UNIVERSITE DE STRASBOURG

ECOLE DOCTORALE 414 – SCIENCES DE LA VIE ET DE LA SANTE

Institut de Biologie Moléculaire des Plantes (CNRS)

THÈSE présentée par :

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soutenue le **15 octobre 2019**

pour obtenir le grade de : **Docteur de l'université de Strasbourg**

Discipline/ Spécialité : Aspects moléculaires et cellulaires de la biologie

Deciphering the interactions between plants and bacteria in the context of isoprenoid biosynthesis in *Arabidopsis thaliana*

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Acknowledgments

Je voudrais tout d'abord exprimer ma gratitude à mes co-directeurs de thèse, Florence Arsène-Ploetze et Dimitri Heintz, de m'avoir donné la possibilité de faire cette thèse. Merci au grand chef, Hubert Schaller, de m'avoir accueillie dans son équipe. Démarrer cette nouvelle thématique au laboratoire n'était pas toujours facile, mais j'ai pris du plaisir à travailler sur ce sujet, malgré un parcours semé d'embuches.

Merci aux membres du jury, Claire Prigent-Combaret, Valérie Geoffroy, Pierre Peyret et Hermann Heipieper d'avoir accepté d'évaluer mon travail de thèse.

Je remercie également les membres de l'équipe, en particulier Anne et Andréa pour votre bonne humeur et votre implication dans la vie du labo. C'était un plaisir de vous côtoyer pendant ces 3 ans. Merci à Thomas pour les conseils avisés. Merci aussi à Quentin pour ton grain de folie, ton ingéniosité, ton optimisme et les mojitos partagés !

Une mention spéciale à mon collègue préféré qui est devenu un véritable ami. Pierrot, tu as été d'un soutien sans faille, tant d'un point de vue professionnel que d'un point de vue personnel. Merci pour ta bonne humeur, ton optimisme permanent, ton aide sur les grosses manips qui ne veulent pas marcher, et tes conseils qui nous sauvent tous de temps à autres ! Tu mériterais une médaille pour ta patience ! Le labo n'a pas son pareil sans toi (on l'a vu pendant ton absence, le 523 sans toi c'est un peu comme le distributeur de friandises sans gaufres ou galettes aux amandes…). C'était un plaisir de partager ces trois années avec toi, malgré tes playlists du vendredi après-midi. Chaque labo devrait avoir son Pierrot. Tu vas énormément me manquer.

Merci à ceux qui étaient juste de passage dans l'équipe pour quelques mois et avec qui j'ai pris plaisir à travailler, Romaric, Myriam, Clara. Mais tout particulièrement, merci à notre dernière recrue, Loïc. Tu as été d'un soutien énorme toi aussi, particulièrement dans les moments difficiles de cette dernière année de thèse, que tu as su rendre plus agréable par ta bonne humeur et ton humour (douteux parfois…).

Merci pour ton aide à la paillasse, pour les longues discussions de travail, mais aussi les autres, les pauses café/gaufres, les voyages à travers le monde, les apéros et j'en passe. Ne lâche rien, tu trouveras ta voie, sans aucun doute. Je te souhaite plein de réussite pour la suite, tu le mérites.

Un grand merci aussi à tous les copains de l'IBMP et aux voisins avec qui j'ai partagé mon quotidien et qui ont vécu leur thèse en même temps que moi. Vous avez mis des paillettes dans ma thèse ! Adrien, un merci tout particulier pour ton soutien et ta confiance en moi quand je doutais. Lucy, ma première poto ici ; Lulu petit Lu ; Valou, le voisin envahissant auquel on s'attache tellement… Un grand merci à vous d'avoir toujours été là, en toutes circonstances, au labo et en dehors. Je ne pouvais pas espérer faire de plus belles rencontres ! Laeti, ou les montagnes russes émotionnelles ; Cédric, monsieur pause café ; Loukas, meilleur partenaire de badminton ; Hélène, copine de vin ! Merci pour votre bonne humeur, votre pêche, vos éclats de rires. Caro et Magda, merci d'avoir partagé ces moments de doutes et de remotivation en salle de rédaction, et pas que. Boubou, Duygu, Olivier, merci les chouchous de BGM d'être toujours là, et merci Faf pour la relecture. Vous allez me manquer ! La liste est longue, mais il y a des gens dans cet institut qui ont été d'une grande aide dans mon travail, ou qui avaient simplement toujours un mot gentil ou un sourire au quotidien… Laura, Arnaud, Sandrine, Jean-Michel, Juju, les voisins du 520 et j'en passe.

Un énorme merci aussi à mes proches et aux copains du Sundgau d'avoir compris qu'une thèse vole une grande partie de notre temps, et d'avoir quand-même toujours été présents.

Enfin, un merci tout particulier à mes parents et mon frangin, pour votre soutien, vos encouragements et votre patience. Vous m'avez supportée dans les bons comme dans les mauvais moments, vous avez toujours su me faire prendre les bonnes décisions et me rebooster quand j'en avais besoin. C'est grâce à vous que j'en suis là. Je vous aime.

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List of abbreviations

AACT: acetyl-CoA C-acetyltransferase
ABA: abscisic acid
BLAST: basic local alignment search tool
CDP-ME: 4-(cytidine 5'diphospho)-2-*C*-methyl-D-erythritol
CDP-MEP: 2-phospho-4-(cytidine 5'diphospho)-2-*C*-methyl-D-erythritol
Cfu: colony forming unit
chs5: chilling sensitive 5
CMK: 4-(cytidine 5'-diphospho)-2-Cmethylerythritol kinase
Col-0: Columbia-0 ecotype
DAMP: damage-associated molecular pattern
DMAPP: dimethylallyl diphosphate
DNA: deoxyribonucleic acid
DXP: 1-deoxy-D-xylulose 5-phosphate
DXR: 1-deoxy-D-xylulose 5-phosphate reductoisomerase
DXS: 1-deoxy-D-xylulose 5-phosphate synthase
EDTA: Ethylenediaminetetraacetic acid
EPS: exopolysaccharide
ET: ethylene
ETI: effector triggered immunity
ETS: effector triggered susceptibility
FAME: fatty acid methyl esters
FDS: farnesyl diphosphate synthase
FPP: farnesyl diphosphate
G3P: D-glyceraldehyde 3-phosphate
GA: gibberellic acid/gibberellin
GC/FID: gas chromatography with flame ionization detection
GC-MS: gas chromatography coupled to mass spectrometry
gDNA: genomic DNA
GFP: green fluorescent protein
GGPP: geranylgeranyl diphosphate
HDR: 4-hydroxy-3-methylbut-2-enyl diphosphate reductase
HDS: 4-hydroxy-3-methylbut-2-enyl diphosphate synthase
HMBPP: 4-hydroxy-3-methylbut-2-enyldiphosphate
HMG-CoA: 3-hydroxy-3-methylglutaryl-CoA
HMGR: 3-hydroxy-3-methylglutaryl-CoA reductase
HMGS: 3-hydroxy-3-methylglutaryl-CoA synthase
HR: hypersensitive response
HRM: high resolution melt
IDI: isopentenyl diphosphate:dimethylallyl diphosphate isomerase
IPP: isopentenyl diphosphate

