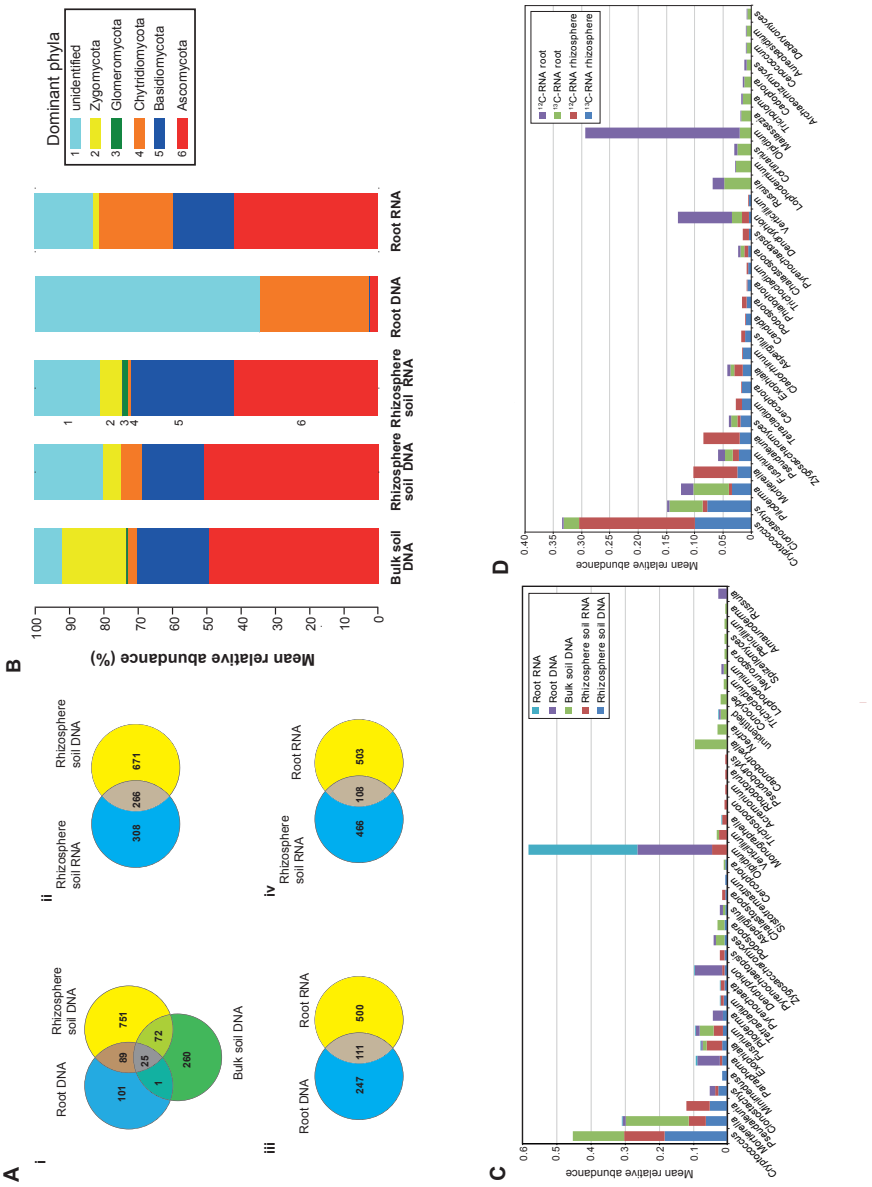

Figure 11. **A**. Venn diagrams showing unique and shared numbers of fungal OTUs. **B**. Mean relative abundances of different fungal phyla in bulk soil DNA, rhizosphere soil DNA & RNA, and root DNA & RNA. **C**. Mean relative abundances of the 20 most abundant fungal genera in bulk soil DNA, rhizosphere soil DNA and RNA, root DNA and RNA. **D**. Mean relative abundances of the top 20 fungal genera found in the ^{13}C-RNA and ^{12}C-RNA in the rhizosphere soil and in the root fractions. (Taxonomic classifications of the ITS region were performed in QIIME using the UNITE reference taxonomy).

that they derive carbon from unlabeled structural pools (Figure 11D). *Clonostachys rosea*, a species of *Clonostachys* has been shown to be an effective biocontrol agent against *B. cinerea*, *S. sclerotiorum*, *Plasmodiophora brassicae* and *F. oxysporum* with mechanisms including mycoparasitism, competition for nutrients and space, antibiosis and induction of systemic resistance through root colonization (Kamou *et al.*, 2016; Lahlali, 2014; Rodriguez *et al.*, 2011). Fungi of the genus *Cryptococcus* have the potential of assisting in nutrient assimilation from soil, thus leading to a competitive advantage against other bacteria and fungi (Vishniac, 2006). *Fusarium* spp. are common soil fungi that can either be pathogens or saprotrophs against other pathogenic fungi (Duffy *et al.*, 2004).

To conclude, the results suggest and further support the idea that there is an active selection from a more diverse rhizosphere community towards the roots, since we observed higher relative dominance of certain microbial taxa in the roots compared with those in rhizosphere soil. Furthermore, the identification of specific genera as incorporators of recently fixed plant carbon points towards their potential as inoculants to improve plant productivity and health and implies that they might be superior competitors in the rhizosphere environment of oilseed rape.

4.3 Paper III: Modification of the *Brassica napus* transcriptome by *Serratia proteamaculans* S4 during interaction with the plant pathogenic fungus *Rhizoctonia solani* AG2-1

The transcriptome responses of *Brassica napus* roots and leaves to root colonization by factorial combinations of the plant pathogenic fungus *Rhizoctonia solani* AG2-1 and the pathogen antagonistic bacterium *Serratia proteamaculans* S4 at 120h (T1) and 240h (T2) post-inoculation were investigated using an *in-vitro* gnotobiotic system and RNA-sequencing. We hypothesized that there would be a greater rearrangement of the plant transcriptome during interaction with *R. solani* alone compared to the S4 bacterial inoculations alone, or in combination with the fungus and that the presence of S4 would alter the plant gene expression patterns and lead to systemic priming

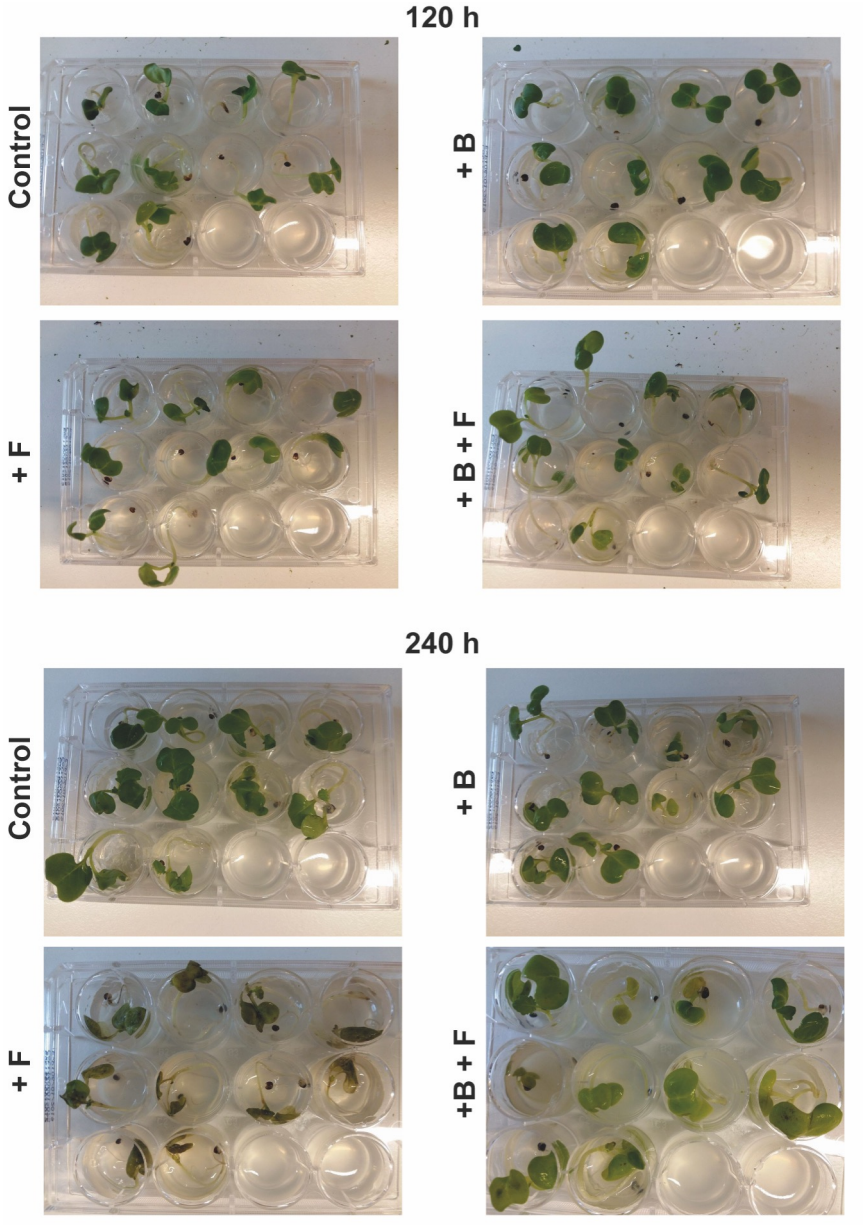

Figure 12. Experimental systems used for challenging roots of *Brassica napus* seedlings with factorial combinations of the plant pathogenic fungus *Rhizoctonia solani* and the pathogen antagonistic bacterium *Serratia proteamaculans* S4.

Table 1. Expression profiles in leaves and roots of *Brassica napus* plants following inoculation of the roots with factorial combinations of the pathogenic fungus *Rhizoctonia solani* and the plant-beneficial bacterium *Serratia proteamaculans* S4.

120 h – Leaves ≥3 fold	120 h – Roots ≥3 fold	240 h – Leaves ≥3 fold	240 h – Roots ≥3 fold
B-C **Up**: 1100 genes **147** **Down**: 1597 genes **577** **Total**: 2697 Unique in **B**: 118 genes Unique in **C**: 228 genes	**B-C** **Up**: 667 genes **210** **Down**: 1055 genes **361** **Total**: 1722 Unique in **B**: 19 genes Unique in **C**: 23 genes	**B-C** **Up**: 77 genes **21** **Down**: 62 genes **43** **Total**: 139 Unique in **B**: 18 genes Unique in **C**: 47 genes	**B-C** **Up**: 172 genes **44** **Down**: 275 genes **75** **Total**: 447 Unique in **B**: 8 genes Unique in **C**: 1 genes
BF-C **Up**: 997 genes **198** **Down**: 2026 genes **579** **Total**: 3023 Unique in BF: 253 genes Unique in C: 221 genes	**BF-C** **Up**: 688 genes **298** **Down**: 649 genes **252** **Total**: 1337 Unique in BF: 44 genes Unique in C: 36 genes	**BF-C** **Up**: 815 genes **485** **Down**: 223 genes **109** **Total**: 1038 Unique in BF: 136 genes Unique in C: 54 genes	**BF-C** **Up**: 1370 genes **629** **Down**: 667 genes **205** **Total**: 2037 Unique in BF: 60 genes Unique in C: 4 genes
F-C **Up**: 1732 genes **263** **Down**: 1019 genes **174** **Total**: 2751 Unique in F: 132 genes Unique in C: 195 genes	**F-C** **Up**: 388 genes **144** **Down**: 303 genes **70** **Total**: 691 Unique in F: 36 genes Unique in C: 25 genes	**F-C** **Up**: 4283 genes **3395** **Down**: 2743 genes **2156** **Total**: 7026 Unique in F: 334 genes Unique in C: 3496 genes	**F-C** **Up**: 4674 genes **2960** **Down**: 3657 genes **2256** **Total**: 8331 Unique in F: 155 genes Unique in C: 4649 genes

of defense responses. Limited information is available about the gene expression patterns of *B. napus* roots and leaves when challenged with a biocontrol bacterium and a necrotrophic fungal pathogen. Therefore, this study provides a global view of genes and potential mechanisms being differentially regulated under the aforementioned conditions, in a crop plant, oilseed rape.

At T1, the phenotypic differences were not yet evident. At T2 however, clear differences were evident between the plants that were inoculated only with *R. solani* and all other treatments. The pathogen-inoculated plants were almost dead, with severe discoloration and tissue degradation, whereas control plants and those inoculated with bacteria only or those inoculated with both bacteria and fungi appeared healthy (Figure 12).

Plant genes that were statistically differently regulated compared to the corresponding control samples had q-value (false discovery rate (FDR) < 0.05 (Table 1, marked with black). However, for all downstream analyses differentially expressed genes (DEGs) were defined if they had a) $\log_2$ fold value > |+/− 3| and b) q-value < 0.05 (Table 1, marked with blue). Interestingly, at T1 the number of statistically differently regulated genes was greater in the leaves than in the roots, indicating that the plant is responding in a systemic way to both microorganisms. However, at T2 the opposite pattern was observed. Additionally, the number of genes responsive to the inoculation with S4 alone reduced dramatically from T1 to T2 in both the roots and the leaves suggesting that the plant is capable of recognizing the beneficial bacterium at an earlier stage and that at the later stage the mutualistic association has already been established. On the other hand, at T2 in both the roots and the leaves of plants inoculated with *R. solani* alone there was a massive increase in the number of differentially expressed genes compared to T1, probably due to the fact that the fungus is growing slower. For the combined treatment with both *R. solani* and S4, the number of differentially expressed genes was intermediate and the pattern was that there was greater downregulation of genes at T1 and greater upregulation of genes at T2 (Table 1). Overall our results further demonstrate earlier findings suggesting that the transcriptome changes that occur in systemic tissues upon root colonization by beneficial microbes are in general relatively mild when compared to the massive transcriptional reprogramming occurring during pathogen attack (Pozo *et al.*, 2008; Van Wees *et al.*, 2008; Alfano *et al.*, 2007; Wang *et al.*, 2005;

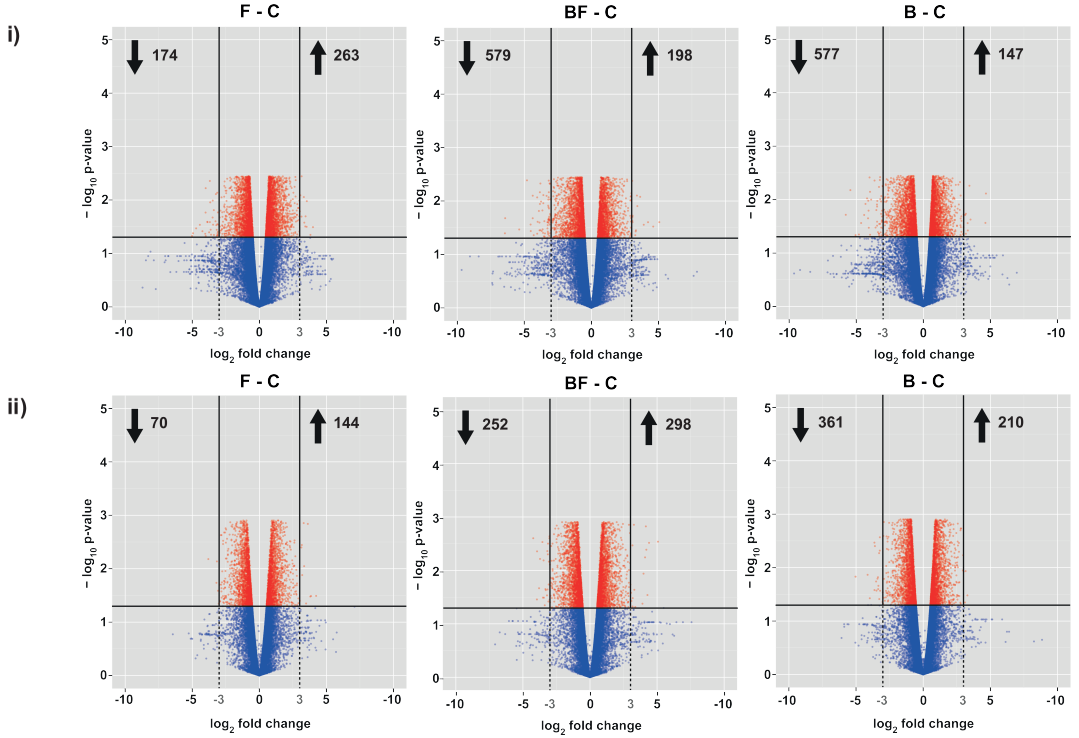

Fig. 13A. Volcano plots showing patterns of comparative gene expression in i) leaves and ii) roots of *Brassica napus* plants 120 hours following exposure to factorial combinations of the plant pathogenic fungus *Rhizoctonia solani* and the pathogen-antagonistic bacterium *Serratia proteamaculans.* Genes were considered to be significantly up or down-regulated if two criteria were fulfilled - ($P<0.05$, FDR corrected) and $\log_2$ fold value > |+/− 3|. Blue dots indicate values not fulfilling the first criterion, red dots indicate significant values.

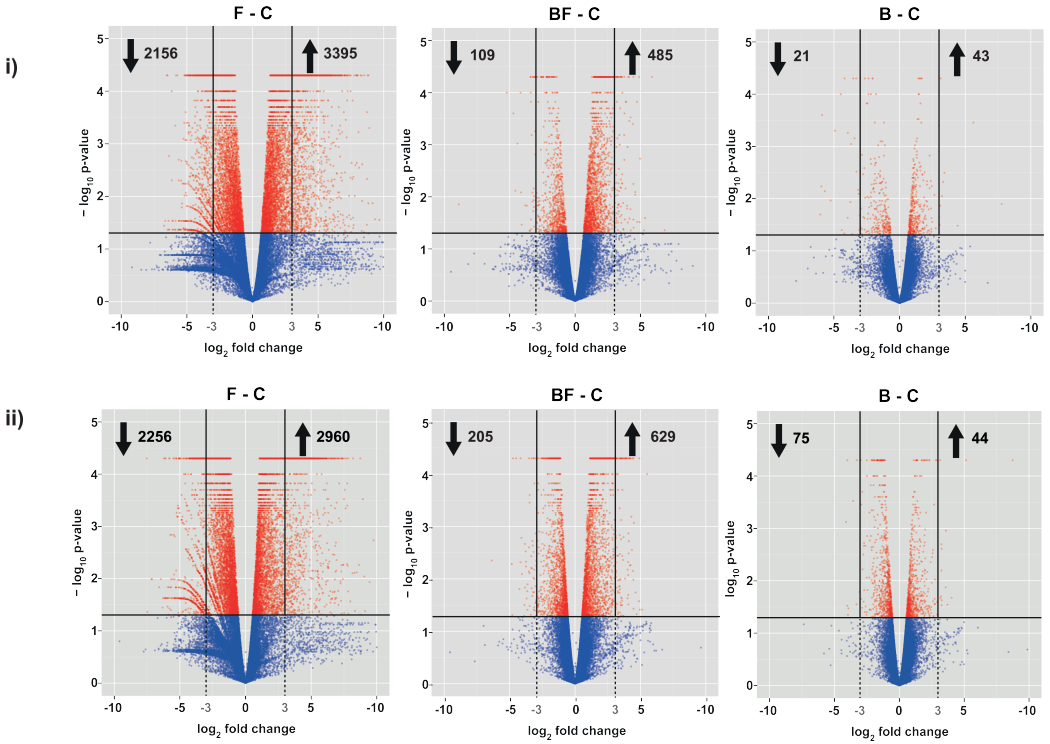

Fig. 13B. Volcano plots showing patterns of comparative gene expression in **i)** leaves and **ii)** roots of *Brassica napus* plants 240 hours following exposure to factorial combinations of the plant pathogenic fungus *Rhizoctonia solani* and the pathogen-antagonistic bacterium *Serratia proteamaculans*. Genes were considered to be significantly up or down-regulated if two criteria were fulfilled - ($P<0.05$, FDR corrected) and $\log_2$ fold value > |+/− 3|. Blue dots indicate values not fulfilling the first criterion, red dots indicate significant values.

Verhagen *et al.*, 2004). A schematic representation of the overall patterns of DEGs is given in Figure 13A, 13B.

Hierarchical clustering of the differentially expressed genes in the roots and in the leaves separated the treatments into three main clusters. Cluster 1 represented genes from samples derived the roots, cluster 2 represented genes from samples derived the leaves and cluster 3 represented genes derived from roots and leaves inoculated with *R. solani* alone at T2, suggesting that the plant response to the different treatments and time points differs and also that the response in the roots varies from that in the leaves (Figure 14).

Analysis of the functional categories (from Gene Ontology annotations) of the top 100 genes that were differentially regulated at T1 and at T2 during the different interactions revealed genes related to hormones to be associated with growth and defense as well as genes related to defense and/or stress responses of the plant (Figures 15A. 15B. 16A. 16B.).

B. napus* response to *R. solani

a) Hormonal crosstalk: Genes associated with ABA, SA and JA were upregulated in the roots at T1, while there was no strong evidence of systemic resistance in the leaves (Figure 15A.). At T2, genes related to SA, JA, ET and glucosinolates were induced in the roots, whereas in the leaves a clear systemic response was evident with upregulation of genes related to SA, ABA, JA, ET, IAA and glucosinolates (Figure 15B.). Until recently it was thought that necrotrophic fungi such as *R. solani* use a quite straightforward approach to overcome host plant defenses, including the use of cell wall degrading enzymes allowing them to enter the plant cell wall with subsequent release of toxins to kill host cells (Oliver & Solomon, 2010). It was also believed that the plant immune system relies preferentially on JA/ET-based defense responses (Glazebrook, 2005) and indeed there are even recent studies pinpointing to the significance of the synergism between JA and ET (Joshi *et al.*, 2016; Wu *et al.*, 2016). On the other hand despite the fact that the interactions between SA and JA are mostly antagonistic (Koornneef *et al.*, 2008), research has also revealed that SA and JA might act in a synergistic way in *Arabidopsis* and *Brassica* (Wang *et al.*, 2012; Schenk *et al.*, 2000; van Wees *et al.*, 2000). Interestingly, the results of a study using multiple plant hormone quantification and expression analysis of marker genes in *B. napus* leaves challenged with *Sclerotinia sclerotiorum* (Novakova *et al.*, 2014) were more

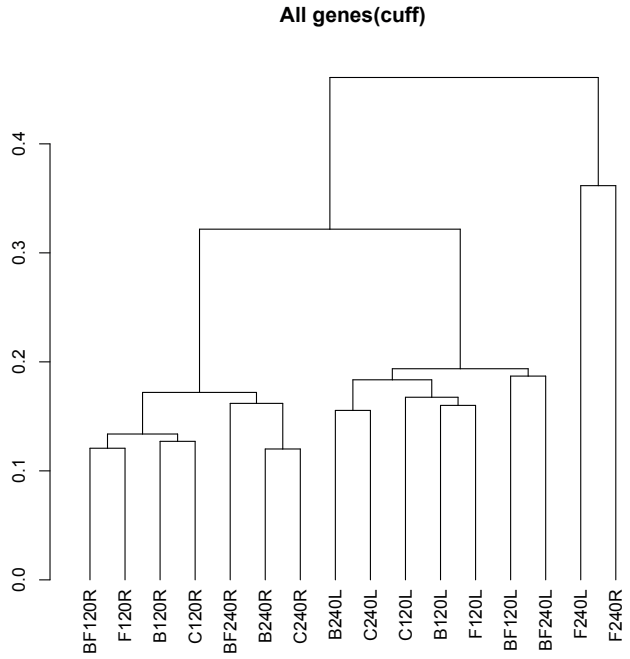

Figure 14. Dendrogram based on Jensen-Shannon distances, showing hierarchical clustering of *Brassica napus* leaf **(L)** and root **(R)** transcriptomes at 120h and 240h following exposure to factorial combinations of the plant pathogenic fungus *Rhizoctonia solani* **(F)** and the pathogen antagonistic bacterium *Serratia proteamaculans* S4 **(B)**. **C** indicates control plants not exposed to either bacteria or the fungal pathogen.

similar to our results on roots and leaves infected with *R. solani* at T2, where SA, JA and ET associated genes were induced and GA related genes were downregulated at T1 in both roots and leaves. Among the induced genes, we found two ET-responsive proteins pathogenesis-related *PR4,* which are chitinases with antifungal activity (Van Loon & Van Strien, 1999). The NPR1 protein, which is a significant transducer of the SA signal, acts as a transcriptional co-activator of *PR* gene expression (Dong, 2004) and is also a key regulator in SA-mediated suppression of JA signaling (Vlot *et al.*, 2009; Spoel *et al.*, 2003) was identified in our list of upregulated genes with log2 fold value >±3. NPR1 has been shown to modulate the antagonistic effect of SA to JA when located in the cytosol, whereas when located in the nucleus it plays a role in the activation of SA-responsive genes (Leon-Reyes *et al.*, 2009; Spoel *et al.*, 2003), but the cellular location of this factor in our study was not determined, thus its role remains unclear. Moreover, at T1 in the roots we found two genes members of the NINJA family, being negative regulators of JA signaling (Pauwels *et al.*, 2010), to be repressed. The identification of ET associated, upregulated genes in roots and leaves at T2 in our study, potentially suggests earlier findings that ET acts as a key component during SA and JA interactions (Leon-Reyes *et al.*, 2009). In addition, at T1 in the roots and at T2 in the leaves ABA-responsive genes were upregulated in our study (Figure 15A. B.), in accordance to the results of Novakova *et al.,* 2014, who suggested reduction of disease symptoms in *B. napus* plants infected with *S. sclerotiorum* after pretreatment with ABA. The importance of ABA as a positive defense regulator via different mechanisms (activation of stomatal closure or callose accumulation) has been demonstrated in other studies too (Mauch-Mani & Mauch, 2005; Ton & Mauch-Mani, 2004). On the other hand, we found ABA associated genes to be strongly downregulated in all treatments including plants challenged with *R. solani* and it has been shown that ABA treatment can suppress SAR induction, indicating an antagonistic interaction between SAR and ABA in *Arabidopsis* (Yasuda *et al.*, 2008). The secondary metabolites glucosinolates, which were induced in our study at T2 have shown enhanced expression in other studies examining the defense of *B. napus* to *S. sclerotiorum* (Wei *et al.*, 2016; Wu *et al.*, 2016). Our results thus imply that SA, JA, ET and ABA commonly regulate the defense response of *B. napus* to *R. solani*.

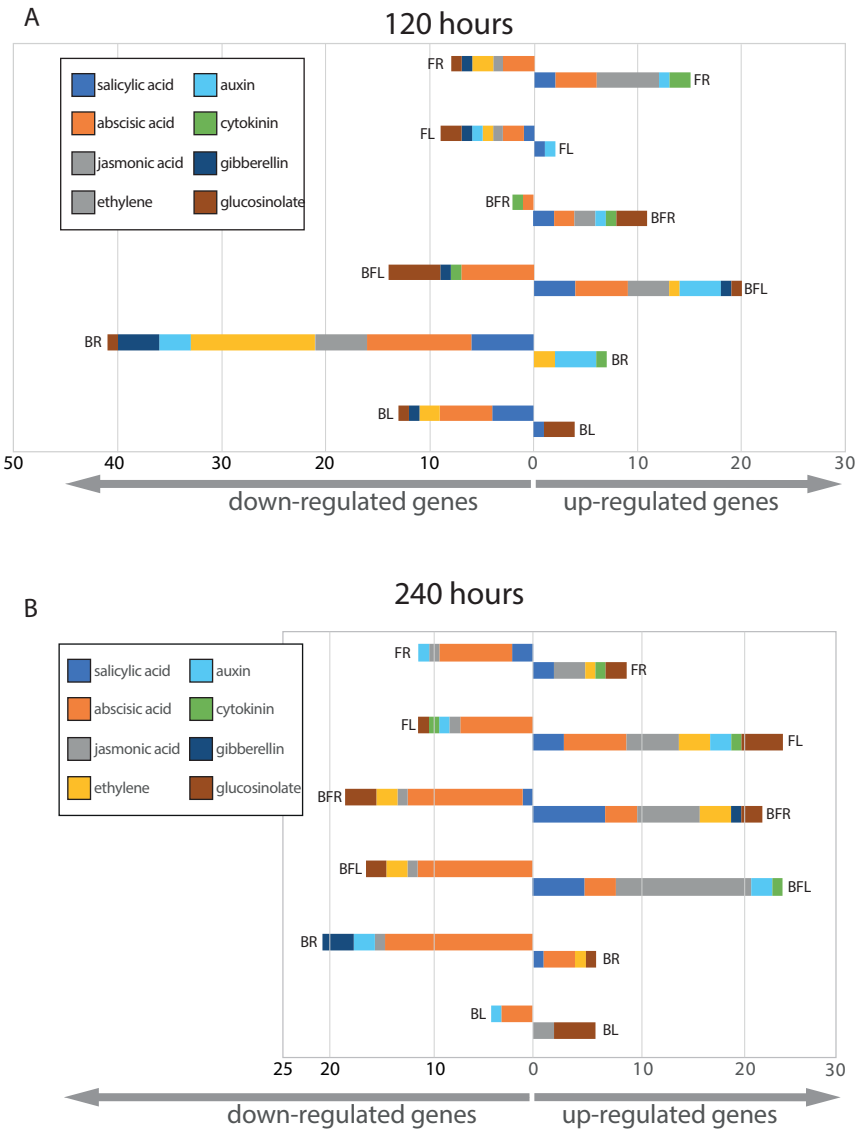

Figure 15. Histogram showing numbers of significantly up- and downregulated genes related to hormone regulation and response in roots (R) and leaves (L) of *Brassica napus* seedlings with factorial combinations of the plant pathogenic fungus *Rhizoctonia solani* (F) and the pathogen antagonistic bacterium *Serratia proteamaculans* S4 (B). The genes were selected among the top 50 within the gene ontology (GO) categories. **A)** 120 h, **B)** 240 h

b) Stress and defense mechanisms: At both time points, in the roots there was an induction of defense mechanisms and a stronger repression of stress mechanisms, however in the leaves at T2 there was stronger induction of stress mechanisms, consistent with the phenotypic observations of the plants (Figure 16A. B.). At both T1 and T2 in both the roots and the leaves a considerable number of upregulated genes was related to oxidation-reduction process and detoxification. Oxidative burst activation has been observed as a defense mechanism in interactions between different plants and necrotrophic fungi, including *R. solani* (Foley *et al.*, 2016; Pietrowska *et al.*, 2015; Foley *et al.*, 2013; Asai & Yoshioka, 2009). Among these genes, members of the cytochrome P450 and peroxidases were well represented. Peroxidases are enzymes that catalyze the formation of lignin, so they contribute to defense and they were found to be induced in another study where *B. napus* was challenged with *S. sclerotiorum* (Joshi *et al.*, 2016). MAPK signaling cascades and WRKY transcription factors play pivotal roles in the regulation of defenses responses against the necrotrophic fungal pathogen *S. sclerotiorum* to *Brassica* and *Arabidopsis* as it has been suggested in other studies (Wu *et al.*, 2016; Sun *et al.*, 2014; Wang *et al.*, 2009b; Yang *et al.*, 2009), and in accordance in our study WRKY transcription factors were induced at both time points in the leaves. Mitochondrial energy metabolism is a known defense mechanism to maintain cellular homeostasis (Schwarzlander & Finkemeier, 2013) and at T1 in the roots we found two related genes that were induced. Defense-related genes such as PR4, PR1 and a lectin were highly induced as Wu *et al.,* 2016, have previously demonstrated. At T2 in the leaves a transcription factor (JUNGBRUNNEN 1-like) associated with the biosynthesis of camalexin was upregulated and camalexins are low molecular weight antimicrobial peptides produced in response to stress (Ahuja *et al.*, 2012; Ferrari *et al.*, 2007). It was not surprising that at T1 and T2 in the leaves and at T2 in the roots, among the upregulated genes, we identified genes related to desiccation and leaf senescence.

On the other hand, reduced development of the plant at T1 in the leaves and at T2 in the roots was confirmed by the significant repression of genes involved in ribosome biogenesis since the ribosome is tightly linked to development (Weis *et al.*, 2015). A mitogen activated kinase kinase kinase 18 (MAPKKK) was repressed at T1 in the leaves, with significant roles in growth and

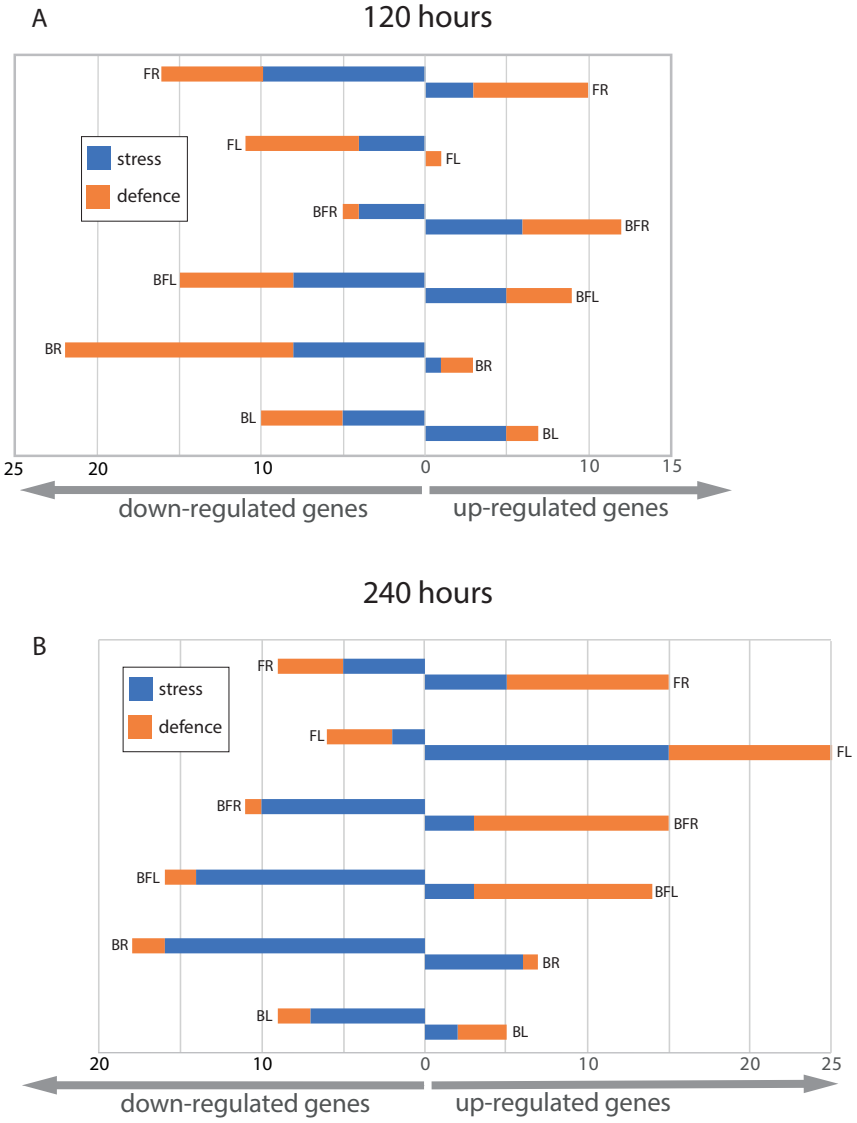

Figure 16. Histogram showing numbers of significantly up- and downregulated genes related to stress and defense in roots (R) and leaves (L) of *Brassica napus* seedlings with factorial combinations of the plant pathogenic fungus *Rhizoctonia solani* (F) and the pathogen antagonistic bacterium *Serratia proteamaculans* S4 (B). The genes were selected among the top 50 within the gene ontology (GO) categories. **A)** 120 h, **B)** 240 h

development as well as in abiotic and biotic stress responses (Virk *et al.*, 2015). At T1 in the roots five of the top 50 downregulated genes were assigned to transmembrane transport, which are potential stress responses, since the transport of toxic substances was potentially repressed. At T2 in the roots 3 aquaporin genes related to hydrogen-peroxide transmembrane transport were highly repressed, indicating a repression of stress mechanisms. A jacalin lectin that has been previously reported to be induced upon pathogen challenge (Joshi *et al.*, 2016) was found repressed in our study at T1 in the leaves and at T2 in the roots. Chaperones are essential for the stabilization of proteins, thus for cell survival under stress, with special roles in the stabilization of R proteins during effector triggered immunity (ETI) (Park & Seo, 2015; Shirasu & Schulze-Lefert, 2003) and in our study two chaperones were highly downregulated at T2 in the leaves. Overall, our results imply that, during pathogen challenge, there is an interplay between defense and stress responses of the plant. These mainly include genes related to oxidation reduction processes, transcription reprogramming and pathogenesis-related proteins (including chitinases and lectins). Compromised development of the plant, mainly due to repression of genes involved in ribosome biogenesis, plant cell wall assembly and induction of genes associated with leaf senescence and desiccation were also observed.

***B. napus* response to *R. solani* and *S. proteamaculans* S4**

a) Hormonal crosstalk: At T1, exposure of roots to both *R. solani* and S4 resulted in a systemic response in the leaves involving upregulation of larger numbers of genes associated with SA, ABA, JA, ET, IAA, GA and glucosinolates (Figure 15A). At T2 the combined upregulation of genes associated with SA, ABA, JA and ET appeared stronger in roots exposed to both *R. solani* and S4 than in roots exposed to these organisms individually. A clear systemic response was evident with clear upregulation, in the leaves, of genes associated with SA, ABA and JA in particular (Figure 15B). The identification of induced SA associated genes at both time points and in both plant compartments (roots and leaves) when S4 present, is of great interest because S4 bacteria are known to possess genes for the production of SA (Neupane, 2013). However, it is known that the production of SA by the bacteria is usually not the causal agent of the observed systemic resistance, probably because the SA produced is not released into the rhizosphere, but becomes incorporated into SA moiety-containing siderophores (Bakker *et al.*, 2014). In this

respect, it is interesting that at T1 in the leaves we found induction of a probable 2-oxoglutarate Fe (II)-dependent dioxygenase, an enzyme dependent on ferrous iron as a co-factor, whose activity is usually increased by the addition of the antioxidant ascorbate, which is produced by *R. solani* and is thought to assist with enzymatic cycles by maintaining the ferrous iron state (Farrow & Facchini, 2014). However, despite the production of SA from S4 bacteria, we cannot ensure that the induction of SA-associated genes is not because of a SA-dependent ISR as has been demonstrated in some studies (Vogel *et al.*, 2016; van de Mortel *et al.*, 2012; Audenaert *et al.*, 2002a; De Meyer *et al.*, 1999). In addition, at T2 in the roots there was strong induction of SA, JA and ET associated genes. Eight ethylene responsive transcription factors were induced and it is known that they regulate molecular responses to pathogen attack (Muller & Munne-Bosch, 2015) and are involved in hormonal crosstalk under biotic stress with JA (Lorenzo *et al.*, 2003). In the leaves at T2, ET was not induced, however JA was primarily induced, followed by SA and ABA responsive genes and finally by IAA associated genes. We found strong induction of the MYC2 transcription factor at T2 in the leaves, which is nuclear-localized and has been identified as a key regulator in priming for enhanced JA-dependent responses (Kazan & Manners, 2013; Pozo *et al.*, 2008), potentially implying that the underlying ISR used by S4 bacteria in *B. napus* is dependent on JA signaling. On the other hand, an alpha-dioxygenase1 was highly induced at T2 in the leaves and this gene is known to act as a promoter of local and systemic plant defense in a SA-dependent manner, including the establishment of systemic acquired resistance (SAR) (Vicente *et al.*, 2012). Additionally, upregulation of the MYB44 transcription factor was found at T2 in the roots, having effects on JA- and SA-mediated defense responses (Shim & Choi, 2013). The MYB72 transcription factor was also induced, required for the signaling steps of rhizobacteria-induced ISR (Ent, 2008). There is strong evidence suggesting that ISR induction to necrotrophs depends on JA and/or ET signaling (Zamioudis & Pieterse, 2012; Sarosh *et al.*, 2009; Van der Ent *et al.*, 2009; Van Wees *et al.*, 2008). Furthermore, IAA is involved in all aspects of plant development, but also in plant-microbe interactions (Dharmasiri & Estelle, 2004). Interestingly, IAA associated genes were induced in *Arabidopsis* leaves colonized by the *P. thivervalensis* rhizobacterium during challenge with *P. syringae*, but

it was concluded that ISR was not due to IAA (Cartieaux *et al.*, 2003). In our study, the IAA-amino acid hydrolase ILR1-like 4 gene was induced at T2 in the leaves playing roles in IAA homeostasis (Cohen & Bandurski, 1982). Based on all the aforementioned results, we speculate that ISR is potentially dependent on JA and IAA, but we cannot exclude that probably ET (at an earlier stage) and SA contribute as well.

b) Stress and defense mechanisms: At T1 in the roots and in the leaves, the number of defense and stress upregulated genes displayed a balance while there was a stronger downregulation of stress-related genes at T1 and T2 in the roots, whereas in the leaves at T2 most downregulation was observed for stress-related genes compared to T1. However, at T2 in the roots and in the leaves the majority of induced genes were associated with defense (Figure 16A. B.).

At both T1 and T2 in the roots and in the leaves, many upregulated genes were related to floral induction and plant growth. One copy of the RVE2 gene that regulates flower development and circadian clock, two copies of the APRR1 gene responsible for controlling photoperiodic flowering response (Matsushika *et al.*, 2000), zinc finger CONSTANS transcription factors with a well-established role in photoperiod sensing in *Arabidopsis* and in flowering induction (Wong *et al.*, 2014) were all induced in our study. Interestingly, LUX-like transcription factors involved in positive regulation of circadian rhythm were also induced, in contrast to previous results where genes related to the regulation of RNA transcription such as circadian clock were downregulated in *Arabidopsis* in the presence of the plant growth promoting *Pseudomonas sp*. G62 (Schwachtje *et al.*, 2011). In addition, phytosulfokines, the MYB transcription factor DIVARICATA, the PIRL8 and EXORDIUM genes are all related to developmental processes and growth (Sauter, 2015; Forsthoefel *et al.*, 2013; Raimundo *et al.*, 2013; Schroder *et al.*, 2011) and Nudix hydrolases assist in the maintenance of cellular homeostasis. Expansins are cell wall loosening agents known for their endogenous function in cell wall extensibility and the *Arabidopsis* expansin-like A2 gene (EXLA2) which is known to be involved in defense against necrotrophic fungi (Abuqamar *et al.*, 2013) was induced in the present study. Interestingly, loosening of plant cell walls, suggests enhanced root exudation. Genes responsible for actin assembly, implicated in the formation of physiological barriers in the site of

infection (Janda *et al.*, 2014) as well as genes related to the biosynthesis of plant cell walls were found induced. We additionally identified induction of the fluG-like gene, which is directly linked to the biosynthesis of peptidoglycan, the major constituent of the bacterial cell wall, implying a synergistic effect of the plant to the S4 bacteria. There was still induction of genes involved in oxidation-reduction process such as members of the cytochrome P450, plant defensins, an endochitinase, and WRKY transcription factors. We also found induction of an RLK gene and it is known that such genes are plant pattern recognition effectors (PRRs) that recognize PAMPs and MAMPs, a crucial mechanism in the discrimination between defense or symbiosis (Antolin-Llovera *et al.*, 2014), in accordance to the results obtained by Vogel *et al.,* 2016. From the above, it is suggested that the S4 bacteria assist in floral induction, plant development and growth, play roles in the maintenance of cellular homeostasis as well as in the building up of plant cell walls and potentially create synergistic interactions with oilseed rape plants and help them to tolerate stress.