JA: jasmonic acid/jasmonate
kb: kilobase
LPS: lipopolysaccharide
MAMP: microbial-associated molecular pattern
MAPK: mitogen-activated protein kinase
MAPK: mitogen-activated protein kinase
MCT: 2-*C*-methyl-D-erythritol 4-phosphate cytidyltransferase
MDC: mevalonate diphosphate decarboxylase
MDS: 2-*C*-methylerythritol-2,4-cyclodiphosphate synthase
MEcDP: 2-*C*-methylerythritol 2,4-cyclodiphosphate
MEcPP: 2-*C*-methyl-D-erythritol 2,4-cyclopyrophosphate
MEP: 2-*C*-methyl-D-erythritol 4-phosphate
MUSCLE: MUltiple Sequence Comparison by Log-Expectation
MVA: mevalonate
MVK: mevalonate kinase
NB-LRR: nucleotide-binding and leucine-rich repeat
OD: optical density
OTU: operational taxonomic unit
PAMP: pathogenic-associated molecular pattern
PBS: phosphate buffered saline
PCR: polymerase chain reaction
PGPB: plant growth promoting bacteria
PGPR: plant growth promoting rhizobacteria
PMVK: phosphomevalonate kinase
PR genes: *pathogenesis-related* genes
PRR: pattern recognition receptor
*Pst*DC3000: *Pseudomonas syringae* pv. *tomato* DC3000
PTI: PAMP-triggered immunity
rDNA: ribosomal DNA
ROS: reactive oxygen species
SA: salicylic acid
SAR: systemic acquired resistance
SQS: squalene synthase
T3SS: type III secretion system
UV: ultraviolet
WS2: Wassilewskja ecotype

Introduction

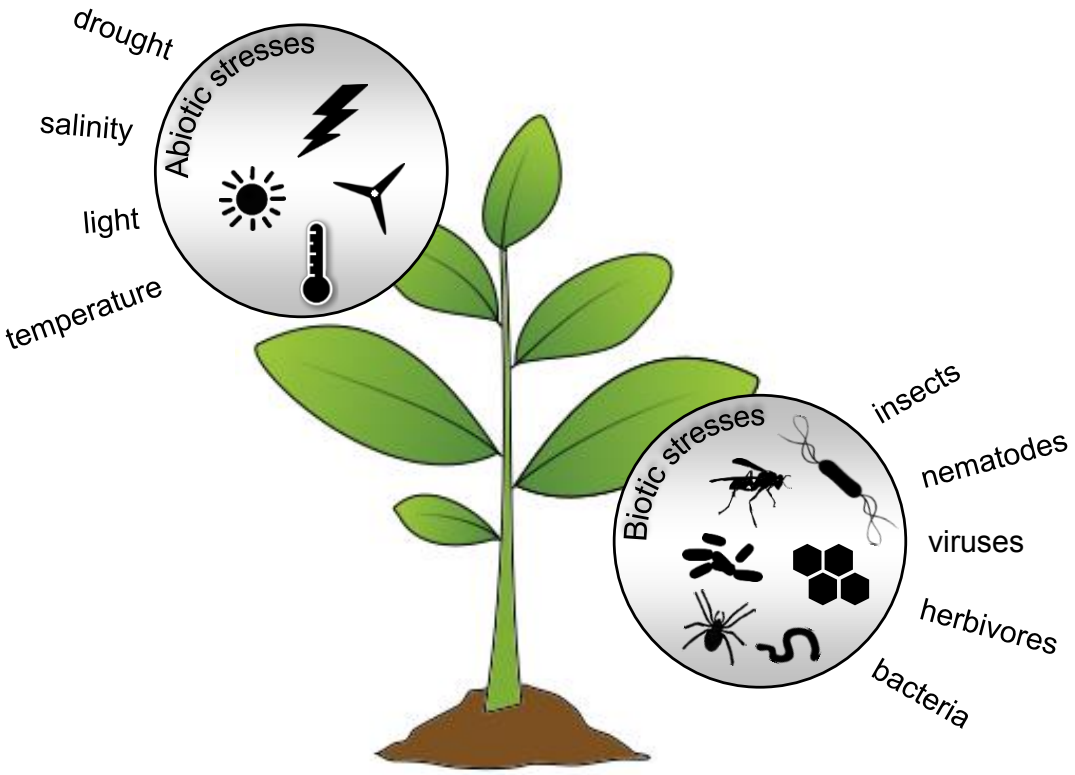

Figure 1. Plants are exposed to a variety of biotic and abiotic stresses. Among several abiotic stresses which can affect the plant, we can mention the most studied that are drought, salinity, light or extreme temperatures. Biotic stresses, on their part, include living organisms such as insects, nematodes, viruses, herbivores or bacteria.

I. Interactions between plants and micro-organisms

1. Generalities

Since plants are sessile organisms, they are constantly exposed to the environment during their whole lifecycle. Thus, both in nature and under culture conditions, they are in contact with multiple organisms and they are exposed to a wide range of abiotic or biotic stresses that can lead to serious damages (**Figure 1**). Basically, a situation is considered as a stress as soon as it is leading to physiological changes in the organism, accompanied by growth inhibition and/or cellular damages for instance. Abiotic stresses are the consequence of some environmental conditions as for example salinity, drought, light or extreme temperatures (Mittler, 2006; Tardieu and Tuberosa, 2010). Plants are also subject to biotic stresses that are caused by other organisms like insects, nematodes, viruses, herbivores or bacteria (Atkinson and Urwin, 2012). In addition, plants are often exposed to several stressful conditions simultaneously. This combination of stresses induces a unique response that is specific to the situation, because the effects of biotic and abiotic stresses are additional (Fujita et al., 2006; Suzuki et al., 2014). Thus, plants interactions with their environment are complex and involve multiple factors.

Interactions between plants and their environment are not necessarily harmful. Plants interact with other organisms among which pollinators ensure their reproduction, or organisms such as fungi or bacteria that are able to provide them some nutrients from environments in which they are not capable to assume a direct uptake, for instance. These interactions are beneficial, and sometimes even crucial for the host plant. During my thesis, I focused my work on the interactions between plants and bacteria.

2. Localization of micro-organisms interacting with the host plant

Plants interact with a wide range of micro-organisms during their whole lifecycle. These micro-organisms interacting with the different parts of the plant (roots, leaves or