Despite the fact that S4 bacteria have efficiently colonized the plant, it was interesting that many downregulated genes were photosynthesis related at both T1 and T2 in the roots and in the leaves probably because photosynthesis is an energy costly mechanism despite of a net energy gain, thus plants try to improve their growth via acting in a synergistic way with the S4 bacteria. While many of the induced genes were related to growth development, compromised growth was a significant trend identified among the repressed genes too. Such genes were either involved in plant growth, developmental and cellular processes or plant cell wall assembly. At the same time we observed downregulation of senescence-associated genes as well as defense-related genes (e.g. genes involved in lipid transport, oxidation-reduction, toxin catabolism, jacalin lectins). Interestingly, at T1 in the leaves an isomerase BH0283-like gene related to nitrogen and its transport was repressed and it has been shown that nitrogen metabolism and nitrogen content were systemically reduced in the leaves of plants in order to reduce the nutritional value of the tissues, re-modeling the primary metabolism in its own right (Schwachtje *et al.*, 2018). In general, compromised growth and repression of defense mechanisms were among the major traits we observed in the fifty genes that were most highly downregulated in the presence of both S4 and *R. solani*.

***B. napus* response to *S. proteamaculans* S4**

a) Hormonal crosstalk: We found strong induction off IAA related genes at T1 in the roots, confirming the growth stimulatory effects of *S. proteamaculans* discussed by Neupane, 2013. However, SA, ABA, JA, ET, IAA, GA and glucosinolates associated genes were downregulated (Figure 15A), suggesting that at this time point the bacteria may already be recognized as mutualists by the plant. At T2 in the roots, ABA related genes were both up- and downregulated (Figure 15B). IAA-related genes have been found to be induced in other studies using *Arabidopsis, Bacillus subtilis* and *P. fluorescens* (Lakshmanan *et al.*, 2013; Wang *et al.*, 2005) and in the latter study, ET-responsive genes were downregulated. Interestingly, a recent study has demonstrated that the positive effects on plant growth and root architecture in *Arabidopsis* when colonized by the beneficial rhizobacterium *P. simiae* WCS417 are regulated by auxin (Stringlis *et al.*, 2018). Moreover, IAA is capable of increasing root surface area thus providing greater access to soil nutrients by the plant as well as of loosening of plant cell walls, thereby enhancing root exudation (Glick, 2012).

b) Stress and defense mechanisms: At T2 the presence of S4 was primarily associated with differential regulation of stress-related genes, rather than defense-related genes (Figure 16A). This pattern was less clear at T1 and there was more even downregulation of defense- and stress-related genes in both roots and leaves (Figure 16B).

Taken together, these results indicate that under the gnotobiotic conditions used in this experiment, the S4 bacteria can protect *B. napus* seedlings against the damaging effects of the necrotrophic fungal pathogen *R. solani*. When plants were exposed to *R. solani* alone, a massive transcriptional reprogramming was observed, involving hormonal signaling, defense and stress responses, similar to those revealed in other pathosystems. In the presence of S4 and *R. solani* the plant response was modulated, resulting in greatly reduced numbers of differentially expressed genes with a proportional increase in the downregulation of stress-related genes and upregulation of defense-related genes.

5 Conclusions

Paper I: The results suggest that the response of the phytopathogenic fungus *R. solani* to two antagonistic bacterial strains of the genus *Serratia*, S4 and AS13, is associated with a large transcriptional rearrangement of the fungus, affecting a wide repertoire of mechanisms.

- The observed inhibition of fungal growth is consistent with the downregulation of genes controlling DNA-replication, transcription, translation, chitinase and ergosterol production.

- Defense response in the fungus is associated to the production of antioxidants and the degradation of xenobiotics in order to counter the oxidative stress caused by the bacteria, or by altering their environment.

- *R. solani* is capable of attacking the bacteria, by producing toxins and lectins.

Paper II: The results suggest that in *B. napus*, root- and rhizosphere soil-associated communities of bacteria and fungi, as well as their DNA and RNA-based community profiles differ significantly from each other.

- Among the predominant abundant and active bacterial phyla identified, *Proteobacteria* and *Actinobacteria* have previously been reported to be associated with disease suppression.

- There was higher relative dominance of certain microbial taxa in the roots than in the rhizosphere soil, supporting the idea of active selection from the more diverse rhizosphere environment.

- Among the active bacterial and fungal genera identified, *Streptomyces*, *Rhizobium*, *Clonostachys* and *Fusarium* might have the potential to be used as bioinoculants to improve health and productivity of oilseed rape.

Paper III: In the presence of the pathogenic fungus *R. solani* visible damage to oilseed rape plants was observed, combined with massive transcriptional reprogramming. However, when the pathogen-antagonistic bacterium *S. proteamaculans* S4 was present, the plants looked healthy and the transcriptional response was moderate. Significant systemic responses were observed in all cases.

- The transcriptional profiling of oilseed rape plants inoculated with *R. solani* and S4 alone or in combination, is significantly different between roots and leaves, as well as between the different inoculation treatments.

- SA, JA, ET and ABA commonly regulate the defense response of *B. napus* to *R. solani*. Interplay between defense and stress is evident, including genes related to oxidation-reduction, transcription reprogramming, ribosome biogenesis, plant cell wall assembly and PR proteins.

- Co-inoculation of roots with *R. solani* and S4 resulted in induced systemic resistance. ISR is potentially dependent on JA, IAA and probably SA. The presence of S4 causes downregulation of stress-related genes and upregulation of defense-related genes and changes in genes associated with floral induction and plant development. However, downregulation of some genes associated with photosynthesis was also observed.

6 Future prospects

The rhizosphere is an extremely complex environment in which plants and microorganisms (pathogenic, beneficial or neutral) interact in varied ways, however the knowledge of the functional mechanisms underlying interactions in the rhizosphere is still limited.

Naturally occurring soil microorganisms have the potential to control fungal pathogens of plants, but their successful use in sustainable agriculture requires further understanding of the molecular processes and modes of action underlying their ability to promote plant growth and act as biocontrol agents. It is also crucial to understand the impacts of such biocontrol organisms on indigenous microbial communities, as well as to elucidate how the reciprocal signaling between diverse microbial consortia and plants is functioning.

The results presented in this thesis provide insight into the functional basis of the responses that fungal pathogens, such as *R. solani*, use in order to counteract antagonistic bacteria. We identified several fungal genes required for survival and defense in the presence of antagonistic bacteria using *in-vitro* experimental conditions. However, the controlled environment used in our study lacks the complexity of real-life soil and rhizosphere habitats. In order to gain an understanding of the *in-situ* competitive interactions it is essential to study the gene expression of the fungus in more natural soil systems and to complement the present studies with modern molecular biological tools such as the use of fungal mutant lines and protein localization.

The results presented in this thesis, also provide a picture of the global differentiation in gene expression of *B. napus* when

challenged with a biocontrol bacterium and a necrotrophic fungal pathogen. Most research up to date has been conducted on *Arabidopsis*. To the best of our knowledge this is the first report examining such tripartite interactions in a crop pathosystem with a recent publicly available genome. Further studies involving protein expression, hormone quantification and gene mutagenesis will help us to get a more complete picture of this specific plant pathosystem. It will also be interesting to examine how *B. napus* responds to other biocontrol bacteria and to compare the transcript profiles obtained. This might support the idea that bacteria trigger many of the known pathogen-related responses more broadly and is thereby interesting from an evolutionary point of view.

Furthermore, in this thesis we identified the active microbiomes of bacteria and fungi colonizing oilseed rape plants using stable isotope probing and targeting the conserved 16S rRNA bacterial region. The identification of microbial taxa that are not only present in the rhizosphere, but that are also capable of assimilating plant-derived carbon sheds insight into the active microbiome of a plant. This helps to determine microorganisms that are superior competitors for recently fixed carbon, and thus have the potential to be used as bioinoculants to improve plant productivity and health. However, the use of metagenomics and especially metatranscriptomics approaches will be even more valuable and probably unbiased, in order to answer the question 'what genes are collectively expressed in an environmental sample' and to assign the functional traits involved in different processes in different taxa.

Nowadays, crop production is being intensified in order to fulfill a) the food demands of an increasing population and b) an increased demand for energy using bioenergy crops such as oilseed rape. However, despite the plethora of biotic and abiotic stresses that plants have to cope with, it is important that increases of crop yields should be achieved in a sustainable way. In this context, the EU directive 2009/128/EC to achieve the sustainable use of pesticides involves mandatory Integrated Pest Management (IPM) for all agricultural production and natural pest control mechanisms are preferred before responsible use of chemical pesticides. Therefore, in a long-term perspective, the findings presented in this thesis might assist in the design of future strategies for sustainable crop production, with reduced input of chemicals.

References

Abramovitch, R.B., Anderson, J.C. & Martin, G.B. (2006). Bacterial elicitation and evasion of plant innate immunity. *Nature Reviews Molecular Cell Biology,* 7(8), pp. 601-611.

Abuamsha, R., Salman, M. & Ehlers, R.U. (2011). Differential resistance of oilseed rape cultivars (Brassica napus ssp oleifera) to *Verticillium longisporum* infection is affected by rhizosphere colonisation with antagonistic bacteria, *Serratia plymuthica* and *Pseudomonas chlororaphis*. *Biocontrol,* 56(1), pp. 101-112.

Abuqamar, S., Ajeb, S., Sham, A., Enan, M.R. & Iratni, R. (2013). A mutation in the expansin-like A2 gene enhances resistance to necrotrophic fungi and hypersensitivity to abiotic stress in *Arabidopsis thaliana*. *Molecular Plant Pathology,* 14(8), pp. 813-827.

Achard, P., Cheng, H., De Grauwe, L., Decat, J., Schoutteten, H., Moritz, T., Van Der Straeten, D., Peng, J.R. & Harberd, N.P. (2006). Integration of plant responses to environmentally activated phytohormonal signals. *Science,* 311(5757), pp. 91-94.

Achard, P., Renou, J.P., Berthome, R., Harberd, N.P. & Genschik, P. (2008). Plant DELLAs restrain growth and promote survival of adversity by reducing the levels of reactive oxygen species. *Current Biology,* 18(9), pp. 656-660.

Adesemoye, A.O., Torbert, H.A. & Kloepper, J.W. (2009). Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. *Microb Ecol,* 58(4), pp. 921-9.

Adie, B.A., Perez-Perez, J., Perez-Perez, M.M., Godoy, M., Sanchez-Serrano, J.J., Schmelz, E.A. & Solano, R. (2007). ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defenses in *Arabidopsis*. *Plant Cell,* 19(5), pp. 1665-81.

Ahn, I.P., Lee, S.W. & Suh, S.C. (2007). Rhizobacteria-induced priming in *Arabidopsis* is dependent on ethylene, jasmonic acid, and NPR1. *Mol Plant Microbe Interact,* 20(7), pp. 759-68.

Ahuja, I., Kissen, R. & Bones, A.M. (2012). Phytoalexins in defense against pathogens. *Trends in Plant Science,* 17(2), pp. 73-90.

Alfano, G., Ivey, M.L.L., Cakir, C., Bos, J.I.B., Miller, S.A., Madden, L.V., Kamoun, S. & Hoitink, H.A.J. (2007). Systemic modulation of gene expression in tomato by *Trichoderma hamatum* 382. *Phytopathology,* 97(4), pp. 429-437.

Alstrom, S. (1991). Induction of Disease Resistance in Common Bean Susceptible to Halo Blight Bacterial Pathogen after Seed Bacterization with Rhizosphere *Pseudomonads*. *Journal of General and Applied Microbiology,* 37(6), pp. 495-501.

Alstrom, S. (2001). Characteristics of bacteria from oilseed rape in relation to their biocontrol activity against *Verticillium dahliae*. *Journal of Phytopathology-Phytopathologische Zeitschrift,* 149(2), pp. 57-64.

Alstrom, S. & Gerhardson, B. (1987). Characteristics of a *Serratia-Plymuthica* Isolate from Plant Rhizospheres. *Plant and Soil,* 103(2), pp. 185-189.

Altenbach, D. & Robatzek, S. (2007). Pattern recognition receptors: From the cell surface to intracellular dynamics. *Molecular Plant-Microbe Interactions,* 20(9), pp. 1031-1039.

Anderson, J.P., Badruzsaufari, E., Schenk, P.M., Manners, J.M., Desmond, O.J., Ehlert, C., Maclean, D.J., Ebert, P.R. & Kazan, K. (2004). Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in *Arabidopsis*. *Plant Cell,* 16(12), pp. 3460-3479.

Anderson, N.A. (1982). The Genetics and Pathology of *Rhizoctonia-Solani*. *Annual Review of Phytopathology,* 20, pp. 329-347.

Antolin-Llovera, M., Petutsching, E.K., Ried, M.K., Lipka, V., Nurnberger, T., Robatzek, S. & Parniske, M. (2014). Knowing your friends and foes - plant receptor-like kinases as initiators of symbiosis or defence. *New Phytologist,* 204(4), pp. 791-802.

Arand, M., Cronin, A., Oesch, F., Mowbray, S.L. & Jones, T.A. (2003). The telltale structures of epoxide hydrolases. *Drug Metabolism Reviews,* 35(4), pp. 365-383.

Asai, S. & Yoshioka, H. (2009). Nitric Oxide as a Partner of Reactive Oxygen Species Participates in Disease Resistance to Necrotrophic Pathogen *Botrytis cinerea* in *Nicotiana benthamiana*. *Molecular Plant-Microbe Interactions,* 22(6), pp. 619-629.

Audenaert, K., De Meyer, G.B. & Hofte, M.M. (2002a). Abscisic acid determines basal susceptibility of tomato to *Botrytis cinerea* and suppresses salicylic acid-dependent signaling mechanisms. *Plant Physiology,* 128(2), pp. 491-501.

Audenaert, K., Pattery, T., Cornelis, P. & Hofte, M. (2002b). Induction of systemic resistance to *Botrytis cinerea* in tomato by *Pseudomonas aeruginosa* 7NSK2: Role of salicylic acid, pyochelin, and pyocyanin. *Molecular Plant-Microbe Interactions,* 15(11), pp. 1147-1156.

Badri, D.V., Quintana, N., El Kassis, E.G., Kim, H.K., Choi, Y.H., Sugiyama, A., Verpoorte, R., Martinoia, E., Manter, D.K. & Vivanco, J.M. (2009a). An ABC Transporter Mutation Alters Root Exudation of Phytochemicals That Provoke an Overhaul of Natural Soil Microbiota. *Plant Physiology,* 151(4), pp. 2006-2017.

Badri, D.V. & Vivanco, J.M. (2009). Regulation and function of root exudates. *Plant Cell and Environment,* 32(6), pp. 666-681.

Badri, D.V., Weir, T.L., van der Lelie, D. & Vivanco, J.M. (2009b). Rhizosphere chemical dialogues: plant-microbe interactions. *Current Opinion in Biotechnology,* 20(6), pp. 642-650.

Badri, D.V., Zolla, G., Bakker, M.G., Manter, D.K. & Vivanco, J.M. (2013). Potential impact of soil microbiomes on the leaf metabolome and on herbivore feeding behavior. *New Phytologist,* 198(1), pp. 264-273.

Bakker, P.A.H.M., Pieterse, C.M.J. & van Loon, L.C. (2007). Induced systemic resistance by fluorescent *Pseudomonas* spp. *Phytopathology,* 97(2), pp. 239-243.

Bakker, P.A.H.M., Ran, L.X. & Mercado-Blanco, J. (2014). Rhizobacterial salicylate production provokes headaches! *Plant and Soil,* 382(1-2), pp. 1-16.

Baldwin, I.T. (2001). An ecologically motivated analysis of plant-herbivore interactions in native tobacco. *Plant Physiology,* 127(4), pp. 1449-1458.

Bari, R. & Jones, J. (2009). Role of plant hormones in plant defence responses. *Plant Molecular Biology,* 69(4), pp. 473-488.

Barzman, M., Barberi, P., Birch, A.N.E., Boonekamp, P., Dachbrodt-Saaydeh, S., Graf, B., Hommel, B., Jensen, J.E., Kiss, J., Kudsk, P., Lamichhane, J.R., Messean, A., Moonen, A.C., Ratnadass, A., Ricci, P., Sarah, J.L. & Sattin, M. (2015). Eight principles of integrated pest management. *Agronomy for Sustainable Development,* 35(4), pp. 1199-1215.

Bates, S.T., Cropsey, G.W.G., Caporaso, J.G., Knight, R. & Fierer, N. (2011). Bacterial Communities Associated with the Lichen Symbiosis. *Applied and Environmental Microbiology,* 77(4), pp. 1309-1314.

Bent, A.F. & Mackey, D. (2007). Elicitors, effectors, and R genes: The new paradigm and a lifetime supply of questions. *Annual Review of Phytopathology,* 45, pp. 399-436.

Berendsen, R.L., Pieterse, C.M. & Bakker, P.A. (2012). The rhizosphere microbiome and plant health. *Trends Plant Sci,* 17(8), pp. 478-86.

Berg, G. (2000). Diversity of antifungal and plant-associated *Serratia plymuthica* strains. *Journal of Applied Microbiology,* 88(6), pp. 952-960.

Berg, G. (2009). Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Appl Microbiol Biotechnol,* 84(1), pp. 11-8.

Berger, S., Sinha, A.K. & Roitsch, T. (2007). Plant physiology meets phytopathology: plant primary metabolism and plant-pathogen interactions. *Journal of Experimental Botany,* 58(15-16), pp. 4019-4026.

Besset-Manzoni, Y., Rieusset, L., Joly, P., Comte, G. & Prigent-Combaret, C. (2018). Exploiting rhizosphere microbial cooperation for developing sustainable agriculture strategies. *Environ Sci Pollut Res Int*.

Bilski, P., Li, M.Y., Ehrenshaft, M., Daub, M.E. & Chignell, C.F. (2000). Vitamin B-6 (pyridoxine) and its derivatives are efficient singlet oxygen quenchers and potential fungal antioxidants. *Photochemistry and Photobiology,* 71(2), pp. 129-134.

Blacutt, A.A., Mitchell, T.R., Bacon, C.W. & Gold, S.E. (2016). *Bacillus mojavensis* RRC101 Lipopeptides Provoke Physiological and Metabolic Changes During Antagonism Against *Fusarium verticillioides*. *Molecular Plant-Microbe Interactions,* 29(9), pp. 713-723.

Blair, D.F. (1995). How Bacteria Sense and Swim. *Annual Review of Microbiology,* 49, pp. 489-522.

Blake, J.J., Spink, J. H. & Bullard, M. J. Successful establishment of oilseed rape. In: *Proceedings of HGCA conference 2004: Managing soil and roots for profitable production.*2004.

Bogino, P.C., Oliva, M.D., Sorroche, F.G. & Giordano, W. (2013). The Role of Bacterial Biofilms and Surface Components in Plant-Bacterial Associations. *International Journal of Molecular Sciences,* 14(8), pp. 15838-15859.

Boller, T. & Felix, G. (2009). A Renaissance of Elicitors: Perception of Microbe-Associated Molecular Patterns and Danger Signals by Pattern-Recognition Receptors. *Annual Review of Plant Biology,* 60, pp. 379-406.

Bolton, M.D. (2009). Primary Metabolism and Plant Defense-Fuel for the Fire. *Molecular Plant-Microbe Interactions,* 22(5), pp. 487-497.

Bowler, C. & Fluhr, R. (2000). The role of calcium and activated oxygens as signals for controlling cross-tolerance. *Trends in Plant Science,* 5(6), pp. 241-246.

Bowman, S.M. & Free, S.J. (2006). The structure and synthesis of the fungal cell wall. *Bioessays,* 28(8), pp. 799-808.

Bressan, M., Roncato, M.A., Bellvert, F., Comte, G., Haichar, F.E., Achouak, W. & Berge, O. (2009). Exogenous glucosinolate produced by *Arabidopsis thaliana* has an impact on microbes in the rhizosphere and plant roots. *Isme Journal,* 3(11), pp. 1243-1257.

Broeckling, C.D., Broz, A.K., Bergelson, J., Manter, D.K. & Vivanco, J.M. (2008). Root exudates regulate soil fungal community composition and diversty. *Applied and Environmental Microbiology,* 74(3), pp. 738-744.

Buckley, D.H., Huangyutitham, V., Hsu, S.F. & Nelson, T.A. (2007). Stable isotope probing with N-15(2) reveals novel noncultivated diazotrophs in soil. *Applied and Environmental Microbiology,* 73(10), pp. 3196-3204.

Bulgarelli, D., Garrido-Oter, R., Munch, P.C., Weiman, A., Droge, J., Pan, Y., McHardy, A.C. & Schulze-Lefert, P. (2015). Structure and Function of the Bacterial Root Microbiota in Wild and Domesticated Barley. *Cell Host & Microbe,* 17(3), pp. 392-403.

Bulgarelli, D., Rott, M., Schlaeppi, K., van Themaat, E.V.L., Ahmadinejad, N., Assenza, F., Rauf, P., Huettel, B., Reinhardt, R., Schmelzer, E., Peplies, J., Gloeckner, F.O., Amann, R., Eickhorst, T. & Schulze-Lefert, P. (2012). Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. *Nature,* 488(7409), pp. 91-95.

Bulgarelli, D., Schlaeppi, K., Spaepen, S., van Themaat, E.V.L. & Schulze-Lefert, P. (2013). Structure and Functions of the Bacterial Microbiota of Plants. *Annual Review of Plant Biology, Vol 64,* 64, pp. 807-838.

Capiati, D.A., Pais, S.M. & Tellez-Inon, M.T. (2006). Wounding increases salt tolerance in tomato plants: evidence on the participation of calmodulin-like activities in cross-tolerance signalling. *Journal of Experimental Botany,* 57(10), pp. 2391-2400.

Caporaso, J.G., Bittinger, K., Bushman, F.D., DeSantis, T.Z., Andersen, G.L. & Knight, R. (2010a). PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics,* 26(2), pp. 266-267.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Tumbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J. & Knight, R. (2010b). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods,* 7(5), pp. 335-336.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N. & Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences of the United States of America,* 108, pp. 4516-4522.

Cartieaux, F., Contesto, C., Gallou, A., Desbrosses, G., Kopka, J., Taconnat, L., Renou, J.P. & Touraine, B. (2008). Simultaneous interaction of *Arabidopsis thaliana* with *Bradyrhizobium* sp strain ORS278 and *Pseudomonas syringae* pv. *tomato* DC3000 leads to complex transcriptome changes. *Molecular Plant-Microbe Interactions,* 21(2), pp. 244-259.

Cartieaux, F., Thibaud, M.C., Zimmerli, L., Lessard, P., Sarrobert, C., David, P., Gerbaud, A., Robaglia, C., Somerville, S. & Nussaume, L. (2003). Transcriptome analysis of *Arabidopsis* colonized by a plant-growth promoting rhizobacterium reveals a general effect on disease resistance. *Plant J,* 36(2), pp. 177-88.

Carvalhais, L.C., Dennis, P.G., Badri, D.V., Kidd, B.N., Vivanco, J.M. & Schenk, P.M. (2015). Linking Jasmonic Acid Signaling, Root Exudates, and Rhizosphere Microbiomes. *Molecular Plant-Microbe Interactions,* 28(9), pp. 1049-1058.

Castrillo, G., Teixeira, P.J.P.L., Paredes, S.H., Law, T.F., de Lorenzo, L., Feltcher, M.E., Finkel, O.M., Breakfield, N.W., Mieczkowski, P., Jones, C.D., Paz-Ares, J. & Dangl, J.L. (2017). Root microbiota drive direct integration of phosphate stress and immunity. *Nature,* 543(7646), pp. 513-+.

Cazorla, F.M., Duckett, S.B., Bergstrom, E.T., Noreen, S., Odijk, R., Lugtenberg, B.J.J., Thomas-Oates, J.E. & Bloemberg, G.V. (2006). Biocontrol of avocado dematophora root rot by antagonistic

Pseudomonas fluorescens PCL1606 correlates with the production of 2-hexyl 5-propyl resorcinol. *Molecular Plant-Microbe Interactions,* 19(4), pp. 418-428.

Chalhoub, B., Denoeud, F., Liu, S., Parkin, I.A., Tang, H., Wang, X., Chiquet, J., Belcram, H., Tong, C., Samans, B., Correa, M., Da Silva, C., Just, J., Falentin, C., Koh, C.S., Le Clainche, I., Bernard, M., Bento, P., Noel, B., Labadie, K., Alberti, A., Charles, M., Arnaud, D., Guo, H., Daviaud, C., Alamery, S., Jabbari, K., Zhao, M., Edger, P.P., Chelaifa, H., Tack, D., Lassalle, G., Mestiri, I., Schnel, N., Le Paslier, M.C., Fan, G., Renault, V., Bayer, P.E., Golicz, A.A., Manoli, S., Lee, T.H., Thi, V.H., Chalabi, S., Hu, Q., Fan, C., Tollenaere, R., Lu, Y., Battail, C., Shen, J., Sidebottom, C.H., Wang, X., Canaguier, A., Chauveau, A., Berard, A., Deniot, G., Guan, M., Liu, Z., Sun, F., Lim, Y.P., Lyons, E., Town, C.D., Bancroft, I., Wang, X., Meng, J., Ma, J., Pires, J.C., King, G.J., Brunel, D., Delourme, R., Renard, M., Aury, J.M., Adams, K.L., Batley, J., Snowdon, R.J., Tost, J., Edwards, D., Zhou, Y., Hua, W., Sharpe, A.G., Paterson, A.H., Guan, C. & Wincker, P. (2014). Plant genetics. Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. *Science,* 345(6199), pp. 950-3.

Chamoun, R. & Jabaji, S. (2011). Expression of genes of *Rhizoctonia solani* and the biocontrol *Stachybotrys elegans* during mycoparasitism of hyphae and sclerotia. *Mycologia,* 103(3), pp. 483-493.

Chamoun, R., Samsatly, J., Pakala, S.B., Cubeta, M.A. & Jabaji, S. (2015). Suppression subtractive hybridization and comparative expression of a pore-forming toxin and glycosyl hydrolase genes in *Rhizoctonia solani* during potato sprout infection. *Mol Genet Genomics,* 290(3), pp. 877-900.

Chen, X.T., Liu, J., Lin, G.F., Wang, A.R., Wang, Z.H. & Lu, G.D. (2013). Overexpression of AtWRKY28 and AtWRKY75 in *Arabidopsis* enhances resistance to oxalic acid and *Sclerotinia sclerotiorum*. *Plant Cell Reports,* 32(10), pp. 1589-1599.

Chen, Y.F., Etheridge, N. & Schaller, G.E. (2005). Ethylene signal transduction. *Ann Bot,* 95(6), pp. 901-15.

Chin-A-Woeng, T.F.C., Bloemberg, G.V., van der Bij, A.J., van der Drift, K.M.G.F., Schripsema, J., Kroon, B., Scheffer, R.J., Keel, C., Bakker, P.A.H.M., Tichy, H.V., de Bruijn, F.J., Thomas-Oates, J.E. & Lugtenberg, B.J.J. (1998). Biocontrol by phenazine-1-carboxamide-producing *Pseudomonas chlororaphis* PCL1391 of tomato root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Molecular Plant-Microbe Interactions,* 11(11), pp. 1069-1077.

Chin-A-Woeng, T.F.C., Bloemberg, G.V., Lugtenberg, B.J.J. (2003). *Mechanisms of biological control of phytopathogenic fungi by Pseudomonas spp.*6). St.Paul, MN: Am. Phytopathol. Soc.

Chisholm, S.T., Coaker, G., Day, B. & Staskawicz, B.J. (2006). Host-microbe interactions: Shaping the evolution of the plant immune response. *Cell,* 124(4), pp. 803-814.

Cohen, J.D. & Bandurski, R.S. (1982). Chemistry and Physiology of the Bound Auxins. *Annual Review of Plant Physiology and Plant Molecular Biology,* 33, pp. 403-430.

Compant, S., Duffy, B., Nowak, J., Clement, C. & Barka, E.A. (2005). Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action, and future prospects. *Applied and Environmental Microbiology,* 71(9), pp. 4951-4959.

Conesa, A., Gotz, S., Garcia-Gomez, J.M., Terol, J., Talon, M. & Robles, M. (2005). Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics,* 21(18), pp. 3674-3676.

Conrath, U., Beckers, G.J.M., Flors, V., Garcia-Agustin, P., Jakab, G., Mauch, F., Newman, M.A., Pieterse, C.M.J., Poinssot, B., Pozo, M.J., Pugin, A., Schaffrath, U., Ton, J., Wendehenne, D., Zimmerli, L. & Mauch-Mani, B. (2006). Priming: Getting ready for battle. *Molecular Plant-Microbe Interactions,* 19(10), pp. 1062-1071.

Conrath, U., Pieterse, C.M.J. & Mauch-Mani, B. (2002). Priming in plant-pathogen interactions. *Trends in Plant Science,* 7(5), pp. 210-216.

Cook, R.J., Thomashow, L.S., Weller, D.M., Fujimoto, D., Mazzola, M., Bangera, G. & Kim, D. (1995). Molecular Mechanisms of Defense by Rhizobacteria against Root Disease. *Proceedings of the National Academy of Sciences of the United States of America,* 92(10), pp. 4197-4201.

Cordovez, V., Carrion, V.J., Etalo, D.W., Mumm, R., Zhu, H., van Wezel, G.P. & Raaijmakers, J.M. (2015). Diversity and functions of volatile organic compounds produced by *Streptomyces* from a disease-suppressive soil. *Frontiers in Microbiology,* 6.

Costa, R., Gotz, M., Mrotzek, N., Lottmann, J., Berg, G. & Smalla, K. (2006). Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds. *Fems Microbiology Ecology,* 56(2), pp. 236-249.

Costerton, J.W., Stewart, P. S. & Greenberg, E. P. (1999). Bacterial biofilms: a common cause of persistent infections. *Science,* 284, pp. 1318-1322.

Crowe, J.D. & Olsson, S. (2001). Induction of laccase activity in *Rhizoctonia solani* by antagonistic *Pseudomonas fluorescens* strains and a range of chemical treatments. *Applied and Environmental Microbiology,* 67(5), pp. 2088-2094.

Cubeta, M.A., Thomas, E., Dean, R.A., Jabaji, S., Neate, S.M., Tavantzis, S., Toda, T., Vilgalys, R., Bharathan, N., Fedorova-Abrams, N., Pakala, S.B., Pakala, S.M., Zafar, N., Joardar, V., Losada, L. & Nierman, W.C. (2014). Draft Genome Sequence of the Plant-Pathogenic Soil Fungus *Rhizoctonia solani* Anastomosis Group 3 Strain Rhs1AP. *Genome Announc,* 2(5).

Cui, H., Tsuda, K. & Parker, J.E. (2015). Effector-triggered immunity: from pathogen perception to robust defense. *Annu Rev Plant Biol,* 66, pp. 487-511.

Daval, S., Lebreton, L., Gazengel, K., Boutin, M., Guillerm-Erckelboudt, A.Y. & Sarniguet, A. (2011). The biocontrol bacterium *Pseudomonas fluorescens* Pf29Arp strain affects the pathogenesis-related gene expression of the take-all fungus *Gaeumannomyces graminis* var. *tritici* on wheat roots. *Molecular Plant Pathology,* 12(9), pp. 839-854.

de Bruijn, I., de Kock, M.J.D., Yang, M., de Waard, P., van Beek, T.A. & Raaijmakers, J.M. (2007). Genome-based discovery, structure prediction and functional analysis of cyclic lipopeptide antibiotics in *Pseudomonas* species. *Molecular Microbiology,* 63(2), pp. 417-428.

De Meyer, G., Capieau, K., Audenaert, K., Buchala, A., Metraux, J.P. & Hofte, M. (1999). Nanogram amounts of salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 activate the systemic acquired resistance pathway in bean. *Molecular Plant-Microbe Interactions,* 12(5), pp. 450-458.

de Torres-Zabala, M., Truman, W., Bennett, M.H., Lafforgue, G., Mansfield, J.W., Egea, P.R., Bogre, L. & Grant, M. (2007). *Pseudomonas syringae* pv. *tomato* hijacks the *Arabidopsis* abscisic acid signalling pathway to cause disease. *Embo Journal,* 26(5), pp. 1434-1443.

De Vos, M., Van Zaanen, W., Koornneef, A., Korzelius, J.P., Dicke, M., Van Loon, L.C. & Pieterse, C.M.J. (2006). Herbivore-induced resistance against microbial pathogens in *Arabidopsis*. *Plant Physiology,* 142(1), pp. 352-363.

De Wit, P.J.G.M. (1997). Pathogen avirulence and plant resistance: a key role for recognition. *Trends i8n Plant Science,* 2, pp. 452-458.

Defago, G. (1993). 2,4-Diacetylphloroglucinol, a Promising Compound in Biocontrol. *Plant Pathology,* 42(3), pp. 311-312.

Dekkers, L.C., Mulders, I.H.M., Phoelich, C.C., Chin-A-Woeng, T.F.C., Wijfjes, A.H.M. & Lugtenberg, B.J.J. (2000). The sss colonization gene of the tomato-*Fusarium oxysporum* f. sp *radicislycopersici* biocontrol strain *Pseudomonas fluorescens* WCS365 can improve root colonization of other wild-type *Pseudomonas* spp. bacteria. *Molecular Plant-Microbe Interactions,* 13(11), pp. 1177-1183.

Denance, N., Sanchez-Vallet, A., Goffner, D. & Molina, A. (2013). Disease resistance or growth: the role of plant hormones in balancing immune responses and fitness costs. *Frontiers in Plant Science,* 4.

Dennis, P.G., Miller, A.J. & Hirsch, P.R. (2010). Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? *Fems Microbiology Ecology,* 72(3), pp. 313-327.

Deveau, A., Barret, M., Diedhiou, A.G., Leveau, J., de Boer, W., Martin, F., Sarniguet, A. & Frey-Klett, P. (2015). Pairwise Transcriptomic Analysis of the Interactions Between the Ectomycorrhizal Fungus *Laccaria bicolor* S238N and Three Beneficial, Neutral and Antagonistic Soil Bacteria. *Microbial Ecology,* 69(1), pp. 146-159.

Dharmasiri, N. & Estelle, M. (2004). Auxin signaling and regulated protein degradation. *Trends in Plant Science,* 9(6), pp. 302-308.

Dias, A.C.F., Dini-Andreote, F., Hannula, S.E., Andreote, F.D., Silva, M.D.P.E., Salles, J.F., de Boer, W., van Veen, J. & van Elsas, J.D. (2013). Different Selective Effects on Rhizosphere Bacteria Exerted by Genetically Modified versus Conventional Potato Lines. *Plos One,* 8(7).

Dodds, P.N. & Rathjen, J.P. (2010). Plant immunity: towards an integrated view of plant-pathogen interactions. *Nature Reviews Genetics,* 11(8), pp. 539-548.

Dong, X.N. (2004). NPR1, all things considered. *Current Opinion in Plant Biology,* 7(5), pp. 547-552.

Dubey, M.K., Broberg, A., Sooriyaarachchi, S., Ubhayasekera, W., Jensen, D.F. & Karlsson, M. (2013). The glyoxylate cycle is involved in pleotropic phenotypes, antagonism and induction of plant defence responses in the fungal biocontrol agent *Trichoderma atroviride*. *Fungal Genetics and Biology,* 58-59, pp. 33-41.

Duffy, B., Keel, C. & Defago, G. (2004). Potential role of pathogen signaling in multitrophic plant-microbe interactions involved in disease protection. *Applied and Environmental Microbiology,* 70(3), pp. 1836-1842.

Dumont, M.G. & Murrell, J.C. (2005). Stable isotope probing - linking microbial identity to function. *Nature Reviews Microbiology,* 3(6), pp. 499-504.

Durrant, W.E. & Dong, X. (2004). Systemic acquired resistance. *Annual Review of Phytopathology,* 42, pp. 185-209.

Dutton, M.V. & Evans, C.S. (1996). Oxalate production by fungi: Its role in pathogenicity and ecology in the soil environment. *Canadian Journal of Microbiology,* 42(9), pp. 881-895.

Edgar, R.C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics,* 26(19), pp. 2460-2461.

Edwards, J., Johnson, C., Santos-Medellin, C., Lurie, E., Podishetty, N.K., Bhatnagar, S., Eisen, J.A. & Sundaresan, V. (2015). Structure, variation, and assembly of the root-associated microbiomes of rice. *Proceedings of the National Academy of Sciences of the United States of America,* 112(8), pp. E911-E920.

Ent, S.V.D.e.a. (2008). MYB72 Is Required in Early Signaling Steps of Rhizobacteria-Induced Systemic Resistance in *Arabidopsis*. *Plant Physiol.,* 146, pp. 1293-1304.

Eulgem, T. & Somssich, I.E. (2007). Networks of WRKY transcription factors in defense signaling. *Current Opinion in Plant Biology,* 10(4), pp. 366-371.

Evangelisti, E., Rey, T. & Schornack, S. (2014). Cross-interference of Plant development and plant-microbe interactions. *Current Opinion in Plant Biology,* 20, pp. 118-126.

Farrow, S.C. & Facchini, P.J. (2014). Functional diversity of 2-oxoglutarate/Fe(II)-dependent dioxygenases in plant metabolism. *Frontiers in Plant Science,* 5.

Ferguson, B.J. & Mathesius, U. (2014). Phytohormone Regulation of Legume-Rhizobia Interactions. *Journal of Chemical Ecology,* 40(7), pp. 770-790.

Fernandez-Calvo, P., Chini, A., Fernandez-Barbero, G., Chico, J.M., Gimenez-Ibanez, S., Geerinck, J., Eeckhout, D., Schweizer, F., Godoy, M., Franco-Zorrilla, J.M., Pauwels, L., Witters, E., Puga, M.I., Paz-Ares, J., Goossens, A., Reymond, P., De Jaeger, G. & Solano, R. (2011). The *Arabidopsis* bHLH Transcription Factors MYC3 and MYC4 Are Targets of JAZ Repressors and Act Additively with MYC2 in the Activation of Jasmonate Responses. *Plant Cell,* 23(2), pp. 701-715.

Ferrari, S., Galletti, R., Denoux, C., De Lorenzo, G., Ausubel, F.M. & Dewdney, J. (2007). Resistance to *Botrytis cinerea* induced in *Arabidopsis* by elicitors is independent of salicylic acid, ethylene, or jasmonate signaling but requires PHYTOALEXIN DEFICIENT3. *Plant Physiology,* 144(1), pp. 367-379.

Ferrari, S., Plotnikova, J.M., De Lorenzo, G. & Ausubel, F.M. (2003). *Arabidopsis* local resistance to *Botrytis cinerea* involves salicylic acid and camalexin and requires EDS4 and PAD2, but not SID2, EDS5 or PAD4. *Plant Journal,* 35(2), pp. 193-205.

Fineran, P.C., Slater, H., Everson, L., Hughes, K. & Salmond, G.P.C. (2005). Biosynthesis of tripyrrole and beta-lactam secondary metabolites in *Serratia*: integration of quorum sensing with multiple new regulatory components in the control of prodigiosin and carbapenem antibiotic production. *Molecular Microbiology,* 56(6), pp. 1495-1517.

Finkel, O.M., Castrillo, G., Paredes, S.H., Gonzalez, I.S. & Dangl, J.L. (2017). Understanding and exploiting plant beneficial microbes. *Current Opinion in Plant Biology,* 38, pp. 155-163.

Foley, R.C., Gleason, C.A., Anderson, J.P., Hamann, T. & Singh, K.B. (2013). Genetic and Genomic Analysis of *Rhizoctonia solani* Interactions with *Arabidopsis*; Evidence of Resistance Mediated through NADPH Oxidases. *Plos One,* 8(2).

Foley, R.C., Kidd, B.N., Hane, J.K., Anderson, J.P. & Singh, K.B. (2016). Reactive Oxygen Species Play a Role in the Infection of the Necrotrophic Fungi, *Rhizoctonia solani* in Wheat. *Plos One,* 11(3).

Forsthoefel, N.R., Klag, K.A., Simeles, B.P., Reiter, R., Brougham, L. & Vernon, D.M. (2013). The *Arabidopsis* Plant Intracellular Ras-group LRR (PIRL) Family and the Value of Reverse Genetic Analysis for Identifying Genes that Function in Gametophyte Development. *Plants (Basel),* 2(3), pp. 507-20.

Frankowski, J., Lorito, M., Scala, F., Schmid, R., Berg, G. & Bahl, H. (2001). Purification and properties of two chitinolytic enzymes of *Serratia plymuthica* HRO-C48. *Archives of Microbiology,* 176(6), pp. 421-426.

Frost, C.J., Mescher, M.C., Carlson, J.E. & De Moraes, C.M. (2008). Plant defense priming against herbivores: Getting ready for a different battle. *Plant Physiology,* 146(3), pp. 818-824.

Fu, S.F., Wei, J.Y., Chen, H.W., Liu, Y.Y., Lu, H.Y. & Chou, J.Y. (2015). Indole-3-acetic acid: A widespread physiological code in interactions of fungi with other organisms. *Plant Signaling & Behavior,* 10(8).

Fu, Z.Q., Yan, S.P., Saleh, A., Wang, W., Ruble, J., Oka, N., Mohan, R., Spoel, S.H., Tada, Y., Zheng, N. & Dong, X.N. (2012). NPR3 and NPR4 are receptors for the immune signal salicylic acid in plants. *Nature,* 486(7402), pp. 228-+.

Furukawa, T.J., Koga, T., Adachi, K. K. & Syomo, K. (1996). Efficient conversion of L-tryptophan to indole-3-acetic acid and/or tryptophol by some species of *Rhizoctonia*. *Plant and Cell Physiology,* 37, pp. 899-905.

Gan, S.S. & Amasino, R.M. (1995). Inhibition of Leaf Senescence by Autoregulated Production of Cytokinin. *Science,* 270(5244), pp. 1986-1988.

Gang, W., Kloepper, J.W. & Tuzun, S. (1991). Induction of Systemic Resistance of Cucumber to *Colletotrichum-Orbiculare* by Select Strains of Plant Growth-Promoting Rhizobacteria. *Phytopathology,* 81(12), pp. 1508-1512.

Garbeva, P., Silby, M.W., Raaijmakers, J.M., Levy, S.B. & Boer, W. (2011). Transcriptional and antagonistic responses of *Pseudomonas fluorescens* Pf0-1 to phylogenetically different bacterial competitors. *ISME J,* 5(6), pp. 973-85.

Gfeller, A., Dubugnon, L., Liechti, R. & Farmer, E.E. (2010). Jasmonate Biochemical Pathway. *Science Signaling,* 3(109).

Giron, D., Frago, E., Glevarec, G., Pieterse, C.M.J. & Dicke, M. (2013). Cytokinins as key regulators in plant-microbe-insect interactions: connecting plant growth and defence. *Functional Ecology,* 27(3), pp. 599-609.

Gkarmiri, K., Finlay, R.D., Alstrom, S., Thomas, E., Cubeta, M.A. & Hogberg, N. (2015). Transcriptomic changes in the plant pathogenic fungus *Rhizoctonia solani* AG-3 in response to the antagonistic bacteria *Serratia proteamaculans* and *Serratia plymuthica*. *Bmc Genomics,* 16.

Gkarmiri, K., Mahmood, S., Ekblad, A., Alstrom, S., Hogberg, N. & Finlay, R. (2017). Identifying the Active Microbiome Associated with Roots and Rhizosphere Soil of Oilseed Rape. *Applied and Environmental Microbiology,* 83(22).

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology,* 43, pp. 205-227.

Glazebrook, J., Chen, W.J., Estes, B., Chang, H.S., Nawrath, C., Metraux, J.P., Zhu, T. & Katagiri, F. (2003). Topology of the network integrating salicylate and jasmonate signal transduction derived from global expression phenotyping. *Plant Journal,* 34(2), pp. 217-228.

Glick, B.R. (2012). Plant growth-promoting bacteria: mechanisms and applications. *Scientifica (Cairo),* 2012, p. 963401.

Glick, B.R., Cheng, Z., Czarny, J. & Duan, J. (2007). Promotion of plant growth by ACC deaminase-producing soil bacteria. *European Journal of Plant Pathology,* 119(3), pp. 329-339.