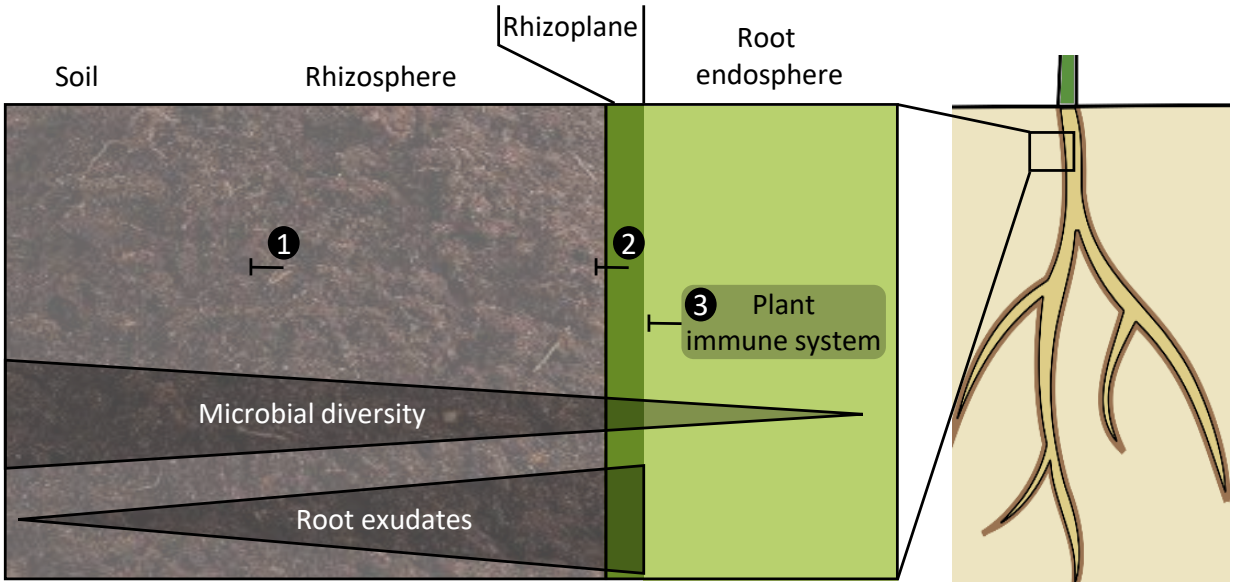

Figure 2. Acquisition of the plant microbiota from soil. Microbiota composition of the underground parts of the plant is highly affected by the secretion of root exudates. The exclusion process is split into different steps. First, in the rhizosphere, there is gradient of root exudates that only allows some bacteria to reach the plant vicinity. Then, in the rhizoplane, bacteria should be able to form biofilms and compete in the presence of high amounts of nutrients. The last step of exclusion is the selection of bacteria from the rhizoplane which can colonize the root endosphere and escape the plant immune system. Adapted from Heijden and Schlaeppi, 2015.

flowers for instance) constitute its microbiota. The term “symbiosis” is also commonly employed to describe a relationship between different organisms, including plants and bacteria (de Bary,1879). Micro-organisms that are localized at the surface of the plant are called epiphytes. In contrast, some of them colonize the internal plant tissues, in which case we talk about endophytes, a term coined by Anton de Bary in 1886 (Porras-Alfaro and Bayman, 2011). Moreover, there is a wide variety of micro-organisms in the surrounding soil, as well as in the atmosphere, around the host. In this symbiose vicinity, molecules can be exchanged between micro-organisms and the plant.

The underground parts of the plant are constituted by the roots themselves, the rhizoplane which is the surface of the roots, and the rhizosphere which is the root-surrounding soil influenced by root exudates, as defined for the first time in 1904 by Hiltner (Hartmann et al., 2008; van der Heijden and Schlaeppi, 2015). First of all, the plant genotype and the soil type are important for the establishment of bacterial communities (Edwards et al., 2015; van der Heijden and Schlaeppi, 2015). The soil type is influenced by root exudates that are composed of a mixture of organic compounds secreted by the plant. Thus, roots highly influence the rhizosphere by impacting the soil structure, but also the pH or the oxygen availability and by providing an energy source and carbon-rich exudates to the surrounding micro-organisms. Each of the compartments that are the rhizosphere, the rhizoplane and the roots, is colonized by a common or specific microbial community, under the influence of this root exudates gradient (**Figure 2**) (van der Heijden and Schlaeppi, 2015). This leads to a first step of exclusion that occurs in the rhizosphere. Then, there is a second step of exclusion in the rhizoplane, where there is a selection of bacteria capable to form biofilms and compete in the presence of high amounts of nutrients. The last step of exclusion is the selection of bacteria from the rhizoplane that are capable to colonize the root endosphere and to evade the recognition by the plant immune system.

In a few numbers, taking in account this selection, the rhizosphere of the model plant *Arabidopsis thaliana (A. thaliana*) is colonized by around 10^6 to 10^9 bacteria.g^{-1} (Spaepen et al., 2009). They are essentially coming from the soil in which we can find the same proportion (Whitman et al., 1998). In the root endosphere, there are 10^4 to 10^8 bacteria.g^{-1} also coming from the soil and influenced by the root exudates secreted by the plant as previously mentioned (van der Heijden and Schlaeppi, 2015; Vandenkoornhuyse et al., 2015; Whitman et al., 1998). This strong connection

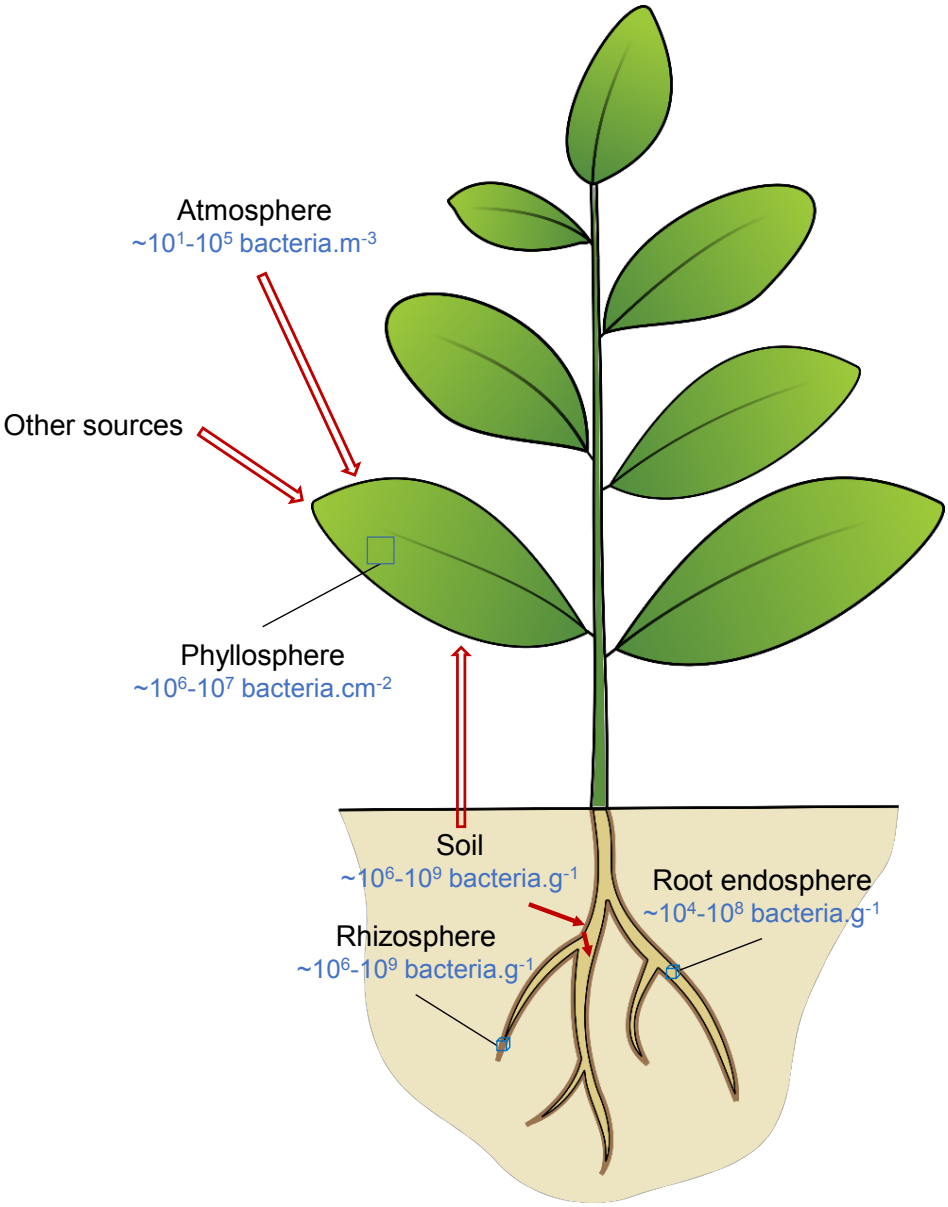

Figure 3. Bacterial titers composing the plant microbiota. Number of bacteria in the phyllosphere (Lindow and Brandl, 2003), atmosphere (Fahlgren et al., 2010), rhizosphere (Spaepen et al., 2009), root and soil (Whitman et al., 1998) are indicated as approximations. Open arrows indicate bacterial sources for the phyllosphere microbiota, and solid arrows represent bacterial sources for the root microbiota. Adapted from Bulgarelli et *al.,* 2013.