Grimont, P.A. & Grimont, F. (1978). The genus *Serratia*. *Annual Review of Microbiology,* 32, pp. 221-48.

Gschwendtner, S., Esperschuetz, J., Buegger, F., Reichmann, M., Muller, M., Munch, J.C. & Schloter, M. (2011). Effects of genetically modified starch metabolism in potato plants on photosynthate fluxes into the rhizosphere and on microbial degraders of root exudates. *Fems Microbiology Ecology,* 76(3), pp. 564-575.

Haas, D. & Defago, G. (2005). Biological control of soil-borne pathogens by fluorescent *pseudomonads*. *Nature Reviews Microbiology,* 3(4), pp. 307-319.

Haas, D. & Keel, C. (2003). Regulation of antibiotic production in root-colonizing *Pseudomonas* spp. and relevance for biological control of plant disease. *Annual Review of Phytopathology,* 41, pp. 117-153.

Haichar, F.E., Marol, C., Berge, O., Rangel-Castro, J.I., Prosser, J.I., Balesdent, J., Heulin, T. & Achouak, W. (2008). Plant host habitat and root exudates shape soil bacterial community structure. *Isme Journal,* 2(12), pp. 1221-1230.

Haichar, F.E., Roncato, M.A. & Achouak, W. (2012). Stable isotope probing of bacterial community structure and gene expression in the rhizosphere of *Arabidopsis* thaliana. *Fems Microbiology Ecology,* 81(2), pp. 291-302.

Halkier, B.A. & Gershenzon, J. (2006). Biology and biochemistry of glucosinolates. *Annual Review of Plant Biology,* 57, pp. 303-333.

Hammer, O., Harper, D. & Ryan, P. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontol Electronica,* 4, pp. 1-9.

Hammer, P.E., Hill, D.S., Lam, S.T., VanPee, K.H. & Ligon, J.M. (1997). Four genes from *Pseudomonas fluorescens* that encode the biosynthesis of pyrrolnitrin. *Applied and Environmental Microbiology,* 63(6), pp. 2147-2154.

Han, S.H., Lee, S.J., Moon, J.H., Park, K.H., Yang, K.Y., Cho, B.H., Kim, K.Y., Kim, Y.W., Lee, M.C., Anderson, A.J. & Kim, Y.C. (2006). GacS-dependent production of 2R, 3R-butanediol by *Pseudomonas chlororaphis* O6 is a major determinant for eliciting systemic resistance against *Erwinia carotovora* but not against *Pseudomonas syringae* pv. *tabaci* in tobacco. *Mol Plant Microbe Interact,* 19(8), pp. 924-30.

Hane, J.K., Anderson, J.P., Williams, A.H., Sperschneider, J. & Singh, K.B. (2014). Genome Sequencing and Comparative Genomics of the Broad Host-Range Pathogen *Rhizoctonia solani* AG8. *Plos Genetics,* 10(5).

Haney, C.H., Samuel, B.S., Bush, J. & Ausubel, F.M. (2015). Associations with rhizosphere bacteria can confer an adaptive advantage to plants. *Nature Plants,* 1(6), pp. 1-9.

Hannula, S.E., Boschker, H.T., de Boer, W. & van Veen, J.A. (2012). 13C pulse-labeling assessment of the community structure of active fungi in the rhizosphere of a genetically starch-modified potato (*Solanum tuberosum*) cultivar and its parental isoline. *New Phytol,* 194(3), pp. 784-99.

Hannula, S.E., de Boer, W. & van Veen, J.A. (2010). In situ dynamics of soil fungal communities under different genotypes of potato, including a genetically modified cultivar. *Soil Biology & Biochemistry,* 42(12), pp. 2211-2223.

Hartman, K., van der Heijden, M.G.A., Wittwer, R.A., Banerjee, S., Walser, J.C. & Schlaeppi, K. (2018). Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. *Microbiome,* 6(1), p. 14.

Hase, S., Takahashi, S., Takenaka, S., Nakaho, K., Arie, T., Seo, S., Ohashi, Y. & Takahashi, H. (2008). Involvement of jasmonic acid signalling in bacterial wilt disease resistance induced by biocontrol agent *Pythium oligandrum* in tomato. *Plant Pathology,* 57(5), pp. 870-876.

Hawes, M.C., Gunawardena, U., Miyasaka, S. & Zhao, X.W. (2000). The role of root border cells in plant defense. *Trends in Plant Science,* 5(3), pp. 128-133.

Heese, A., Hann, D.R., Gimenez-Ibanez, S., Jones, A.M.E., He, K., Li, J., Schroeder, J.I., Peck, S.C. & Rathjen, J.P. (2007). The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. *Proceedings of the National Academy of Sciences of the United States of America,* 104(29), pp. 12217-12222.

Heil, M. & Bostock, R.M. (2002). Induced systemic resistance (ISR) against pathogens in the context of induced plant defences. *Annals of Botany,* 89(5), pp. 503-512.

Hennessy, R.C., Glaring, M.A., Olsson, S. & Stougaard, P. (2017). Transcriptomic profiling of microbe-microbe interactions reveals the specific response of the biocontrol strain *P. fluorescens* In5 to the phytopathogen *Rhizoctonia solani*. *BMC Res Notes,* 10(1), p. 376.

Hernandez-Blanco, C., Feng, D.X., Hu, J., Sanchez-Vallet, A., Deslandes, L., Llorente, F., Berrocal-Lobo, M., Keller, H., Barlet, X., Sanchez-Rodriguez, C., Anderson, L.K., Somerville, S., Marco, Y. & Molina, A. (2007). Impairment of cellulose synthases required for *Arabidopsis* secondary cell wall formation enhances disease resistance. *Plant Cell,* 19(3), pp. 890-903.

Hinsinger, P. & Marschner, P. (2006). Rhizosphere - perspectives and challenges - a tribute to Lorenz Hiltner 12-17 September 2004 - Munich, Germany. *Plant and Soil,* 283(1-2), pp. Vii-Viii.

Hol, W.H.G., Garbeva, P., Hordijk, C., Hundscheid, M.P.J., Gunnewiek, P.J.A.K., van Agtmaal, M., Kuramae, E.E. & de Boer, W. (2015). Non-random species loss in bacterial communities reduces antifungal volatile production. *Ecology,* 96(8), pp. 2042-2048.

Houlden, A., Timms-Wilson, T.M., Day, M.J. & Bailey, M.J. (2008). Influence of plant developmental stage on microbial community structure and activity in the rhizosphere of three field crops. *Fems Microbiology Ecology,* 65(2), pp. 193-201.

Huot, B., Yao, J., Montgomery, B.L. & He, S.Y. (2014). Growth-Defense Tradeoffs in Plants: A Balancing Act to Optimize Fitness. *Molecular Plant,* 7(8), pp. 1267-1287.

Ihrmark, K., Bodeker, I.T.M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandstrom-Durling, M., Clemmensen, K.E. & Lindahl, B.D. (2012). New primers to amplify the fungal ITS2 region - evaluation by 454-sequencing of artificial and natural communities. *Fems Microbiology Ecology,* 82(3), pp. 666-677.

Imperiali, N., Chiriboga, X., Schlaeppi, K., Fesselet, M., Villacres, D., Jaffuel, G., Bender, S.F., Dennert, F., Blanco-Perez, R., van der Heijden, M.G.A., Maurhofer, M., Mascher, F., Turlings, T.C.J., Keel, C.J. & Campos-Herrera, R. (2017). Combined Field Inoculations of *Pseudomonas* Bacteria, Arbuscular Mycorrhizal Fungi, and Entomopathogenic Nematodes and their Effects on Wheat Performance. *Frontiers in Plant Science,* 8.

Inbar, J. & Chet, I. (1991). Evidence That Chitinase Produced by *Aeromonas-Caviae* Is Involved in the Biological-Control of Soil-Borne Plant-Pathogens by This Bacterium. *Soil Biology & Biochemistry,* 23(10), pp. 973-978.

Inceoglu, O., Abu Al-Soud, W., Salles, J.F., Semenov, A.V. & van Elsas, J.D. (2011). Comparative Analysis of Bacterial Communities in a Potato Field as Determined by Pyrosequencing. *Plos One,* 6(8).

Ipcho, S., Sundelin, T., Erbs, G., Kistler, H.C., Newman, M.A. & Olsson, S. (2016). Fungal Innate Immunity Induced by Bacterial Microbe-Associated Molecular Patterns (MAMPs). *G3-Genes Genomes Genetics,* 6(6), pp. 1585-1595.

Janda, M., Matouskova, J., Burketova, L. & Valentova, O. (2014). Interconnection between actin cytoskeleton and plant defense signaling. *Plant Signal Behav,* 9(11), p. e976486.

Jones, D.L., Hodge, A. & Kuzyakov, Y. (2004). Plant and mycorrhizal regulation of rhizodeposition. *New Phytologist,* 163(3), pp. 459-480.

Jones, D.L., Nguyen, C. & Finlay, R.D. (2009). Carbon flow in the rhizosphere: carbon trading at the soil-root interface. *Plant and Soil,* 321(1-2), pp. 5-33.

Jones, J.D.G. & Dangl, J.L. (2006). The plant immune system. *Nature,* 444(7117), pp. 323-329.

Joshi, R.K., Megha, S., Rahman, M.H., Basu, U. & Kav, N.N.V. (2016). A global study of transcriptome dynamics in canola (*Brassica napus* L.) responsive to *Sclerotinia sclerotiorum* infection using RNA-Seq. *Gene,* 590(1), pp. 57-67.

Kai, M., Effmert, U., Berg, G. & Piechulla, B. (2007). Volatiles of bacterial antagonists inhibit mycelial growth of the plant pathogen *Rhizoctonia solani*. *Arch Microbiol,* 187(5), pp. 351-60.

Kalbe, C., Marten, P. & Berg, G. (1996). Strains of the genus *Serratia* as beneficial rhizobacteria of oilseed rape with antifungal properties. *Microbiological Research,* 151(4), pp. 433-439.

Kamensky, M., Ovadis, M., Chet, I. & Chernin, L. (2003). Soil-borne strain IC14 of *Serratia plymuthica* with multiple mechanisms of antifungal activity provides biocontrol of *Botrytis cinerea* and *Sclerotinia sclerotiorum* diseases. *Soil Biology & Biochemistry,* 35(2), pp. 323-331.

Kamilova, F., Lamers, G. & Lugtenberg, B. (2008). Biocontrol strain *Pseudomonas fluorescens* WCS365 inhibits germination of *Fusarium oxysporum* spores in tomato root exudate as well as subsequent formation of new spores. *Environmental Microbiology,* 10(9), pp. 2455-2461.

Kamilova, F., Validov, S., Azarova, T., Mulders, I. & Lugtenberg, B. (2005). Enrichment for enhanced competitive plant root tip colonizers selects for a new class of biocontrol bacteria. *Environmental Microbiology,* 7(11), pp. 1809-1817.

Kamou, N.N., Dubey, M., Tzelepis, G., Menexes, G., Papadakis, E.N., Karlsson, M., Lagopodi, A.L. & Jensen, D.F. (2016). Investigating the compatibility of the biocontrol agent *Clonostachys rosea* IK726 with prodigiosin-producing *Serratia rubidaea* S55 and phenazine-producing *Pseudomonas chlororaphis* ToZa7. *Archives of Microbiology,* 198(4), pp. 369-377.

Kanini, G.S., Katsifas, E.A., Savvides, A.L. & Karagouni, A.D. (2013). *Streptomyces rochei* ACTA1551, an Indigenous Greek Isolate Studied as a Potential Biocontrol Agent against *Fusarium oxysporum* f.sp *lycopersici*. *Biomed Research International*.

Kasim, W.A., Gaafar, R. M., Abou-Ali, R., Omar, M. N. & Hewait, H. M. (2016). Effect of biofilm forming plant growth promoting rhizobacteria on salinity tolerance in barley. *Annals of Agricultural Sciences,* 61(2), pp. 217-227.

Katagiri, F. & Tsuda, K. (2010). Understanding the Plant Immune System. *Molecular Plant-Microbe Interactions,* 23(12), pp. 1531-1536.

Katsir, L., Chung, H.S., Koo, A.J.K. & Howe, G.A. (2008). Jasmonate signaling: a conserved mechanism of hormone sensing. *Current Opinion in Plant Biology,* 11(4), pp. 428-435.

Kaur, R., Macleod, J., Foley, W. & Nayudu, M. (2006). Gluconic acid: An antifungal agent produced by *Pseudomonas* species in biological control of take-all. *Phytochemistry,* 67(6), pp. 595-604.

Kazan, K. & Manners, J.M. (2013). MYC2: the master in action. *Molecular Plant,* 6(3), pp. 686-703.
Kemmerling, B., Schwedt, A., Rodriguez, P., Mazzotta, S., Frank, M., Abu Qamar, S., Mengiste, T., Betsuyaku, S., Parker, J.E., Mussig, C., Thomma, B.P.H.J., Albrecht, C., de Vries, S.C., Hirt, H. & Nurnberger, T. (2007). The BRI1-associated kinase 1, BAK1, has a Brassinolindependent role in plant cell-death control. *Current Biology,* 17(13), pp. 1116-1122.
Kendrick, M.D. & Chang, C. (2008). Ethylene signaling: new levels of complexity and regulation. *Current Opinion in Plant Biology,* 11(5), pp. 479-485.
Kersters, K., De Vos, P., Gillis, M., Swings, J., Vandamme, P. Stackebrandt, E. (2006). Introduction to the Proteobacteria. In: Falkow, S., Shleifer, K. H., Rosenberg, E., Stackebrandt, E. (ed. *Prokaryotes: a handbook on the biology of bacteria*. 3rd. ed6). Berlin, Germany: Springer-Verlag, pp. 3-37.
Khangura, R.K., Barbetti, M.J. & Sweetingham, M.W. (1999). Characterization and pathogenicity of *Rhizoctonia* species on canola. *Plant Disease,* 83(8), pp. 714-721.
Kinkel, L.L., Bakker, M.G. & Schlatter, D.C. (2011). A coevolutionary framework for managing disease-suppressive soils. *Annu Rev Phytopathol,* 49, pp. 47-67.
Kissoudis, C., Chowdhury, R., van Heusden, S., van de Wiel, C., Finkers, R., Visser, R.G.F., Bai, Y.L. & van der Linden, G. (2015). Combined biotic and abiotic stress resistance in tomato. *Euphytica,* 202(2), pp. 317-332.
Kliebenstein, D.J., Rowe, H.C. & Denby, K.J. (2005). Secondary metabolites influence *Arabidopsis*/*Botrytis* interactions: variation in host production and pathogen sensitivity. *Plant Journal,* 44(1), pp. 25-36.
Kline, K.A., Falker, S., Dahlberg, S., Normark, S. & Henriques-Normark, B. (2009). Bacterial Adhesins in Host-Microbe Interactions. *Cell Host & Microbe,* 5(6), pp. 580-592.
Kolton, M., Green, S.J., Harel, Y.M., Sela, N., Elad, Y. & Cytryn, E. (2012). Draft Genome Sequence of *Flavobacterium* sp Strain F52, Isolated from the Rhizosphere of Bell Pepper (Capsicum annuum L. cv. Maccabi). *Journal of Bacteriology,* 194(19), pp. 5462-5463.
Koornneef, A., Leon-Reyes, A., Ritsema, T., Verhage, A., Den Otter, F.C., Van Loon, L.C. & Pieterse, C.M.J. (2008). Kinetics of salicylate-mediated suppression of jasmonate signaling reveal a role for redox modulation. *Plant Physiology,* 147(3), pp. 1358-1368.
Kuc, J. (1982). Induced Immunity to Plant-Disease. *Bioscience,* 32(11), pp. 854-860.
Kunkel, B.N. & Brooks, D.M. (2002). Cross talk between signaling pathways in pathogen defense. *Current Opinion in Plant Biology,* 5(4), pp. 325-331.
Kuzyakov, Y. & Domanski, G. (2000). Carbon input by plants into the soil. Review. *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde,* 163(4), pp. 421-431.
Lahlali, R.P., G. (2014). Suppression of clubroot by *Clonostachys rosea* via antibiosis and induced host resistance. *Plant Pathology,* 63, pp. 447-455.
Lakshmanan, V., Castaneda, R., Rudrappa, T. & Bais, H.P. (2013). Root transcriptome analysis of *Arabidopsis thaliana* exposed to beneficial *Bacillus subtilis* FB17 rhizobacteria revealed genes for bacterial recruitment and plant defense independent of malate efflux. *Planta,* 238(4), pp. 657-668.
Lambrix, V., Reichelt, M., Mitchell-Olds, T., Kliebenstein, D.J. & Gershenzon, J. (2001). The *Arabidopsis* epithiospecifier protein promotes the hydrolysis of glucosinolates to nitriles and influences *Trichoplusia* ni herbivory. *Plant Cell,* 13(12), pp. 2793-807.
Laur, J., Ramakrishnan, G.B., Labbe, C., Lefebvre, F., Spanu, P.D. & Belanger, R.R. (2018). Effectors involved in fungal-fungal interaction lead to a rare phenomenon of hyperbiotrophy in the tritrophic system biocontrol agent-powdery mildew-plant. *New Phytol,* 217(2), pp. 713-725.
Lawton, K.A., Potter, S.L., Uknes, S. & Ryals, J. (1994). Acquired-Resistance Signal-Transduction in *Arabidopsis* Is Ethylene Independent. *Plant Cell,* 6(5), pp. 581-588.
Lebeis, S.L., Paredes, S.H., Lundberg, D.S., Breakfield, N., Gehring, J., McDonald, M., Malfatti, S., del Rio, T.G., Jones, C.D., Tringe, S.G. & Dangl, J.L. (2015). Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science,* 349(6250), pp. 860-864.
Lehr, N.A., Schrey, S.D., Hampp, R. & Tarkka, M.T. (2008). Root inoculation with a forest soil *streptomycete* leads to locally and systemically increased resistance against phytopathogens in Norway spruce. *New Phytologist,* 177(4), pp. 965-976.
Leon-Reyes, A., Spoel, S.H., De Lange, E.S., Abe, H., Kobayashi, M., Tsuda, S., Millenaar, F.F., Welschen, R.A.M., Ritsema, T. & Pieterse, C.M.J. (2009). Ethylene Modulates the Role of

NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1 in Cross Talk between Salicylate and Jasmonate Signaling. *Plant Physiology,* 149(4), pp. 1797-1809.

Liang, W.W., Yang, B., Yu, B.J., Zhou, Z.L., Li, C., Jia, M., Sun, Y., Zhang, Y., Wu, F.F., Zhang, H.F., Wang, B.Y., Deyholos, M.K. & Jiang, Y.Q. (2013). Identification and analysis of MKK and MPK gene families in canola (*Brassica napus* L.). *Bmc Genomics,* 14.

Lillis, L., Doyle, E. & Clipson, N. (2009). Comparison of DNA- and RNA-based bacterial community structures in soil exposed to 2,4-dichlorophenol. *Journal of Applied Microbiology,* 107(6), pp. 1883-1893.

Livak, K.J. & Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(T)(-Delta Delta C) method. *Methods,* 25(4), pp. 402-408.

Llorente, F., Muskett, P., Sanchez-Vallet, A., Lopez, G., Ramos, B., Sanchez-Rodriguez, C., Jorda, L., Parker, J. & Molina, A. (2008). Repression of the auxin response pathway increases *Arabidopsis* susceptibility to necrotrophic fungi. *Molecular Plant,* 1(3), pp. 496-509.

Loake, G. & Grant, M. (2007). Salicylic acid in plant defence-the players and protagonists. *Current Opinion in Plant Biology,* 10(5), pp. 466-472.

Loper, J.E. & Henkels, M.D. (1999). Utilization of heterologous siderophores enhances levels of iron available to *Pseudomonas putida* in the rhizosphere. *Applied and Environmental Microbiology,* 65(12), pp. 5357-5363.

Lorenzo, O., Chico, J.M., Sanchez-Serrano, J.J. & Solano, R. (2004). Jasmonate-insensitive1 encodes a MYC transcription factor essential to discriminate between different jasmonate-regulated defense responses in *Arabidopsis*. *Plant Cell,* 16(7), pp. 1938-1950.

Lorenzo, O., Piqueras, R., Sanchez-Serrano, J.J. & Solano, R. (2003). ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense. *Plant Cell,* 15(1), pp. 165-178.

Lu, W.D., D. (2013). The coevolutionary arms race between pathogen and plant host. *journal of undergraduate Life Sciences,* 7, pp. 73-76.

Lueders, T., Manefield, M. & Friedrich, M.W. (2004). Enhanced sensitivity of DNA- and rRNA-based stable isotope probing by fractionation and quantitative analysis of isopycnic centrifugation gradients. *Environmental Microbiology,* 6(1), pp. 73-78.

Lugtenberg, B. & Kamilova, F. (2009). Plant-Growth-Promoting Rhizobacteria. *Annual Review of Microbiology,* 63, pp. 541-556.

Lugtenberg, B.J., Chin, A.W.T.F. & Bloemberg, G.V. (2002). Microbe-plant interactions: principles and mechanisms. *Antonie Van Leeuwenhoek,* 81(1-4), pp. 373-83.

Lundberg, D.S., Lebeis, S.L., Paredes, S.H., Yourstone, S., Gehring, J., Malfatti, S., Tremblay, J., Engelbrektson, A., Kunin, V., del Rio, T.G., Edgar, R.C., Eickhorst, T., Ley, R.E., Hugenholtz, P., Tringe, S.G. & Dangl, J.L. (2012). Defining the core *Arabidopsis thaliana* root microbiome. *Nature,* 488(7409), pp. 86-+.

Makela, M., Galkin, S., Hatakka, A. & Lundell, T. (2002). Production of organic acids and oxalate decarboxylase in lignin-degrading white rot fungi. *Enzyme and Microbial Technology,* 30(4), pp. 542-549.

Manefield, M., Whiteley, A.S., Griffiths, R.I. & Bailey, M.J. (2002a). RNA stable isotope probing, a novel means of linking microbial community function to Phylogeny. *Applied and Environmental Microbiology,* 68(11), pp. 5367-5373.

Manefield, M., Whiteley, A.S., Ostle, N., Ineson, P. & Bailey, M.J. (2002b). Technical considerations for RNA-based stable isotope probing: an approach to associating microbial diversity with microbial community function. *Rapid Communications in Mass Spectrometry,* 16(23), pp. 2179-2183.

Mantri, N.L., Ford, R., Coram, T.E. & Pang, E.C.K. (2010). Evidence of unique and shared responses to major biotic and abiotic stresses in chickpea. *Environmental and Experimental Botany,* 69(3), pp. 286-292.

Marek-Kozaczuk, M. & Skorupska, A. (2001). Production of B-group vitamins by plant growth-promoting *Pseudomonas fluorescens* strain 267 and the importance of vitamins in the colonization and nodulation of red clover. *Biology and Fertility of Soils,* 33(2), pp. 146-151.

Martinez-Medina, A., Appels, F.V.W. & van Wees, S.C.M. (2017). Impact of salicylic acid- and jasmonic acid-regulated defences on root colonization by *Trichoderma harzianum* T-78. *Plant Signal Behav,* 12(8), p. e1345404.

Massa, A.N., Childs, K.L. & Buell, C.R. (2013). Abiotic and Biotic Stress Responses in *Solanum tuberosum* Group Phureja DM1-3 516 R44 as Measured through Whole Transcriptome Sequencing. *Plant Genome,* 6(3).

Mathioni, S.M., Patel, N., Riddick, B., Sweigard, J.A., Czymmek, K.J., Caplan, J.L., Kunjeti, S.G., Kunjeti, S., Raman, V., Hillman, B.I., Kobayashi, D.Y. & Donofrio, N.M. (2013). Transcriptomics of the Rice Blast Fungus *Magnaporthe oryzae* in Response to the Bacterial Antagonist *Lysobacter enzymogenes* Reveals Candidate Fungal Defense Response Genes. *Plos One,* 8(10).

Matilla, M.A., Ramos, J.L., Bakker, P.A.H.M., Doornbos, R., Badri, D.V., Vivanco, J.M. & Ramos-Gonzalez, M.I. (2010). *Pseudomonas putida* KT2440 causes induced systemic resistance and changes in *Arabidopsis* root exudation. *Environmental Microbiology Reports,* 2(3), pp. 381-388.

Matsushika, A., Makino, S., Kojima, M. & Mizuno, T. (2000). Circadian waves of expression of the APRR1/TOC1 family of pseudo-response regulators in *Arabidopsis thaliana*: Insight into the plant circadian clock. *Plant and Cell Physiology,* 41(9), pp. 1002-1012.

Mauch-Mani, B. & Mauch, F. (2005). The role of abscisic acid in plant-pathogen interactions. *Current Opinion in Plant Biology,* 8(4), pp. 409-414.

Maurhofer, M., Reimmann, C., Schmidli-Sacherer, P., Heeb, S., Haas, D. & Defago, G. (1998). Salicylic acid biosynthetic genes expressed in *Pseudomonas fluorescens* strain P3 improve the induction of systemic resistance in tobacco against tobacco necrosis virus. *Phytopathology,* 88(7), pp. 678-684.

Mavrodi, D.V., Blankenfeldt, W. & Thomashow, L.S. (2006). Phenazine compounds in fluorescent *Pseudomonas* spp. Biosynthesis and regulation. *Annual Review of Phytopathology,* 44, pp. 417-445.

Meier, I.C., Avis, P.G. & Phillips, R.P. (2013). Fungal communities influence root exudation rates in pine seedlings. *Fems Microbiology Ecology,* 83(3), pp. 585-595.

Mela, F., Fritsche, K., de Boer, W., van Veen, J.A., de Graaff, L.H., van den Berg, M. & Leveau, J.H.J. (2011). Dual transcriptional profiling of a bacterial/fungal confrontation: *Collimonas fungivorans* versus *Aspergillus niger*. *Isme Journal,* 5(9), pp. 1494-1504.

Mendes, R., Garbeva, P. & Raaijmakers, J.M. (2013). The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *Fems Microbiology Reviews,* 37(5), pp. 634-663.

Mendes, R., Kruijt, M., de Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J.H., Piceno, Y.M., DeSantis, T.Z., Andersen, G.L., Bakker, P.A. & Raaijmakers, J.M. (2011). Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science,* 332(6033), pp. 1097-100.

Micales, J.A. (1997). Localization and induction of oxalate decarboxylase in the brown-rot wood decay fungus *Postia placenta*. *International Biodeterioration & Biodegradation,* 39(2-3), pp. 125-132.

Millet, Y.A., Danna, C.H., Clay, N.K., Songnuan, W., Simon, M.D., Werck-Reichhart, D. & Ausubel, F.M. (2010). Innate Immune Responses Activated in *Arabidopsis* Roots by Microbe-Associated Molecular Patterns. *Plant Cell,* 22(3), pp. 973-990.

Mishina, T.E. & Zeier, J. (2007). Pathogen-associated molecular pattern recognition rather than development of tissue necrosis contributes to bacterial induction of systemic acquired resistance in *Arabidopsis*. *Plant Journal,* 50(3), pp. 500-513.

Mohr, P.G. & Cahill, D.M. (2007). Suppression by ABA of salicylic acid and lignin accumulation and the expression of multiple genes, in *Arabidopsis* infected with *Pseudomonas syringae* pv. tomato. *Functional & Integrative Genomics,* 7(3), pp. 181-191.

Moriya, Y., Itoh, M., Okuda, S., Yoshizawa, A.C. & Kanehisa, M. (2007). KAAS: an automatic genome annotation and pathway reconstruction server. *Nucleic Acids Research,* 35, pp. W182-W185.

Mou, Z., Fan, W.H. & Dong, X.N. (2003). Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. *Cell,* 113(7), pp. 935-944.

Mozafar, A. & Oertli, J.J. (1992). Uptake of a Microbially-Produced Vitamin-B12 by Soybean Roots. *Plant and Soil,* 139(1), pp. 23-30.

Muller, H., Westendorf, C., Leitner, E., Chernin, L., Riedel, K., Schmidt, S., Eberl, L. & Berg, G. (2009). Quorum-sensing effects in the antagonistic rhizosphere bacterium *Serratia plymuthica* HRO-C48. *Fems Microbiology Ecology,* 67(3), pp. 468-478.

Muller, M. & Munne-Bosch, S. (2015). Ethylene Response Factors: A Key Regulatory Hub in Hormone and Stress Signaling. *Plant Physiology,* 169(1), pp. 32-41.

Mur, L.A.J., Kenton, P., Atzorn, R., Miersch, O. & Wasternack, C. (2006). The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include

synergy, antagonism, and oxidative stress leading to cell death. *Plant Physiology,* 140(1), pp. 249-262.

Nagarajkumara, M., Jayaraj, J., Muthukrishnan, S., Bhaskaran, R. & Velazhahan, R. (2005). Detoxification of oxalic acid by *Pseudomonas fluorescens* strain PfMDU2: Implications for the biological control of rice sheath blight caused by *Rhizoctonia solani*. *Microbiological Research,* 160(3), pp. 291-298.

Nakashita, H., Yasuda, M., Nitta, T., Asami, T., Fujioka, S., Arai, Y., Sekimata, K., Takatsuto, S., Yamaguchi, I. & Yoshida, S. (2003). Brassinosteroid functions in a broad range of disease resistance in tobacco and rice. *Plant Journal,* 33(5), pp. 887-898.

Nallanchakravarthula, S., Mahmood, S., Alstrom, S. & Finlay, R.D. (2014). Influence of Soil Type, Cultivar and *Verticillium dahliae* on the Structure of the Root and Rhizosphere Soil Fungal Microbiome of Strawberry. *Plos One,* 9(10).

Naoumkina, M.A., Zhao, Q.A., Gallego-Giraldo, L., Dai, X.B., Zhao, P.X. & Dixon, R.A. (2010). Genome-wide analysis of phenylpropanoid defence pathways. *Molecular Plant Pathology,* 11(6), pp. 829-846.

Navarro, L., Bari, R., Achard, P., Lison, P., Nemri, A., Harberd, N.P. & Jones, J.D.G. (2008). DELLAs control plant immune responses by modulating the balance and salicylic acid signaling. *Current Biology,* 18(9), pp. 650-655.

Navarro, L., Dunoyer, P., Jay, F., Arnold, B., Dharmasiri, N., Estelle, M., Voinnet, O. & Jones, J.D.G. (2006). A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. *Science,* 312(5772), pp. 436-439.

Nejat, N. & Mantri, N. (2017). Plant Immune System: Crosstalk Between Responses to Biotic and Abiotic Stresses the Missing Link in Understanding Plant Defence. *Curr Issues Mol Biol,* 23, pp. 1-16.

Neupane, S. (2013). *Genomics and Transcriptomics of Plant Beneficial Serratia spp.* Diss. Uppsala, Sweden: Swedish University of Agricultural Sciences.

Neupane, S., Andersson, B., Hogberg, N., Ihrmark, K. & Alstrom, S. (2013a). Fungal communities associated with field grown oilseed rape (*Brassica napus* L.) - their possible role in early crop establishment. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science,* 63(3), pp. 241-252.

Neupane, S., Finlay, R.D., Alstrom, S., Elfstrand, M. & Hogberg, N. (2015). Transcriptional responses of the bacterial antagonist *Serratia plymuthica* to the fungal phytopathogen *Rhizoctonia solani*. *Environ Microbiol Rep,* 7(1), pp. 123-7.

Neupane, S., Finlay, R.D., Alstrom, S., Goodwin, L., Kyrpides, N.C., Lucas, S., Lapidus, A., Bruce, D., Pitluck, S., Peters, L., Ovchinnikova, G., Chertkov, O., Han, J., Han, C., Tapia, R., Detter, J.C., Land, M., Hauser, L., Cheng, J.F., Ivanova, N., Pagani, I., Klenk, H.P., Woyke, T. & Hogberg, N. (2012a). Complete genome sequence of *Serratia plymuthica* strain AS12. *Standards in Genomic Sciences,* 6(2), pp. 165-173.

Neupane, S., Finlay, R.D., Kyrpides, N.C., Goodwin, L., Alstrom, S., Lucas, S., Land, M., Han, J., Lapidus, A., Cheng, J.F., Bruce, D., Pitluck, S., Peters, L., Ovchinnikova, G., Held, B., Han, C., Detter, J.C., Tapia, R., Hauser, L., Ivanova, N., Pagani, I., Woyke, T., Klenk, H.P. & Hogberg, N. (2012b). Complete genome sequence of the plant-associated *Serratia plymuthica* strain AS13. *Standards in Genomic Sciences,* 7(1), pp. 22-30.

Neupane, S., Goodwin, L.A., Hogberg, N., Kyrpides, N.C., Alstrom, S., Bruce, D., Quintana, B., Munk, C., Daligault, H., Teshima, H., Davenport, K., Reitenga, K., Green, L., Chain, P., Erkkila, T., Gu, W., Zhang, X.J., Xu, Y., Kunde, Y., Chertkov, O., Han, J., Han, C., Detter, J.C., Ivanova, N., Pati, A., Chen, A., Szeto, E., Mavromatis, K., Huntemann, M., Nolan, M., Pitluck, S., Deshpande, S., Markowitz, V., Pagani, I., Klenk, H.P., Woyke, T. & Finlay, R.D. (2013b). Non-contiguous finished genome sequence of plant-growth promoting *Serratia proteamaculans* S4. *Standards in Genomic Sciences,* 8(3), pp. 441-449.

Neupane, S., Hogberg, N., Alstrom, S., Lucas, S., Han, J., Lapidus, A., Cheng, J.F., Bruce, D., Goodwin, L., Pitluck, S., Peters, L., Ovchinnikova, G., Lu, M.G., Han, C., Detter, J.C., Tapia, R., Fiebig, A., Land, M., Hauser, L., Kyrpides, N.C., Ivanova, N., Pagani, I., Klenk, H.P., Woyke, T. & Finlay, R.D. (2012c). Complete genome sequence of the rapeseed plant-growth promoting *Serratia plymuthica* strain AS9. *Standards in Genomic Sciences,* 6(1), pp. 54-62.

Newman, M.A., Sundelin, T., Nielsen, J.T. & Erbs, G. (2013). MAMP (microbe-associated molecular pattern) triggered immunity in plants. *Front Plant Sci,* 4, p. 139.

Novakova, M., Sasek, V., Dobrev, P.I., Valentova, O. & Burketova, L. (2014). Plant hormones in defense response of *Brassica napus* to *Sclerotinia sclerotiorum* - Reassessing the role of salicylic acid in the interaction with a necrotroph. *Plant Physiology and Biochemistry,* 80, pp. 308-317.

Nowak, J. & Shulaev, V. (2003). Priming for transplant stress resistance in in vitro propagation. *In Vitro Cellular & Developmental Biology-Plant,* 39(2), pp. 107-124.

Nurnberger, T., Brunner, F., Kemmerling, B. & Piater, L. (2004). Innate immunity in plants and animals: striking similarities and obvious differences. *Immunological Reviews,* 198, pp. 249-266.

Ogoshi, A. (1987). Ecology and Pathogenicity of Anastomosis and Intraspecific Groups of *Rhizoctonia-Solani* Kuhn. *Annual Review of Phytopathology,* 25, pp. 125-143.

Okon, Y., Bloemberg, G. V. & Lugtenberg, B. J. J. (1998). *Biotechnology of biofertilization and phytostimulation.* (Agricultural Biotechnology: A Altman.

Okubara, P.A., Dickman, M.B. & Blechl, A.E. (2014). Molecular and genetic aspects of controlling the soilborne necrotrophic pathogens *Rhizoctonia* and *Pythium*. *Plant Science,* 228, pp. 61-70.

Oldroyd, G.E.D., Harrison, M.J. & Paszkowski, U. (2009). Reprogramming Plant Cells for Endosymbiosis. *Science,* 324(5928), pp. 753-754.

Oliver, R.P. & Solomon, P.S. (2010). New developments in pathogenicity and virulence of necrotrophs. *Current Opinion in Plant Biology,* 13(4), pp. 415-419.

Oliveros, J.C. (2007). VENNY. An interactive tool for comparing lists with Venn Diagrams.

Ovadis, M., Liu, X.G., Gavriel, S., Ismailov, Z., Chet, I. & Chernin, L. (2004). The global regulator genes from biocontrol strain *Serratia plymuthica* IC1270: Cloning, sequencing, and functional studies. *Journal of Bacteriology,* 186(15), pp. 4986-4993.

Ownley, B.H., Weller, D.M. & Thomashow, L.S. (1992). Influence of Insitu and Invitro Ph on Suppression of *Gaeumannomyces-Graminis* Var *Tritici* by *Pseudomonas-Fluorescens* 2-79. *Phytopathology,* 82(2), pp. 178-184.

Panstruga, R. & Dodds, P.N. (2009). Terrific Protein Traffic: The Mystery of Effector Protein Delivery by Filamentous Plant Pathogens. *Science,* 324(5928), pp. 748-750.

Park, C.J. & Seo, Y.S. (2015). Heat Shock Proteins: A Review of the Molecular Chaperones for Plant Immunity. *Plant Pathology Journal,* 31(4), pp. 323-333.

Park, W.J., Kriechbaumer, V., Muller, A., Piotrowski, M., Meeley, R.B., Gierl, A. & Glawischnig, E. (2003). The nitrilase ZmNIT2 converts indole-3-acetonitrile to indole-3-acetic acid. *Plant Physiology,* 133(2), pp. 794-802.

Paulitz, T.C. (2006). Low input no-till cereal production in the Pacific Northwest of the US: The challenges of root diseases. *European Journal of Plant Pathology,* 115(3), pp. 271-281.

Pauwels, L., Barbero, G.F., Geerinck, J., Tilleman, S., Grunewald, W., Perez, A.C., Chico, J.M., Vanden Bossche, R., Sewell, J., Gil, E., Garcia-Casado, G., Witters, E., Inze, D., Long, J.A., De Jaeger, G., Solano, R. & Goossens, A. (2010). NINJA connects the co-repressor TOPLESS to jasmonate signalling. *Nature,* 464(7289), pp. 788-U169.

Pedras, M.S.C. & Ahiahonu, P.W.K. (2002). Probing the phytopathogenic stem rot fungus with phytoalexins and analogues: Unprecedented glucosylation of camalexin and 6-methoxycamalexin. *Bioorganic & Medicinal Chemistry,* 10(10), pp. 3307-3312.

Peiffer, J.A., Spor, A., Koren, O., Jin, Z., Tringe, S.G., Dangl, J.L., Buckler, E.S. & Ley, R.E. (2013). Diversity and heritability of the maize rhizosphere microbiome under field conditions. *Proceedings of the National Academy of Sciences of the United States of America,* 110(16), pp. 6548-6553.

Penninckx, I.A.M.A., Thomma, B.P.H.J., Buchala, A., Metraux, J.P. & Broekaert, W.F. (1998). Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in *Arabidopsis*. *Plant Cell,* 10(12), pp. 2103-2113.

Penuelas, J. & Terradas, J. (2014). The foliar microbiome. *Trends in Plant Science,* 19(5), pp. 278-280.

Philippot, L., Raaijmakers, J.M., Lemanceau, P. & van der Putten, W.H. (2013). Going back to the roots: the microbial ecology of the rhizosphere. *Nature Reviews Microbiology,* 11(11), pp. 789-799.

Pierik, R., Tholen, D., Poorter, H., Visser, E.J. & Voesenek, L.A. (2006). The Janus face of ethylene: growth inhibition and stimulation. *Trends Plant Sci,* 11(4), pp. 176-83.

Pierret, A., Doussan, C., Capowiez, Y., Bastardie, F. & Pages, L. (2007). Root functional architecture: A framework for modeling the interplay between roots and soil. *Vadose Zone Journal,* 6(2), pp. 269-281.

Pieterse, C.M., de Jonge, R. & Berendsen, R.L. (2016). The Soil-Borne Supremacy. *Trends Plant Sci,* 21(3), pp. 171-3.
Pieterse, C.M., van Wees, S.C., Hoffland, E., van Pelt, J.A. & van Loon, L.C. (1996). Systemic resistance in *Arabidopsis* induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. *Plant Cell,* 8(8), pp. 1225-37.
Pieterse, C.M., van Wees, S.C., van Pelt, J.A., Knoester, M., Laan, R., Gerrits, H., Weisbeek, P.J. & van Loon, L.C. (1998). A novel signaling pathway controlling induced systemic resistance in *Arabidopsis*. *Plant Cell,* 10(9), pp. 1571-80.
Pieterse, C.M.J., Leon-Reyes, A., Van der Ent, S. & Van Wees, S.C.M. (2009). Networking by small-molecule hormones in plant immunity. *Nature Chemical Biology,* 5(5), pp. 308-316.
Pieterse, C.M.J., Van der Does, D., Zamioudis, C., Leon-Reyes, A. & Van Wees, S.C.M. (2012). Hormonal Modulation of Plant Immunity. *Annual Review of Cell and Developmental Biology, Vol 28,* 28, pp. 489-521.
Pieterse, C.M.J., Van Pelt, J.A., Ton, J., Parchmann, S., Mueller, M.J., Buchala, A.J., Metraux, J.P. & Van Loon, L.C. (2000). Rhizobacteria-mediated induced systemic resistance (ISR) in *Arabidopsis* requires sensitivity to jasmonate and ethylene but is not accompanied by an increase in their production. *Physiological and Molecular Plant Pathology,* 57(3), pp. 123-134.
Pieterse, C.M.J., Zamioudis, C., Berendsen, R.L., Weller, D.M., Van Wees, S.C.M. & Bakker, P.A.H.M. (2014). Induced Systemic Resistance by Beneficial Microbes. *Annual Review of Phytopathology, Vol 52,* 52, pp. 347-375.
Pietrowska, E., Rozalska, S., Kazmierczak, A., Nawrocka, J. & Malolepsza, U. (2015). Reactive oxygen and nitrogen (ROS and RNS) species generation and cell death in tomato suspension cultures-*Botrytis cinerea* interaction. *Protoplasma,* 252(1), pp. 307-319.
Pliego, C., de Weert, S., Lamers, G., de Vicente, A., Bloemberg, G., Manuel Cazorla, F. & Ramos, C. (2008). Two similar enhanced root-colonizing *Pseudomonas* strains differ largely in their colonization strategies of avocado roots and *Rosellinia necatrix* hyphae. *Environmental Microbiology,* 10(12), pp. 3295-3304.
Powis, G. & Montfort, W.R. (2001). Properties and biological activities of thioredoxins. *Annual Review of Pharmacology and Toxicology,* 41, pp. 261-295.
Pozo, M.J., Van Der Ent, S., Van Loon, L.C. & Pieterse, C.M. (2008). Transcription factor MYC2 is involved in priming for enhanced defense during rhizobacteria-induced systemic resistance in *Arabidopsis thaliana*. *New Phytol,* 180(2), pp. 511-23.
Pozo, M.J., Van Loon, L.C. & Pieterse, C.M.J. (2004). Jasmonates - Signals in plant-microbe interactions. *Journal of Plant Growth Regulation,* 23(3), pp. 211-222.
Pre, M., Atallah, M., Champion, A., De Vos, M., Pieterse, C.M.J. & Memelink, J. (2008). The AP2/ERF domain transcription factor ORA59 integrates jasmonic acid and ethylene signals in plant defense. *Plant Physiology,* 147(3), pp. 1347-1357.
Press, C.M., Wilson, M., Tuzun, S. & Kloepper, J.W. (1997). Salicylic acid produced by *Serratia marcescens* 90-166 is not the primary determinant of induced systemic resistance in cucumber or tobacco. *Molecular Plant-Microbe Interactions,* 10(6), pp. 761-768.
Prosser, J.I., Rangel-Castro, J.I. & Killham, K. (2006). Studying plant-microbe interactions using stable isotope technologies. *Current Opinion in Biotechnology,* 17(1), pp. 98-102.
Raaijmakers, J.M., Paulitz, T.C., Steinberg, C., Alabouvette, C. & Moenne-Loccoz, Y. (2009). The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and Soil,* 321(1-2), pp. 341-361.
Raaijmakers, J.M., Vlami, M. & de Souza, J.T. (2002). Antibiotic production by bacterial biocontrol agents. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology,* 81(1-4), pp. 537-547.
Raaijmakers, J.M. & Weller, D.M. (1998). Natural plant protection by 2,4-diacetylphloroglucinol - Producing *Pseudomonas* spp. in take-all decline soils. *Molecular Plant-Microbe Interactions,* 11(2), pp. 144-152.
Radajewski, S., McDonald, I.R. & Murrell, J.C. (2003). Stable-isotope probing of nucleic acids: a window to the function of uncultured microorganisms. *Current Opinion in Biotechnology,* 14(3), pp. 296-302.
Raimundo, J., Sobral, R., Bailey, P., Azevedo, H., Galego, L., Almeida, J., Coen, E. & Costa, M.M. (2013). A subcellular tug of war involving three MYB-like proteins underlies a molecular antagonism in *Antirrhinum* flower asymmetry. *Plant J,* 75(4), pp. 527-38.