between the soil bacterial communities and those associated with the roots of *A. thaliana* has been highlighted by several studies (Bulgarelli et al., 2012; Lundberg et al., 2012; Schlaeppi et al., 2014). In contrast, the aboveground part of the plant, called the phyllosphere, contains 10^6 to 10^7 bacteria.cm^{-2} (Lindow and Brandl, 2003), among which some come from the atmosphere, that contains itself 10^1 to 10^5 bacteria.m^{-3} (Fahlgren et al., 2010), or other sources like the soil or the interacting macro-organisms for example (**Figure 3**). The microbiota so formed may be composed of a huge variety of bacteria that can have diverse repercussions on the host plant.

3. Different types of plant-bacteria interactions

Interactions between plants and bacteria have an effect on each partner. Micro-organisms can be neutral, beneficial, or sometimes pathogenic for their host (**Figure 4**). We talk about symbiosis if there is a relationship between different organisms of different species (de Bary,1879). This interaction allows each one to complete its lifecycle by providing nutrients or promoting growth, defense or resistance to stress, for instance (Lugtenberg and Kamilova, 2009; Yang et al., 2008). In the case of mutualism, the interaction is beneficial for both organisms. Commensalism, for its part, describes an interaction in which one organism benefits from the situation without affecting the other one, neither positively, nor negatively. Finally, parasitism is a particular relationship in which one organism takes advantage of the interaction to the detriment of the other (Bulgarelli et al., 2013). The interaction between a unique bacteria and its host is variable depending on the presence of other bacteria entering in competition or not, for instance. Thus, the plant and its communities are governed by a complex set of different kinds of interactions involving hundreds of micro-organisms.

4. Plant-pathogen interactions

Plant pathogenic micro-organisms are classified in three main categories according to their lifestyle. The two most common categories are the necrotrophs and the biotrophs, but some pathogens behave as both biotrophs and necrotrophs, depending

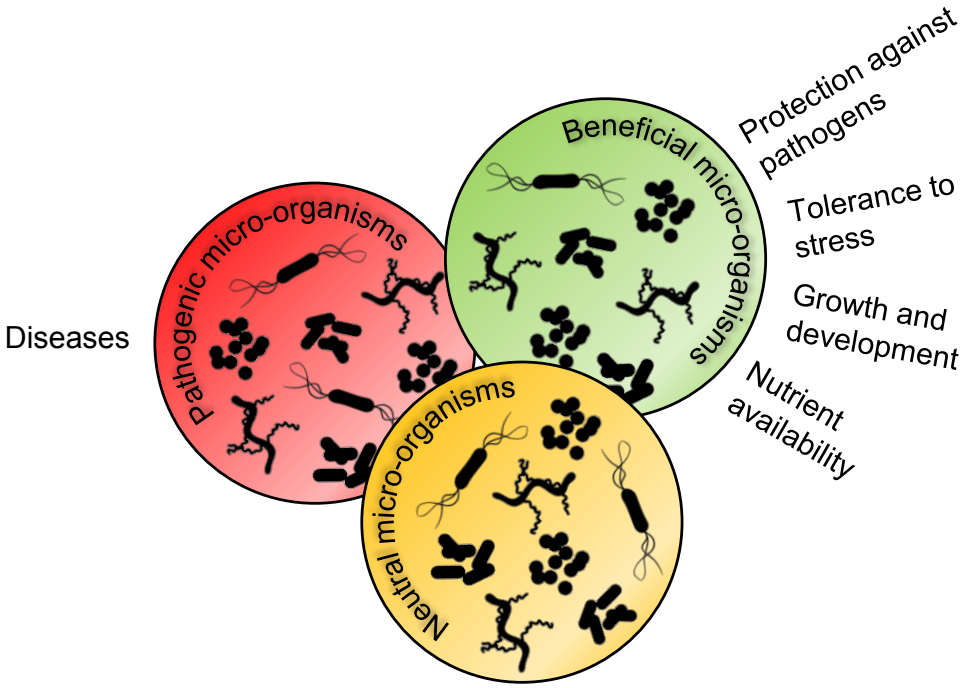

Figure 4. Possible effects of micro-organisms interacting with plants. Most of the micro-organisms composing the plant microbiota are defined as neutral for the host plant, since no beneficial nor harmful effect has been demonstrated yet. Some others can be pathogenic and induce plant diseases. Finally, there are several beneficial micro-organisms that lead to protection against pathogens or better tolerance to stress for instance, but they can also favorize growth and development or nutrient availability.

on the conditions and the stage of their lifecycle, giving rise to a third category called hemibiotrophs (Glazebrook, 2005). Biotrophic pathogens need a living host to complete their lifecycle since they feed on living tissues. Necrotrophic pathogens, for their part, directly kill the infected tissues to retrieve nutrients from dead or dying cells. Finally, hemibiotrophic pathogens are a mix between the two previous categories, as they first act as biotrophic organisms to multiply in the host, and then as nectrotrophic ones in order to kill the plant to retrieve everything they need to proliferate (Pieterse et al., 2009). A well-known hemibiotroph is the phytopathogen *Pseudomonas syringae* (*P. syringae*), that is described in detail below.

Pathogens can enter in the apoplast, which is the intercellular space, through wounds or natural opening like stomata in leaves, for instance. Or they can indirectly affect the interior of the plant via the secretion and injection of some compounds that are harmful for the plant. In some particular situation, bacteria like *Xylella fastidiosa* can enter into the xylem, a water transport network of vessels, which allows it to escape the plant recognition and to cause diseases in plants of economic interest such as grapevine (Bucci, 2018; Cella et al., 2018).

4.1. The plant immune system

The ability of plants to defend themselves against pathogens is one of the key factors determining their fitness. Plants have evolved diverse mechanisms to prevent damages caused by harmful organisms. There is a perpetual struggle between plants and bacteria, because plants can recognize pathogens through different mechanisms; while pathogens developed strategies to counteract plants defenses by secretion of effectors (Dodds and Rathjen, 2010). Finally, there is a co-evolution between pathogen strategies to attack plants and plant defenses.