Ramamoorthy, V., Viswanathan, R., Raguchander, T., Prakasam, V. & Samiyappan, R. (2001). Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. *Crop Protection,* 20(1), pp. 1-11.

Ramirez, V., Van der Ent, S., Garcia-Andrade, J., Coego, A., Pieterse, C.M.J. & Vera, P. (2010). OCP3 is an important modulator of NPR1-mediated jasmonic acid-dependent induced defenses in *Arabidopsis*. *Bmc Plant Biology,* 10.

Rasche, F., Lueders, T., Schloter, M., Schaefer, S., Buegger, F., Gattinger, A., Hood-Nowotny, R.C. & Sessitsch, A. (2009). DNA-based stable isotope probing enables the identification of active bacterial endophytes in potatoes. *New Phytologist,* 181(4), pp. 802-807.

Revillas, J.J., Rodelas, B., Pozo, C., Martinez-Toledo, M.V. & Gonzalez-Lopez, J. (2000). Production of B-group vitamins by two *Azotobacter* strains with phenolic compounds as sole carbon source under diazotrophic and adiazotrophic conditions. *J Appl Microbiol,* 89(3), pp. 486-93.

Reymond, P. & Farmer, E.E. (1998). Jasmonate and salicylate as global signals for defense gene expression. *Current Opinion in Plant Biology,* 1(5), pp. 404-411.

Ritpitakphong, U., Falquet, L., Vimoltust, A., Berger, A., Metraux, J.P. & L'Haridon, F. (2016). The microbiome of the leaf surface of *Arabidopsis* protects against a fungal pathogen. *New Phytologist,* 210(3), pp. 1033-1043.

Robert-Seilaniantz, A., Grant, M. & Jones, J.D.G. (2011). Hormone Crosstalk in Plant Disease and Defense: More Than Just JASMONATE-SALICYLATE Antagonism. *Annual Review of Phytopathology, Vol 49,* 49, pp. 317-343.

Robert-Seilaniantz, A., Navarro, L., Bari, R. & Jones, J.D. (2007). Pathological hormone imbalances. *Current Opinion in Plant Biology,* 10(4), pp. 372-379.

Rodriguez, H., Fraga, R., Gonzalez, T. & Bashan, Y. (2006). Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. *Plant and Soil,* 287(1-2), pp. 15-21.

Rodriguez, M.A., Cabrera, G., Gozzo, F.C., Eberlin, M.N. & Godeas, A. (2011). *Clonostachys rosea* BAFC3874 as a *Sclerotinia sclerotiorum* antagonist: mechanisms involved and potential as a biocontrol agent. *Journal of Applied Microbiology,* 110(5), pp. 1177-1186.

Rowe, H.C. & Kliebenstein, D.J. (2008). Complex Genetics Control Natural Variation in *Arabidopsis thaliana* Resistance to *Botrytis cinerea*. *Genetics,* 180(4), pp. 2237-2250.

Ryan, C.A., Huffaker, A. & Yamaguchi, Y. (2007). New insights into innate immunity in *Arabidopsis*. *Cellular Microbiology,* 9(8), pp. 1902-1908.

Ryu, C.M., Farag, M.A., Hu, C.H., Reddy, M.S., Kloepper, J.W. & Pare, P.W. (2004a). Bacterial volatiles induce systemic resistance in *Arabidopsis*. *Plant Physiology,* 134(3), pp. 1017-1026.

Ryu, C.M., Farag, M.A., Hu, C.H., Reddy, M.S., Wei, H.X., Pare, P.W. & Kloepper, J.W. (2003). Bacterial volatiles promote growth in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America,* 100(8), pp. 4927-4932.

Ryu, C.M., Murphy, J.F., Mysore, K.S. & Kloepper, J.W. (2004b). Plant growth-promoting rhizobacteria systemically protect *Arabidopsis thaliana* against *Cucumber* mosaic virus by a salicylic acid and NPR1-independent and jasmonic acid-dependent signaling pathway. *Plant Journal,* 39(3), pp. 381-392.

Sakakibara, H. (2006). Cytokinins: Activity, biosynthesis, and translocation. *Annual Review of Plant Biology,* 57, pp. 431-449.

Sarosh, B.R., Danielsson, J. & Meijer, J. (2009). Transcript profiling of oilseed rape (*Brassica napus*) primed for biocontrol differentiate genes involved in microbial interactions with beneficial *Bacillus amyloliquefaciens* from pathogenic *Botrytis cinerea*. *Plant Molecular Biology,* 70(1-2), pp. 31-45.

Sato, M., Mitra, R.M., Coller, J., Wang, D., Spivey, N.W., Dewdney, J., Denoux, C., Glazebrook, J. & Katagiri, F. (2007). A high-performance, small-scale microarray for expression profiling of many samples in *Arabidopsis*-pathogen studies. *Plant Journal,* 49(3), pp. 565-577.

Sauter, M. (2015). Phytosulfokine peptide signalling. *Journal of Experimental Botany,* 66(17), pp. 5160-5168.

Schenk, P.M., Kazan, K., Wilson, I., Anderson, J.P., Richmond, T., Somerville, S.C. & Manners, J.M. (2000). Coordinated plant defense responses in *Arabidopsis* revealed by microarray analysis. *Proceedings of the National Academy of Sciences of the United States of America,* 97(21), pp. 11655-11660.

Schippers, B., Bakker, A.W. & Bakker, P.A.H.M. (1987). Interactions of Deleterious and Beneficial Rhizosphere Microorganisms and the Effect of Cropping Practices. *Annual Review of Phytopathology,* 25, pp. 339-358.

Schlaeppi, K. & Bulgarelli, D. (2015). The Plant Microbiome at Work. *Molecular Plant-Microbe Interactions,* 28(3), pp. 212-217.

Schlaeppi, K., Dombrowski, N., Oter, R.G., van Themaat, E.V.L. & Schulze-Lefert, P. (2014). Quantitative divergence of the bacterial root microbiota in *Arabidopsis thaliana* relatives. *Proceedings of the National Academy of Sciences of the United States of America,* 111(2), pp. 585-592.

Schleper, C. & Nicol, G.W. (2010). Ammonia-Oxidising Archaea - Physiology, Ecology and Evolution. *Advances in Microbial Physiology, Vol 57,* 57, pp. 1-41.

Schmidt, R., Etalo, D.W., de Jager, V., Gerards, S., Zweers, H., de Boer, W. & Garbeva, P. (2015). Microbial Small Talk: Volatiles in Fungal-Bacterial Interactions. *Front Microbiol,* 6, p. 1495.

Schmidt, R., Jager, V., Zuhlke, D., Wolff, C., Bernhardt, J., Cankar, K., Beekwilder, J., Ijcken, W.V., Sleutels, F., Boer, W., Riedel, K. & Garbeva, P. (2017). Fungal volatile compounds induce production of the secondary metabolite Sodorifen in *Serratia plymuthica* PRI-2C. *Sci Rep,* 7(1), p. 862.

Schroder, F., Lisso, J. & Mussig, C. (2011). EXORDIUM-LIKE1 Promotes Growth during Low Carbon Availability in *Arabidopsis*. *Plant Physiology,* 156(3), pp. 1620-1630.

Schroeckh, V., Scherlach, K., Nutzmann, H.W., Shelest, E., Schmidt-Heck, W., Schuemann, J., Martin, K., Hertweck, C. & Brakhage, A.A. (2009). Intimate bacterial-fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*. *Proceedings of the National Academy of Sciences of the United States of America,* 106(34), pp. 14558-14563.

Schwachtje, J. & Baldwin, I.T. (2008). Why does herbivore attack reconfigure primary metabolism? *Plant Physiology,* 146(3), pp. 845-851.

Schwachtje, J., Fischer, A., Erban, A. & Kopka, J. (2018). Primed primary metabolism in systemic leaves: a functional systems analysis. *Sci Rep,* 8(1), p. 216.

Schwachtje, J., Karojet, S., Thormahlen, I., Bernholz, C., Kunz, S., Brouwer, S., Schwochow, M., Kohl, K. & van Dongen, J.T. (2011). A naturally associated rhizobacterium of *Arabidopsis thaliana* induces a starvation-like transcriptional response while promoting growth. *Plos One,* 6(12), p. e29382.

Schwarzlander, M. & Finkemeier, I. (2013). Mitochondrial Energy and Redox Signaling in Plants. *Antioxidants & Redox Signaling,* 18(16), pp. 2122-2144.

Sheehan, D.J., Hitchcock, C.A. & Sibley, C.M. (1999). Current and emerging azole antifungal agents. *Clinical Microbiology Reviews,* 12(1), pp. 40-+.

Shim, J.S. & Choi, Y.D. (2013). Direct regulation of WRKY70 by AtMYB44 in plant defense responses. *Plant Signal Behav,* 8(6), p. e20783.

Shirasu, K. & Schulze-Lefert, P. (2003). Complex formation, promiscuity and multi-functionality: protein interactions in disease-resistance pathways. *Trends in Plant Science,* 8(6), pp. 252-258.

Soberon, M., Lopez-Diaz, J.A. & Bravo, A. (2013). Cyt toxins produced by *Bacillus thuringiensis*: A protein fold conserved in several pathogenic microorganisms. *Peptides,* 41, pp. 87-93.

Somers, E., Vanderleyden, J. & Srinivasan, M. (2004). Rhizosphere bacterial signalling: a love parade beneath our feet. *Crit Rev Microbiol,* 30(4), pp. 205-40.

Spaepen, S. & Vanderleyden, J. (2011). Auxin and plant-microbe interactions. *Cold Spring Harb Perspect Biol,* 3(4).

Spoel, S.H., Koornneef, A., Claessens, S.M.C., Korzelius, J.P., Van Pelt, J.A., Mueller, M.J., Buchala, A.J., Metraux, J.P., Brown, R., Kazan, K., Van Loon, L.C., Dong, X.N. & Pieterse, C.M.J. (2003). NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. *Plant Cell,* 15(3), pp. 760-770.

Stibal, M., Schostag, M., Cameron, K.A., Hansen, L.H., Chandler, D.M., Wadham, J.L. & Jacobsen, C.S. (2015). Different bulk and active bacterial communities in cryoconite from the margin and interior of the Greenland ice sheet. *Environmental Microbiology Reports,* 7(2), pp. 293-300.

Stotz, H.U., Sawada, Y., Shimada, Y., Hirai, M.Y., Sasaki, E., Krischke, M., Brown, P.D., Saito, K. & Kamiya, Y. (2011). Role of camalexin, indole glucosinolates, and side chain modification of glucosinolate-derived isothiocyanates in defense of *Arabidopsis* against *Sclerotinia sclerotiorum*. *Plant Journal,* 67(1), pp. 81-93.

Strange, R.N. & Scott, P.R. (2005). Plant disease: A threat to global food security. *Annual Review of Phytopathology,* 43, pp. 83-116.

Stringlis, I.A., Proietti, S., Hickman, R., Van Verk, M.C., Zamioudis, C. & Pieterse, C.M.J. (2018). Root transcriptional dynamics induced by beneficial rhizobacteria and microbial immune elicitors reveal signatures of adaptation to mutualists. *Plant Journal,* 93(1), pp. 166-180.

Sun, Y., Wang, C., Yang, B., Wu, F.F., Hao, X.Y., Liang, W.W., Niu, F.F., Yan, J.L., Zhang, H.F., Wang, B.Y., Deyholos, M.K. & Jiang, Y.Q. (2014). Identification and functional analysis of mitogen-activated protein kinase kinase kinase (MAPKKK) genes in canola (*Brassica napus* L.). *Journal of Experimental Botany,* 65(8), pp. 2171-2188.

Syu, M.J. (2001). Biological production of 2,3-butanediol. *Appl Microbiol Biotechnol,* 55(1), pp. 10-8.

Tada, Y., Spoel, S.H., Pajerowska-Mukhtar, K., Mou, Z.L., Song, J.Q., Wang, C., Zuo, J.R. & Dong, X.N. (2008). Plant immunity requires conformational charges of NPR1 via S-nitrosylation and thioredoxins. *Science,* 321(5891), pp. 952-956.

Taghavi, S., Garafola, C., Monchy, S., Newman, L., Hoffman, A., Weyens, N., Barac, T., Vangronsveld, J. & van der Lelie, D. (2009). Genome Survey and Characterization of Endophytic Bacteria Exhibiting a Beneficial Effect on Growth and Development of Poplar Trees. *Applied and Environmental Microbiology,* 75(3), pp. 748-757.

Tanaka, H. & Phaff, H.J. (1965). Enzymatic Hydrolysis of Yeast Cell Walls .I. Isolation of Wall-Decomposing Organisms and Separation and Purification of Lytic Enzymes. *Journal of Bacteriology,* 89(6), pp. 1570-&.

Thaler, J.S. & Bostock, R.M. (2004). Interactions between abscisic-acid-mediated responses and plant resistance to pathogens and insects. *Ecology,* 85(1), pp. 48-58.

Thilmony, R., Underwood, W. & He, S.Y. (2006). Genome-wide transcriptional analysis of the *Arabidopsis thaliana* interaction with the plant pathogen *Pseudomonas syringae* pv. tomato DC3000 and the human pathogen *Escherichia coli* O157 : H7. *Plant Journal,* 46(1), pp. 34-53.

Thomma, B.P., Nelissen, I., Eggermont, K. & Broekaert, W.F. (1999). Deficiency in phytoalexin production causes enhanced susceptibility of *Arabidopsis thaliana* to the fungus *Alternaria brassicicola*. *Plant J,* 19(2), pp. 163-71.

Thomson, N.R., Crow, M.A., McGowan, S.J., Cox, A. & Salmond, G.P.C. (2000). Biosynthesis of carbapenem antibiotic and prodigiosin pigment in *Serratia* is under quorum sensing control. *Molecular Microbiology,* 36(3), pp. 539-556.

Thrane, C., Olsson, S., Nielsen, T.H. & Sorensen, J. (1999). Vital fluorescent stains for detection of stress in *Pythium ultimum* and *Rhizoctonia solani* challenged with viscosinamide from *Pseudomonas fluorescens* DR54. *Fems Microbiology Ecology,* 30(1), pp. 11-23.

Tierens, K.F.M.J., Thomma, B.P.H., Brouwer, M., Schmidt, J., Kistner, K., Porzel, A., Mauch-Mani, B., Cammue, B.P.A. & Broekaert, W.F. (2001). Study of the role of antimicrobial glucosinolate-derived isothiocyanates in resistance of *Arabidopsis* to microbial pathogens. *Plant Physiology,* 125(4), pp. 1688-1699.

Tjamos, S.E., Flemetakis, E., Paplomatas, E.J. & Katinakis, P. (2005). Induction of resistance to *Verticillium dahliae* in *Arabidopsis thaliana* by the biocontrol agent K-165 and pathogenesis-related proteins gene expression. *Molecular Plant-Microbe Interactions,* 18(6), pp. 555-561.

Ton, J. & Mauch-Mani, B. (2004). Beta-amino-butyric acid-induced resistance against necrotrophic pathogens is based on ABA-dependent priming for callose. *Plant J,* 38(1), pp. 119-30.

Tranier, M.S., Pognant-Gros, J., Quiroz, R.D., Gonzalez, C.N.A., Mateille, T. & Roussos, S. (2014). Commercial Biological Control Agents Targeted Against Plant-Parasitic Root-knot Nematodes. *Brazilian Archives of Biology and Technology,* 57(6), pp. 831-841.

Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D.R., Pimentel, H., Salzberg, S.L., Rinn, J.L. & Pachter, L. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nature Protocols,* 7(3), pp. 562-578.

Trda, L., Boutrot, F., Claverie, J., Brule, D., Dorey, S. & Poinssot, B. (2015). Perception of pathogenic or beneficial bacteria and their evasion of host immunity: pattern recognition receptors in the frontline. *Frontiers in Plant Science,* 6.

Tsuda, K., Sato, M., Glazebrook, J., Cohen, J.D. & Katagiri, F. (2008). Interplay between MAMP-triggered and SA-mediated defense responses (vol 53, pg 763, 2008). *Plant Journal,* 55(6), pp. 1061-1061.

Turan, M. & Bringu, A. (2007). Phytoremediation based on canola (*Brassica napus* L.) and Indian mustard (*Brassica Juncea* L.) planted on spiked soil by aliquot amount of Cd, Cu, Pb, and Zn. *Plant Soil and Environment,* 53(1), pp. 7-15.

Turner, T.R., Ramakrishnan, K., Walshaw, J., Heavens, D., Alston, M., Swarbreck, D., Osbourn, A., Grant, A. & Poole, P.S. (2013). Comparative metatranscriptomics reveals kingdom level changes in the rhizosphere microbiome of plants. *Isme Journal,* 7(12), pp. 2248-2258.

Vacheron, J., Desbrosses, G., Bouffaud, M.L., Touraine, B., Moenne-Loccoz, Y., Muller, D., Legendre, L., Wisniewski-Dye, F. & Prigent-Combaret, C. (2013). Plant growth-promoting rhizobacteria and root system functioning. *Front Plant Sci,* 4, p. 356.

van de Mortel, J.E., de Vos, R.C.H., Dekkers, E., Pineda, A., Guillod, L., Bouwmeester, K., van Loon, J.J.A., Dicke, M. & Raaijmakers, J.M. (2012). Metabolic and Transcriptomic Changes Induced in *Arabidopsis* by the Rhizobacterium *Pseudomonas fluorescens* SS101. *Plant Physiology,* 160(4), pp. 2173-2188.

van der Ent, S., Van Wees, S.C.M. & Pieterse, C.M.J. (2009). Jasmonate signaling in plant interactions with resistance-inducing beneficial microbes. *Phytochemistry,* 70(13-14), pp. 1581-1588.

van der Ent, S., Verhagen, B.W., Van Doorn, R., Bakker, D., Verlaan, M.G., Pel, M.J., Joosten, R.G., Proveniers, M.C., Van Loon, L.C., Ton, J. & Pieterse, C.M. (2008). MYB72 is required in early signaling steps of rhizobacteria-induced systemic resistance in *Arabidopsis*. *Plant Physiology,* 146(3), pp. 1293-304.

van der Heijden, M.G.A., de Bruin, S., Luckerhoff, L., van Logtestijn, R.S.P. & Schlaeppi, K. (2016). A widespread plant-fungal-bacterial symbiosis promotes plant biodiversity, plant nutrition and seedling recruitment. *Isme Journal,* 10(2), pp. 389-399.

van Elsas, J.D., Chiurazzi, M., Mallon, C.A., Elhottova, D., Kristufek, V. & Salles, J.F. (2012). Microbial diversity determines the invasion of soil by a bacterial pathogen. *Proceedings of the National Academy of Sciences of the United States of America,* 109(4), pp. 1159-1164.

van Houdt, R., Givskov, M. & Michiels, C.W. (2007). Quorum sensing in *Serratia*. *Fems Microbiology Reviews,* 31(4), pp. 407-424.

van Hulten, M., Pelser, M., van Loon, L.C., Pieterse, C.M.J. & Ton, J. (2006). Costs and benefits of priming for defense in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America,* 103(14), pp. 5602-5607.

van Loon, L.C., Geraats, B.P.J. & Linthorst, H.J.M. (2006a). Ethylene as a modulator of disease resistance in plants. *Trends in Plant Science,* 11(4), pp. 184-191.

van Loon, L.C., Rep, M. & Pieterse, C.M.J. (2006b). Significance of inducible defense-related proteins in infected plants. *Annual Review of Phytopathology,* 44, pp. 135-162.

van Loon, L.C. & Van Strien, E.A. (1999). The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. *Physiological and Molecular Plant Pathology,* 55(2), pp. 85-97.

van Wees, S.C.M., de Swart, E.A.M., van Pelt, J.A., van Loon, L.C. & Pieterse, C.M.J. (2000). Enhancement of induced disease resistance by simultaneous activation of salicylate- and jasmonate-dependent defense pathways in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the United States of America,* 97(15), pp. 8711-8716.

van Wees, S.C.M., Van der Ent, S. & Pieterse, C.M.J. (2008). Plant immune responses triggered by beneficial microbes. *Current Opinion in Plant Biology,* 11(4), pp. 443-448.

Vandenkoornhuyse, P., Mahe, S., Ineson, P., Staddon, P., Ostle, N., Cliquet, J.B., Francez, A.J., Fitter, A.H. & Young, J.P.W. (2007). Active root-inhabiting microbes identified by rapid incorporation of plant-derived carbon into RNA. *Proceedings of the National Academy of Sciences of the United States of America,* 104(43), pp. 16970-16975.

Vanpeer, R., Niemann, G.J. & Schippers, B. (1991). Induced Resistance and Phytoalexin Accumulation in Biological-Control of Fusarium-Wilt of Carnation by *Pseudomonas* Sp Strain Wcs417r. *Phytopathology,* 81(7), pp. 728-734.

Vanrhijn, P. & Vanderleyden, J. (1995). The Rhizobium-Plant Symbiosis. *Microbiological Reviews,* 59(1), pp. 124-142.