4.1.1. Constitutive barriers against pathogens

Plants first defense mechanisms are independent of the pathogen, and they are based on the plant constitutive physical barriers which are the cuticle, the wax or pecto-cellulosic walls, but also the stomatal closure (Chisholm et al., 2006). Other actors of

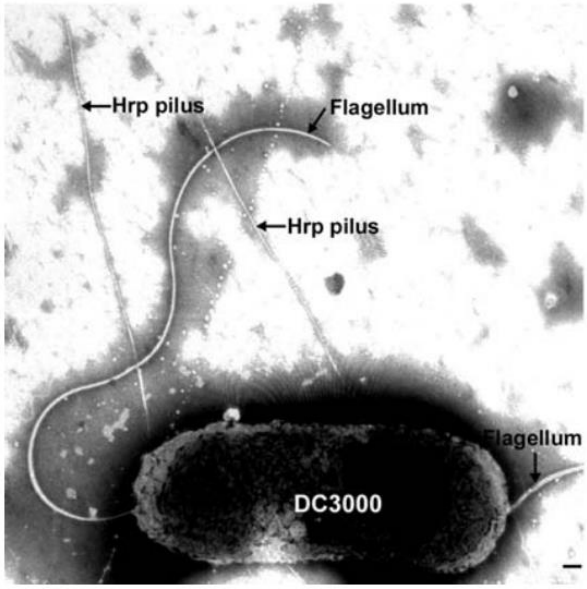

Figure 5. Transmission electron microscope image of *Pseudomonas syringae* pv. *tomato* DC3000. *Pst*DC3000 produces polar flagella (15 nm in diameter) and a few Hrp pili (8 nm in diameter). The flagella and Hrp pili are indicated with arrows. Flagella enable bacteria to swim toward or away from specific chemical stimuli. Hrp pili are involved in type III secretion of avirulence and virulence proteins. Extracted from Katagiri *et al.,* 2002.

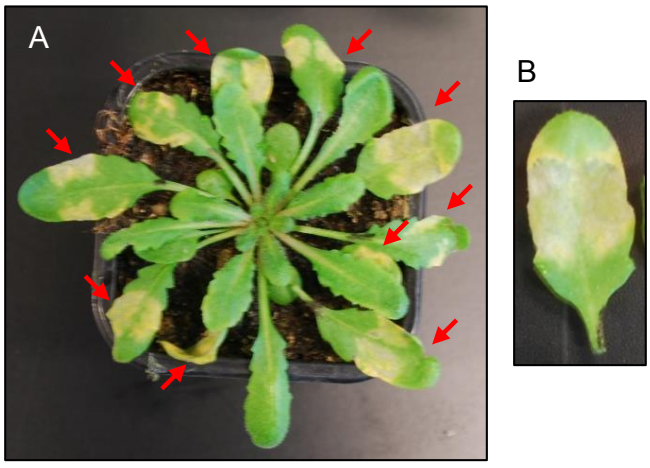

Figure 6. Disease symptoms in *Arabidopsis thaliana* Col-0 leaves following the infection by *Pseudomonas syringae* pv. *tomato* DC3000. (A) Leaves indicated with red arrows were syringe-infiltrated with 10^5 cfu. mL^{-1} of *Pst*DC3000. Picture was taken 6 days post infection. (B) A close-up of an infected leaf clearly shows the chlorotic lesion due to the pathogen.

this first line of defense are some anti-microbial compounds such as flavonoids for example (Cowan, 1999; Cushnie and Lamb, 2005, 2011) that can be excreted and which are efficient against a wide range of bacteria (Jones and Dangl, 2001). These barriers may stop the establishment of the infection. However, sometimes bacteria manage to overcome these constitutive defenses. Thus, plants have evolved strategies to detect them and activate their immune system in order to resist the attack (Chisholm et al., 2006; Jones and Dangl, 2006). A good system to study the plant defense is the *Arabidopsis thaliana*/*Pseudomonas syringae* system (Tsuda et al., 2008). This model of interaction is sometimes unappreciated because there is no naturally occurring infection of *A. thaliana* by *P. syringae* in nature, and we have to employ artificial inoculation methods in the laboratory, which are not exactly representative of a natural infection (Katagiri et al., 2002). However, this is a good model for understanding the plant immune system and it allows a comparative study of the sensitivity of two plants to the same pathogen, in the same conditions.

4.1.2. *Pseudomonas syringae*, a model pathogen

The gamma Proteobacteria *P. syringae* is probably one of the most studied bacteria in the context of plant-microbe interactions (Baltrus et al., 2017). It is a Gram-negative bacteria, rod-shaped and with a polar flagella (**Figure 5**) (Hirano and Upper, 2000; Katagiri et al., 2002). *P. syringae* is considered as a hemibiotroph. It is first an epiphyte present in the phyllosphere before entering quickly in the plant through natural openings such as stomata on leaves. Afterward, it becomes an endophyte localized in the apoplast which is the intercellular space, capable to infect plant tissues (Hirano and Upper, 2000).

There are 255 strains of *P. syringae* (Lifemap, NCBI), that have evolved to interact with a wide range of plants. The specialization within the species led each one to interact preferentially with a specific host (Hirano and Upper, 2000), leading to a classification in pathovars. *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*DC3000) is a pathovar responsible for bacterial speck of tomato, which is at the origin of its name. Since it is also virulent against the model plant *A. thaliana*, this phytopathogen is widely used in laboratory (Lewis et al., 2015; Whalen et al., 1991). *Pst*DC3000 enters

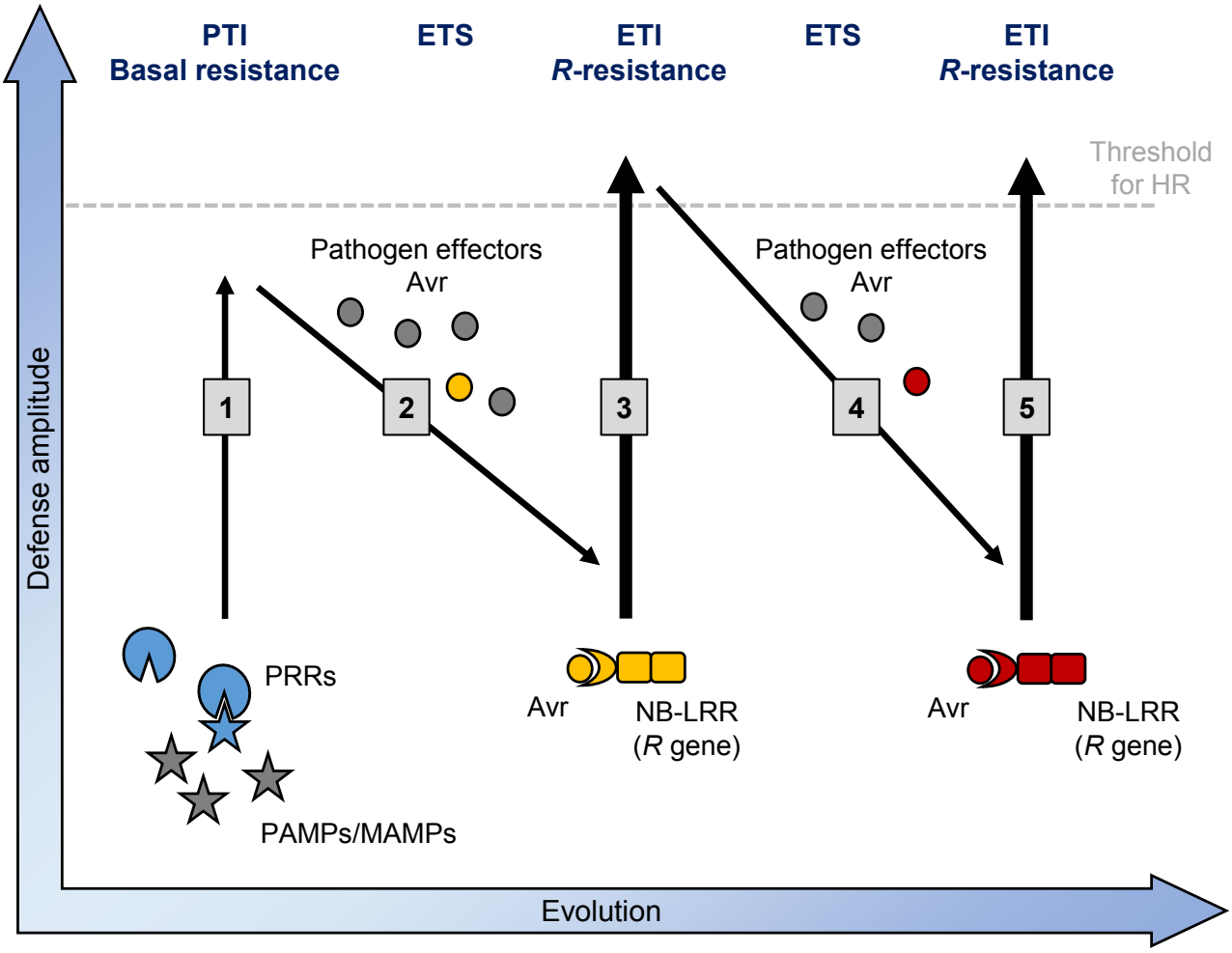

Figure 7. Zigzag model representation of the plant immune system. In phase 1, plants recognize pathogen/microbial-associated molecular patterns (PAMPs/MAMPs) via their PRRs (in blue) to trigger PAMP-triggered immunity (PTI). In phase 2, some pathogens are able to counteract the PTI by secreting effectors (Avr) (in yellow), resulting in effector-triggered susceptibility (ETS). In phase 3, an effector is recognized by an NB-LRR protein encoded by a Resistance gene (*R*) (in yellow), resulting in effector-triggered immunity (ETI). The ETI is an amplified PTI that reaches a threshold allowing the induction of hypersensitive response (HR). In phase 4, pathogens have evolved to produce new effectors (in red) allowing to suppress ETI. In phase 5, plants have in turn evolved to fight pathogens and allow ETI again thanks to new NB-LRR proteins (in red). Adapted from Jones and Dangl, 2006.

the plant leaves and then multiplies in the apoplast. After multiplication, we can observe symptoms on the infected leaves that are characterized by “water-soaked” patches only 2 days post infection. The patches look like necrotic lesions after 3 days post infection and the surrounding tissue becomes chlorotic (**Figure 6**). These symptoms that are characteristic of speck disease are clearly visible on *A. thaliana* (Cuppels, 1986; Katagiri et al., 2002; Melotto et al., 2006).

The complete genome sequence of *Pst*DC3000 was obtained in 2003 and is about 6.5 megabases. It contains a circular chromosome and two plasmids, encoding a total of 5 763 genes. Among them, genes encoding type III secretion system (T3SS) actively participate in the pathogenicity of the bacteria; and a total of 298 genes were identified as implicated in its virulence (Buell et al., 2003). Interestingly, loss-of-function mutations in the T3SS abrogates the disease formation, demonstrating that effectors injection into the cells is necessary for *P. syringae* pathogenesis (Collmer et al., 2000).

4.2. The zigzag model of the plant immunity

The current view of the plant immune system is usually represented as a zigzag model (Jones and Dangl, 2006) showing the perpetual confrontation between plants and pathogens that can be compared to an armament race. The evolution of the plant defense mechanisms and pathogen attack mechanisms can be split into 5 steps described below (**Figure 7**). It is important to remember that these different phases appeared during evolution to improve the persistence of each organism.

4.2.1. Phase 1: recognition of pathogens inducing the PTI

Following the infection by pathogens, plant resistance is driven by two distinct perception mechanisms (Jones and Dangl, 2006). These perception mechanisms can act together to induce the plant resistance. Both are based on the recognition of pathogen motifs or effectors by plant receptors, and they lead to two different levels of defense. The first level is the pathogenic associated molecular patterns (PAMP)-triggered immunity (PTI), and the second one is the effector triggered immunity (ETI) mentioned below.

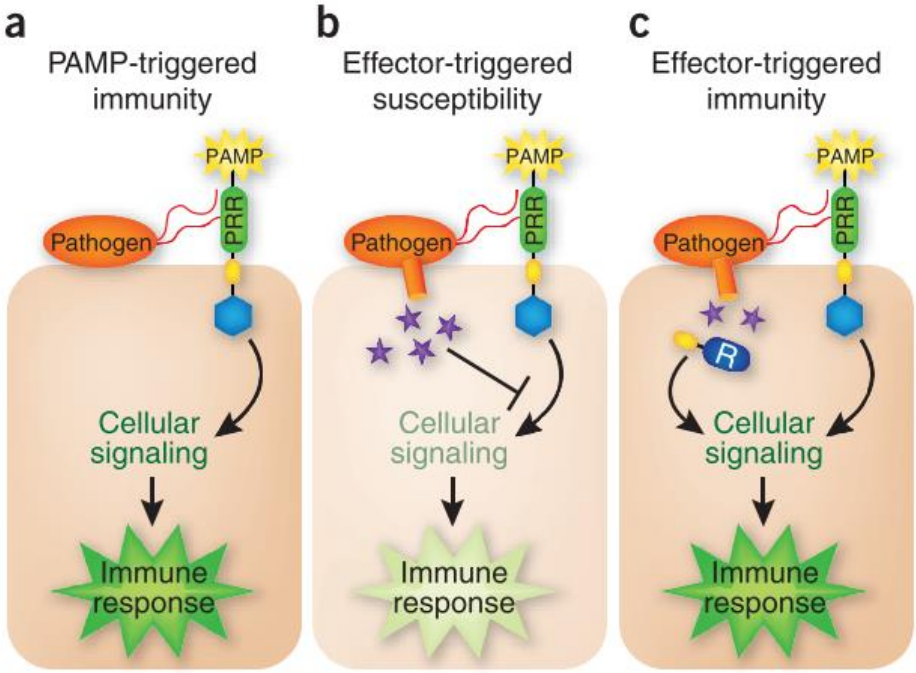

Figure 8. Simplified schematic representation of the plant immune system. (a) Upon pathogen attack, pathogen-associated molecular patterns (PAMPs) activate pattern-recognition receptors (PRRs) in the host, resulting in a downstream signaling cascade that leads to PAMP-triggered immunity (PTI). (b) Virulent pathogens have acquired effectors (purple stars) that suppress PTI, resulting in effector-triggered susceptibility (ETS). (c) In turn, some resistant plants have acquired resistance (R) proteins that recognize these specific effectors, resulting in a secondary immune response called effector-triggered immunity (ETI). Extracted from Pieterse et *al.,* 2009.

Pathogen motifs that can be recognized by plants correspond to some conserved bacterial components, referred as pathogenic associated molecular patterns (PAMPs). These patterns are also referred as microbial associated molecular patterns (MAMPs) because they are present not only in pathogenic but also in non-pathogenic microbes. These molecular signatures, such as flagellin, lipopolysaccharide (LPS), peptidoglycan or elongation factor Tu (EF-Tu) for instance, are recognized as non-self by transmembrane pattern recognition receptors (PRRs) found at the surface of the host cells (Jones and Dangl, 2006; Nürnberger and Lipka, 2005). Cell-surface receptors can also recognize damage associated molecular patterns (DAMPs) representing altered-self molecules, resulting from damages caused by microbes (Boller and Felix, 2009).