Vassilev, N., Vassileva, M. & Nikolaeva, I. (2006). Simultaneous P-solubilizing and biocontrol activity of microorganisms: potentials and future trends. *Appl Microbiol Biotechnol,* 71(2), pp. 137-44.

Venturi, V. & Keel, C. (2016). Signaling in the Rhizosphere. *Trends Plant Sci,* 21(3), pp. 187-98.

Verbon, E.H. & Liberman, L.M. (2016). Beneficial Microbes Affect Endogenous Mechanisms Controlling Root Development. *Trends in Plant Science,* 21(3), pp. 218-229.

Verhagen, B.W.M., Glazebrook, J., Zhu, T., Chang, H.S., van Loon, L.C. & Pieterse, C.M.J. (2004). The transcriptome of rhizobacteria-induced systemic resistance in *Arabidopsis*. *Molecular Plant-Microbe Interactions,* 17(8), pp. 895-908.

Vicente, J., Cascon, T., Vicedo, B., Garcia-Agustin, P., Hamberg, M. & Castresana, C. (2012). Role of 9-Lipoxygenase and alpha-Dioxygenase Oxylipin Pathways as Modulators of Local and Systemic Defense. *Molecular Plant,* 5(4), pp. 914-928.

Vilgalys, R. & Cubeta, M.A. (1994). Molecular Systematics and Population Biology of *Rhizoctonia*. *Annual Review of Phytopathology,* 32, pp. 135-155.

Virk, N., Li, D.Y., Tian, L.M., Huang, L., Hong, Y.B., Li, X.H., Zhang, Y.F., Liu, B., Zhang, H.J. & Song, F.M. (2015). *Arabidopsis* Raf-Like Mitogen-Activated Protein Kinase Kinase Kinase Gene Raf43 Is Required for Tolerance to Multiple Abiotic Stresses. *Plos One,* 10(7).

Vishniac, H.S. (2006). A multivariate analysis of soil yeasts isolated from a latitudinal gradient. *Microbial Ecology,* 52(1), pp. 90-103.

Vlot, A.C., Dempsey, D.A. & Klessig, D.F. (2009). Salicylic Acid, a Multifaceted Hormone to Combat Disease. *Annual Review of Phytopathology,* 47, pp. 177-206.

Vogel, C., Bodenhausen, N., Gruissem, W. & Vorholt, J.A. (2016). The *Arabidopsis* leaf transcriptome reveals distinct but also overlapping responses to colonization by phyllosphere commensals and pathogen infection with impact on plant health. *New Phytol,* 212(1), pp. 192-207.

Vorholt, J.A. (2012). Microbial life in the phyllosphere. *Nature Reviews Microbiology,* 10(12), pp. 828-840.

Walker, M.A., Volpi, S., Sims, K.B., Walter, J.E. & Traggiai, E. (2014). Powering the Immune System: Mitochondria in Immune Function and Deficiency. *Journal of Immunology Research*.

Walters, D.R., Ratsep, J. & Havis, N.D. (2013). Controlling crop diseases using induced resistance: challenges for the future. *Journal of Experimental Botany,* 64(5), pp. 1263-1280.

Wang, D., Amornsiripanitch, N. & Dong, X.N. (2006). A genomic approach to identify regulatory nodes in the transcriptional network of systemic acquired resistance in plants. *Plos Pathogens,* 2(11), pp. 1042-1050.

Wang, Y., Bao, Z.L., Zhu, Y. & Hua, J. (2009a). Analysis of Temperature Modulation of Plant Defense Against Biotrophic Microbes. *Molecular Plant-Microbe Interactions,* 22(5), pp. 498-506.

Wang, Y.Q., Ohara, Y., Nakayashiki, H., Tosa, Y. & Mayama, S. (2005). Microarray analysis of the gene expression profile induced by the endophytic plant growth-promoting rhizobacteria, *Pseudomonas fluorescens* FPT9601-T5 in *Arabidopsis*. *Molecular Plant-Microbe Interactions,* 18(5), pp. 385-396.

Wang, Z., Fang, H., Chen, Y., Chen, K., Li, G., Gu, S. & Tan, X. (2014). Overexpression of BnWRKY33 in oilseed rape enhances resistance to *Sclerotinia sclerotiorum*. *Mol Plant Pathol,* 15(7), pp. 677-89.

Wang, Z., Mao, H., Dong, C.H., Ji, R.Q., Cai, L., Fu, H. & Liu, S.Y. (2009b). Overexpression of *Brassica napus* MPK4 Enhances Resistance to *Sclerotinia sclerotiorum* in Oilseed Rape. *Molecular Plant-Microbe Interactions,* 22(3), pp. 235-244.

Wang, Z., Tan, X.L., Zhang, Z.Y., Gu, S.L., Li, G.Y. & Shi, H.F. (2012). Defense to *Sclerotinia sclerotiorum* in oilseed rape is associated with the sequential activations of salicylic acid signaling and jasmonic acid signaling. *Plant Science,* 184, pp. 75-82.

Wasilewska, A., Vlad, F., Sirichandra, C., Redko, Y., Jammes, F., Valon, C., Frei dit Frey, N. & Leung, J. (2008). An update on abscisic acid signaling in plants and more. *Molecular Plant,* 1(2), pp. 198-217.

Wasternack, C. (2007). Jasmonates: An update on biosynthesis, signal transduction and action in plant stress response, growth and development. *Annals of Botany,* 100(4), pp. 681-697.

Wei, L.J., Jian, H.J., Lu, K., Filardo, F., Yin, N.W., Liu, L.Z., Qu, C.M., Li, W., Du, H. & Li, J.N. (2016). Genome-wide association analysis and differential expression analysis of resistance to *Sclerotinia* stem rot in *Brassica napus*. *Plant Biotechnology Journal,* 14(6), pp. 1368-1380.

Weis, B.L., Kovacevic, J., Missbach, S. & Schleiff, E. (2015). Plant-Specific Features of Ribosome Biogenesis. *Trends Plant Sci,* 20(11), pp. 729-40.

Weller, D.M., Landa, B.B., Mavrodi, O.V., Schroeder, K.L., De La Fuente, L., Bankhead, S.B., Molar, R.A., Bonsall, R.F., Mavrodi, D.V. & Thomashow, L.S. (2007). Role of 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* spp. in the defense of plant roots. *Plant Biology,* 9(1), pp. 4-20.

Whipps, J.M. (2001). Microbial interactions and biocontrol in the rhizosphere. *Journal of Experimental Botany,* 52, pp. 487-511.

Whiteley, A.S., Thomson, B., Lueders, T. & Manefield, M. (2007). RNA stable-isotope probing. *Nature Protocols,* 2(4), pp. 838-844.

Wong, A.C.S., Hecht, V.F.G., Picard, K., Diwadkar, P., Laurie, R.E., Wen, J., Mysore, K., Macknight, R.C. & Weller, J.L. (2014). Isolation and functional analysis of CONSTANS-LIKE genes suggests that a central role for CONSTANS in flowering time control is not evolutionarily conserved in *Medicago truncatula*. *Frontiers in Plant Science,* 5.

Wu, J., Zhao, Q., Yang, Q.Y., Liu, H., Li, Q.Y., Yi, X.Q., Cheng, Y., Guo, L., Fan, C.C. & Zhou, Y.M. (2016). Comparative transcriptomic analysis uncovers the complex genetic network for resistance to *Sclerotinia sclerotiorum* in *Brassica napus*. *Scientific Reports,* 6.

Wu, W.X., Liu, W., Lu, H.H., Chen, Y.X., Devare, M. & Thies, J. (2009). Use of C-13 labeling to assess carbon partitioning in transgenic and nontransgenic (parental) rice and their rhizosphere soil microbial communities. *Fems Microbiology Ecology,* 67(1), pp. 93-102.

Yang, B., Jiang, Y.Q., Rahman, M.H., Deyholos, M.K. & Kav, N.N.V. (2009). Identification and expression analysis of WRKY transcription factor genes in canola (*Brassica napus* L.) in response to fungal pathogens and hormone treatments. *Bmc Plant Biology,* 9.

Yasuda, M., Ishikawa, A., Jikumaru, Y., Seki, M., Umezawa, T., Asami, T., Maruyama-Nakashita, A., Kudo, T., Shinozaki, K., Yoshida, S. & Nakashita, H. (2008). Antagonistic interaction between systemic acquired resistance and the abscisic acid-mediated abiotic stress response in *Arabidopsis*. *Plant Cell,* 20(6), pp. 1678-1692.

Ye, J., Fang, L., Zheng, H.K., Zhang, Y., Chen, J., Zhang, Z.J., Wang, J., Li, S.T., Li, R.Q., Bolund, L. & Wang, J. (2006). WEGO: a web tool for plotting GO annotations. *Nucleic Acids Research,* 34, pp. W293-W297.

Zamioudis, C. & Pieterse, C.M.J. (2012). Modulation of Host Immunity by Beneficial Microbes. *Molecular Plant-Microbe Interactions,* 25(2), pp. 139-150.

Zhang, F., Zhang, F., Huang, L.Y., Cruz, C.V., Ali, J.H., Xu, J.L., Zhou, Y.L. & Li, Z.K. (2016). Overlap between Signaling Pathways Responsive to *Xanthomonas oryzae* pv. *oryzae* Infection and Drought Stress in Rice Introgression Line Revealed by RNA-Seq. *Journal of Plant Growth Regulation,* 35(2), pp. 345-356.

Zhou, X.G. & Wu, F.Z. (2012). p-Coumaric Acid Influenced Cucumber Rhizosphere Soil Microbial Communities and the Growth of *Fusarium oxysporum* f.sp *cucumerinum* Owen. *Plos One,* 7(10).

Zhu, J.K. (2016). Abiotic Stress Signaling and Responses in Plants. *Cell,* 167(2), pp. 313-324.

Zhulin, I.B. & Armitage, J.P. (1992). The Role of Taxis in the Ecology of *Azospirillum*. *Symbiosis,* 13(1-3), pp. 199-206.

Zipfel, C. (2008). Pattern-recognition receptors in plant innate immunity. *Current Opinion in Immunology,* 20(1), pp. 10-16.

Zipfel, C. & Robatzek, S. (2010). Pathogen-Associated Molecular Pattern-Triggered Immunity: Veni, Vidi ... ? *Plant Physiology,* 154(2), pp. 551-554.

Popular science summary

In nature, all plants grow in soil. The soil is an environment where microorganisms such as bacteria and fungi live and interact. Of particular interest from an agricultural and ecological perspective, is the area of soil immediately next to plant roots, which is called the 'rhizosphere'. The microorganisms that live in the rhizosphere are called the 'rhizosphere microbiome'. There, microorganisms are especially abundant and are influenced to a great extent by the plants, especially by the plant roots, because roots release organic compounds into the soil. Some members of the microbiome are more active, and better able to compete for this source of energy than others. These compounds are known as 'exudates or rhizodeposits' and can increase the availability of nutrients in the rhizosphere and provide a food source for the microorganisms. This causes the number of microorganisms to be far higher in the rhizosphere than in the soil environment away from the plant roots (known as 'bulk soil'). In return the rhizosphere microbiome influences the plants, either in a positive or a negative way, depending on whether they are beneficial or pathogenic.

Some beneficial rhizosphere soil or root microbiome members are capable of creating mutualistic associations with plants. During mutualism, microorganisms can help the plant to acquire nutrients from the soil, provide indirect pathogen protection or release phytohormones to stimulate plant growth. Some very well studied examples of mutualistic interactions are: a) the establishment of arbuscular mycorrhizal fungi in most flowering plants, b) nitrogen-fixing rhizobia bacteria in legumes and c) interactions of plants with Biological Control Agents (BCAs), which can be either bacteria promoting plant growth (PGPRs) and providing resistance to

pathogens (known as 'biocontrol'), or biocontrol fungi. These so called BCAs are free-living microorganisms and exist naturally in the rhizosphere, on the root surface or even inside the roots (known as 'endophytes'). Such microorganisms can be utilized in sustainable agriculture to reduce inputs of chemical pesticides or fertilizers.

Plants are faced with continuous biotic stress caused by pathogens and pests that are capable of exploiting highly specialized mechanisms in order to damage the plant. In this context, plants have evolved a plethora of complex and sophisticated immune responses that enable them to limit pathogen growth and protect themselves at the same time, while pathogens need to suppress plant immune responses in order to proliferate.

All the aforementioned interactions take place in the rhizosphere, which can be likened to a battlefield between soilborne pathogens and antagonistic microorganisms.

Within this thesis, we approached some of these issues from different angles by working under more controlled, *in-vitro*, conditions, but also using more realistic soil-based systems, exploiting modern molecular and –omics approaches.

We focused on the interactions between specific bacterial isolates of the genus *Serratia*, which are known for their plant growth promoting effects on oilseed rape plants as well as for their antagonistic potential against the fungal phytopathogen *Rhizoctonia solani* causing damping off and root rot diseases in oilseed rape.

We firstly tried to get an insight into the defense of the fungal pathogen *R. solani* against *Serratia* bacteria, because in order to develop efficient biocontrol agents, it is crucial to understand not only how biocontrol agents interact with the pathogens, but also what mechanisms fungal pathogens use to counteract the bacteria. We saw that large changes in the gene expression of the fungus occur during antagonism. The fungus restricts its growth by modifying fundamental processes when it recognizes the toxic environment surrounding the bacteria. *R. solani* is also capable of detoxifying itself by producing antioxidants and a number of enzymes that neutralize the toxic metabolites produced by the bacteria. Finally, the fungus also counter-attacks bacterial antagonism by producing harmful toxins.

Then we tried to identify bacterial and fungal members in the rhizosphere of oilseed rape that are capable of assimilating plant-derived carbon, thus rendering them active and not just present. It is

known that 20-50% of the carbon produced during photosynthesis is transferred to the roots and half of this is then released into the soil and serves as a food source for the competent rhizosphere microbiome members. Interestingly, we could see that some of the active genera that were superior competitors for plant-derived carbon have documented abilities to promote plant growth and act as biocontrol agents against pathogens (e.g. *Streptomyces*, *Rhizobium*, *Clonostachys*). This finding suggests that such genera have the potential to strongly antagonize others for effective root colonization and renders them promising candidates as bioinoculants to improve plant productivity and health of oilseed rape.

Finally, we performed an experiment in controlled conditions in the laboratory, where we inoculated roots of oilseed rape seedlings with *R. solani* alone or in combination with a *Serratia* isolate. We observed that the appearance of the plants differed a lot. Plants that were inoculated only with *R. solani* were almost dead 240h after inoculation, whereas plants that had been root inoculated with both microorganisms appeared healthy in the roots, but also systemically in the leaves. So, we tried to understand what changes in the gene expression and defense mechanisms of oilseed rape plants occur when an antagonistic bacterium colonizes the plant. We found a massive number of genes to be differentially expressed. Plant hormones are known to play a crucial role in defense against pathogens and are also important in Induced Systemic Resistance (ISR), a term explaining the induced state of resistance in plants, triggered by biological agents that protect the whole plant against future pathogen challenge. Indeed, we identified genes related to key hormonal regulators such as jasmonic acid, auxin and salicylic acid to be involved. Moreover, we found that during pathogen challenge there is an interplay between defense and stress responses, but in the presence of the biocontrol bacterium more genes related to stress responses were downregulated and more genes related to defense were upregulated. We suggest that the S4 bacteria assist in floral induction, plant development and growth and play roles in the assembly of plant cell walls.

Overall, within this thesis work we gained more knowledge on the complex tripartite interactions that take place in the rhizosphere by using a novel combination of a crop plant and microorganisms. Many questions remain unanswered, but the results gained contribute to further research.

Populärvetenskaplig sammanfattning

Jorden kryllar av mikroorganismer där bland annat bakterier och svampar lever och interagerar. Jordskiktet som finns närmast levande växters rötter, rhizosfären, är särskilt intressant på grund av sitt mycket rika mikrobiella liv, speciellt inuti rötterna eftersom växterna utsöndrar energi i form av diverse organiska kemiska föreningar, kallas rotexudat. Mikroorganismerna som lever i rhizosfären benämns ¨rhizosfärens mikrobiom¨. Några organismer i mikrobiomet är mer aktiva än andra och har förmågan att effektivt konkurrera om denna energiresurs. Den energirika miljön gör att rhizosfären är mycket rikare på förekomsten av mikroorganimser än den jordmiljö som ligger längre ifrån rötterna. Rhizosfärens mikrobiom kan påverka växter på ett positivt eller på ett negativt sätt, beroende på om mikroorganismen är nyttig för växten eller om den är en växtpatogen.

Några mikroorganismer som förekommer i rhizosfären eller i rötterna har förmåga att bilda mutualistiska förhållanden med växten där bägge parter gynnas. I ett mutualistiskt förhållande kan en mikroorganism förse växten med näring, skydd mot växtpatogener samt stimulera växtens tillväxt genom att utsöndra växthormoner. Bland några väl undersökta mutualistiska interaktioner kan nämnas: a) arbuskulära mykorrhizasvampar i de flesta blomster växtarter, b) kvävefixerande bakterier i baljväxter samt c) interaktioner mellan växter och frilevande växtstimulerande bakterier och/eller antagonister som skyddar mot patogener. Mikroorganismerna kan leva på eller i roten, de som lever inuti rötterna kallas endofyter. Mutualistiska mikroorganismer erbjuder stora möjligheter för uthållig växtproduktion med minskad kemikalieanvändningen i jordbruket.

Växter utsätts kontinuerligt för biologisk stress av växtpatogener och andra skadegörare som gör allt i sin makt för att utnyttja och skada växten. Växterna har i sin tur utvecklat en mängd komplexa och sofistikerade mekanismer för dels att hindra patogeners framfart och dels att skydda sig själva. Samtidigt är det viktigt för patogenen att dämpa växternas försvars mekanismer för att kunna växa snabbt och för att sprida sig. Alla dessa interaktioner utspelar sig i rhizosfären som kan liknas vid ett slagfält t.ex. mellan jordburna patogener och mikrobiella antagonister.

Inom ramen för denna avhandling belyses några av dessa frågor ur olika vinklar genom att utföra studier under kontrollerade förhållanden men även i den mer realistiska jordbaserade miljön, med hjälp av moderna molekylärbiologiska och genomikbaserade metoder. Vi använde specifika bakteriestammar av släktet *Serratia* som tidigare påvisats förbättra rapsens tillväxt och som också är antagonister till rapspatogenen *Rhizoctonia solani.*

I den första studien försökte vi att få förståelse för hur *R. solani* försvarar sig mot *Serratia* antagonister. För att kunna ta fram nya effektiva biokontrollorganismer är det nödvändigt att förstå hur de påverkar patogenen men även att förstå hur patogenen försvarar sig mot antagonister. Vi såg omfattande förändringar i svampens genuttryck när den exponerades för antagonistiska bakterier. Svampen begränsade sin egen tillväxt genom att förändra sina grundläggande metaboliska processer när den känner igen den för svampen giftiga miljön kring bakterierna. Svampen verkar ha förmåga att avgifta sin omgivning genom att utsöndra antioxidanter och enzymer för att neutralisera giftiga ämnen som bakterierna bildat. Slutligen kan svampen motverka bakteriell antagonism genom att bilda toxiska ämnen.

I en separat studie försökte vi identifiera de bakterie- och svampsamhällen som lever på växters rotexudat och således utgör den aktiva komponenten av rhizosfärsmikrobiomet. Så mycket som 20-50% av den energi i form av kol som bildas i växter av fotosyntesen transporteras ned till rötterna och ungefär hälften av den släpps sedan ut i jorden där den fungerar som en energikälla för rhizosfärkompetenta svampar och bakterier. Intressant nog såg vi att några av de aktiva rhizosfärorganismerna som konkurrerade effektivt om rotexudaten var de som tidigare är kända för att ha förmåga till biokontroll och tillhör släktena *Fusarium*, *Streptomyces*, *Rhizobium*, *Clonostachys*. Resultaten tyder på att

dessa organismer med potential för antagonism är konkurrenskraftiga med avseende på effektiv kolonisering av växtrötter. De är också lovande kandidater som biokontrollorganismer mot groddbrand, och som kan förbättra rapsens tillväxt vid en tidig etablering.

Slutligen utfördes experiment under kontrollerade förhållanden där rapsfröplantor tillfördes enbart *R. solani* eller i kombination med Serratiabakterien. Vi försökte således förstå vilka förändringar i genuttryck och försvarsmekanismer som rapsplantor uppvisar när en antagonistisk bakterie koloniserar växten i samspel med patogenen. Vi observerade stora skillnader i växternas utseende mellan de olika behandlingarna. Plantorna var nästan döda tio dagar efter ympning med enbart *R. solani*, medan plantorna saminokulerade med både svampen och bakterien höll sig friska i rötterna, men också i bladen. Vid koloniseringen av rapsen med bakterien S4, hittade vi ett stort antal gener som uttrycktes annorlunda. Växthormoner är kända för sin avgörande roll i försvaret mot patogener och är också viktiga i inducerad systemisk resistens (ISR), ett begrepp som förklaras med framkallad motståndskraft hos växten, utlöst av organismer som skyddar hela växten mot senare patogenangrepp. Vi identifierade gener som reglerar viktiga växthormoner såsom jasmonsyra, auxin och salicylsyra. Vi fann också ett samspel mellan försvar- och stressresponsassocierade geners vid saminokulering med patogenen. Däremot nedreglerades av flera gener relaterade till stressrespons medan fler gener relaterade till försvarsrespons var uppreglerade i plantor behandlade med Serratiabakterien. Det ser ut som om att bakterien har en roll i att stimulera blomning, rapsens tillväxt och utveckling samt uppbyggnaden av växtcellens väggar.

Inom ramen för studier presenterade i denna avhandling har vi fått ny kunskap om den komplicerade trepartsinteraktionen som pågår i rhizosfären av en jordbruksgröda. Många frågor förblir obesvarade och hittills erhållna resultat ger upphov till nya spännande frågor för fortsatt forskning.

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Appendix 1. Supplementary information

Supplementary information to Paper I is available at the following link: https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-015-1758-z#Sec26