A good example of recognition mechanism is the recognition of the flagellin, the major component of the bacterial flagellum. This protein contains a conserved peptide of 22 amino acids (flg22), that is recognized as a MAMP by leucine-rich repeat (LRR) domains of the plant PRR FLAGELLIN-SENSING 2 (FLS2) (Boller and He, 2009). This small peptide is sufficient for the plant to induce many cellular responses, among which the induction of the expression of more than 1 000 genes in *A. thaliana* (Jones and Dangl, 2006). Interestingly, the same flagellin recognition mechanism occurs in animals thanks to the Toll-like receptor TLR5 (Boller and Felix, 2009) which recognizes another domain of flagellin. We can also mention another well-known PRR of *A. thaliana*, EF-Tu receptor (EFR), that recognizes a peptide of 18 amino acids (elf18) of the pathogen elongation factor Tu (EF-Tu) which is one of the most abundant protein in bacterial cells (Abramovitch et al., 2006). FLS2 and EFR activation both trigger the association with LRR (leucine rich repeat) kinase BAK1 (BRI1-ASSOCIATED RECEPTOR KINASE1), which then participates in the subsequent signal initiation.

The pathogen recognition initiates the basal immunity of the plant, referred as PAMP-triggered immunity (PTI) (Cunnac et al., 2009). PTI is efficient against non-adapted pathogens, those that did not evolve in such a way to resist to the plant defenses (**Figure 8, A**). This response exhibits similarities with the innate immunity in animals and can also be called non-host resistance (Jones and Dangl, 2006; Nürnberger and Lipka, 2005).

The first detectable modification following the pathogen detection is the modification of ion channel activities leading to ions fluxes across the plasma membrane. This is accompanied by the oxidative burst characterized by the production

of reactive oxygen species (ROS) such as superoxide (O_2^-) for instance (Mcdowell and Dangl, 2000; Scheel, 1998). These ROS can act as antimicrobial compounds at high concentrations, or signal molecules at low concentrations. But PTI is also characterized by the quick induction of defense responses such as callose and lignin deposition to reinforce the cell membrane; activation of mitogen-activated protein kinases (MAPKs) cascades for the signal transduction after pathogen recognition; and expression of a broad range of defense-related genes (Boller and Felix, 2009; Deslandes and Rivas, 2012; Tsuda et al., 2008).

Moreover, *A. thaliana* is also known to produce molecules called phytoalexins such as camalexin, which represent an additional defense pathway (Thomma et al., 2001). Camalexin has a direct toxicity against a wide range of pathogens by disrupting the integrity of bacterial membranes (Glawischnig, 2007; Nawrath and Métraux, 1999). Phytoalexins deficient *A. thaliana* mutant, *pad3-1* that are impaired in the synthesis of camalexin, are more susceptible to infection by the necrotrophic fungus *Alternaria brassicicola*, even if they do not show increased susceptibility to other pathogens such as *P. syringae* (Glazebrook and Ausubel, 1994; Thomma et al., 1999). However, *A. thaliana* affected in *pad1* and *pad2* are more susceptible to *P. syringae*. Thus, phytoalexins themselves could be required for the limitation of the pathogen growth, or the precursor of phytoalexins could also have antimicrobial activities, for instance (Glazebrook and Ausubel, 1994).

In addition, other molecules may be indirectly involved in the plant response to pathogens. For instance, it has been shown that in *Nicotiana benthamiana*, phytosterols are probably implicated in resistance against some pathogens such as *P. syringae* or *Xanthomonas campestri*. Actually, a deficit in phytosterols leads to an increased nutrient efflux into the apoplast, allowing pathogens to grow easily (Wang et al., 2012a). Sterol biosynthesis in *A. thaliana* is also important for the plant immunity against some pathogens, as there is an increased amount of stigmasterol resulting from β-sitosterol conversion upon inoculation with non-host pathogen (Griebel and Zeier, 2010; Wang et al., 2012a).

4.2.2. Phase 2: pathogen effectors leading to ETS

During evolution, well-adapted pathogens such as *P. syringae* have developed strategies to overcome the plant defense mechanisms (**Figure 7**). To successfully colonize the plant, *Pst*DC3000 has evolved a wide variety of virulent effectors, also called elicitors, able to antagonize the PTI, resulting in enhanced virulence called effector triggered susceptibility (ETS) (**Figure 8, B**) (Deslandes and Rivas, 2012; Jones and Dangl, 2006).

Pathogen effectors promote pathogenicity thanks to diverse enzymatic activities. Some effectors are cell wall degrading enzymes, or small molecules such as toxins that are produced and secreted by bacteria to overcome the immune responses. Some bacteria also produce exopolysaccharides (EPS) in order to mask their PAMPs to escape the plant immune system. The most effective strategy for bacteria to deliver effectors into the plant cell is their injection via T3SS (Abramovitch et al., 2006), giving rise to another name, type III effectors (Chang et al., 2005; Lewis et al., 2015; Petnicki-Ocwieja et al., 2002). Once they are in the cells, effectors can contribute to pathogen virulence, bacterial multiplication and the development of disease symptoms (Guo et al., 2009; Staskawicz et al., 2001). Thus, pathogen effectors provide a beneficial environment for bacteria to complete their lifecycle. Every strain of pathogenic bacteria can deliver 15-30 effectors into host cells, increasing possibilities to bypass the plant defense (Jones and Dangl, 2006). For example, *Pst*DC3000 possesses 37 T3SS effectors (Xin et al., 2018), among which a subset of 8 effectors is sufficient to confer nearly full virulence to the bacteria (Cunnac et al., 2009; Wei et al., 2015). To deepen about T3SS, they are encoded by *hypersensitive response and pathogenicity* (*hrp*) and *hrp conserved* (*hrc*) genes; and effector proteins are encoded by *Hrp outer protein* (*hop*) genes or avirulence genes (*avr*) (Collmer et al., 2000; Nomura et al., 2005). These *hrp/hrc* genes are responsible for the HR response in nonhost or resistant plants that are able to recognize them, but they are also required for pathogenesis in susceptible plants (Cunnac et al., 2009). Indeed, *Pst*DC3000 *hrp* mutants do not multiply or cause disease in *A. thaliana*, which confirms the essential role of these genes in the pathogen successful attack (Hauck et al., 2003).

Among *Pst*DC3000 effectors or avirulence proteins, AvrPto is one of the best-studied protein acting at the beginning of the infection by interfering with callose

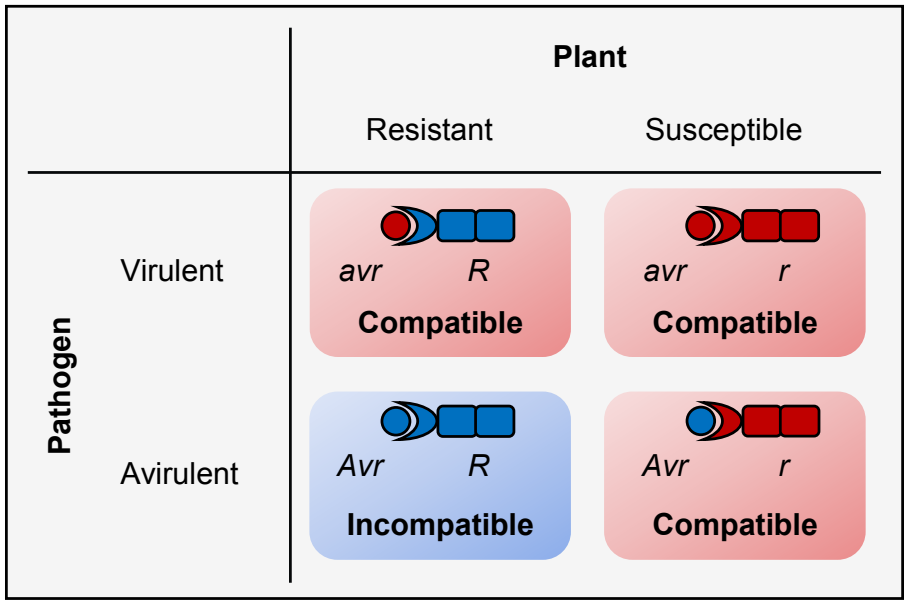

Figure 9. Gene-for-gene concept introduced by Flor in 1971. The interaction between a pathogen avirulent (*Avr*) gene product and a host resistant (*R*) gene product leads to an incompatible interaction, the plant is able to recognize the pathogen and is resistant (in blue). If the same pathogen avirulent (*Avr*) gene product interacts with a host susceptible (*r*) product, or if the pathogen is virulent, carrying *avr* gene product, the pathogen is able to escape the plant immune defense, the interaction is compatible, leading to plant disease (in red).

deposition in the plant cell wall to suppress basal defense in a salicylic acid-independent manner (Collmer et al., 2000; Hauck et al., 2003; Nomura et al., 2005). Thus, if this effector triggers resistance in plants carrying the protein Pto and the associated resistance protein Prf, it promotes infection in susceptible plants. Basically, AvrPto acts upstream of the MAPK cascade to inhibit the establishment of the PTI (Xiang et al., 2008). This effector is capable to interact with FLS2/BAK1 and EFR, two well-known PRRs of plants which recognize bacterial flagellar peptide flg22 and EF-Tu peptide elf18, respectively, normally leading to the activation of the plant defense (Chisholm et al., 2006; Deslandes and Rivas, 2012; Xiang et al., 2008).

4.2.3. Phase 3: evolution of the plant defense leading to the ETI

Pressure from pathogens effectors led plants to develop a second line of defense during evolution if PTI is not sufficient to resist against pathogens attack. This defense mechanism called effector triggered immunity (ETI) is able to counteract ETS (**Figures 7 and 8, C**). This response formerly called *R*-gene based resistance (Boller and Felix, 2009) is highly specific, and has similarities with the adaptative immunity in animals. ETI mechanism involves the interaction between pathogen effectors and the products of the plant specific disease resistance *R*-genes. Most of these *R*-genes code for NB-LRR proteins, because of characteristic nucleotide binding (NB) and leucine rich repeat domain (LRR) (Jones and Dangl, 2006). This concept is also known as the gene-for-gene concept (**Figure 9**) (Flor, 1971; Glazebrook, 2005). There are about 125 *R*-genes in *A. thaliana* Columbia-0 (Col-0) ecotype that can be involved in the pathogen recognition (Jones and Dangl, 2006). Pathogens carrying avirulence genes (*Avr*) that are recognized by the host plant carrying resistance *R*-gene and therefore fail to induce disease are called avirulent pathogens, and the interaction is incompatible (Glazebrook, 2005). Thanks to this gene-for-gene recognition, the ETI will stop the avirulent pathogen growth (Chisholm et al., 2006; Jones and Dangl, 2006; Mcdowell and Dangl, 2000).

PTI and ETI are responsible of the same kind of response by the plant, but this response is qualitatively stronger and accelerated in the case of ETI. ETI induces the resistance and usually, the response is strong enough to reach a threshold inducing

the Hypersensitive Response (HR), characterized by programmed cell death at the infection site (Greenberg and Yao, 2004; Jones and Dangl, 2006). This cell death allows to limit the access of the pathogen to water and nutrients necessary for its growth (Glazebrook, 2005). As for the PTI, MAPK cascades are essential in the transmission of the pathogen recognition signal to the plant cell to activate the defense mechanisms (Abramovitch et al., 2006; Asai et al., 2002; Pedley and Martin, 2005).

4.2.4. Perpetual co-evolution of plants defense and pathogens effectors

In response to the ETI, the evolution led some virulent pathogens to acquire additional effectors or to diversify the ones they already had to escape the plant defense and suppress the ETI, leading to a new phase of ETS (**Figure 7**). These effectors could have enzymatic activities modifying or degrading targets in signaling pathways, such as phosphatase activity against MAPKs for instance (Abramovitch et al., 2006; Pedley and Martin, 2005). At this stage, pathogens are well adapted and the plant developed specific responses to defend. As for the first occurrence of ETS, effectors are commonly injected inside the host cells via T3SS.

Successful pathogens that managed to evade the plant detection in turn led the host plant to evolve in order to respond back by selection for novel *R*-genes that should be able to recognize other effectors, so that ETI can be triggered a second time to definitely eliminate the pathogen (**Figure 7**).

In this model, we can clearly understand that there is a coevolution between plants and pathogens, that led plants to develop defenses against pathogens, which then developed ways to bypass these defenses, leading plants to react back and so on. The final outcome of this battle depends on the abilities of each interactant to fight against the other, which are defined by their genetic features.

4.3. Systemic defenses of the plant

Once the pathogen is recognized by the plant at the infection site thanks to the mechanisms explained above, systemic defenses are deployed quickly to protect other

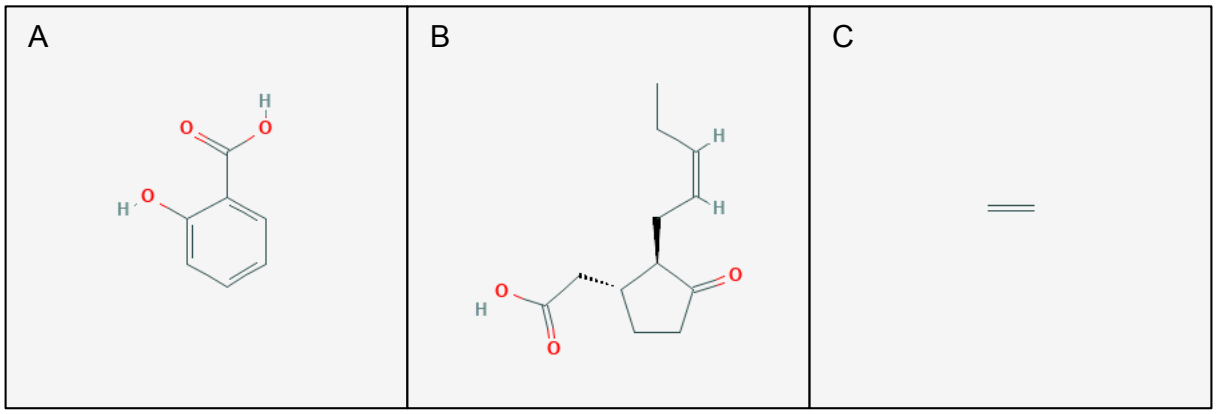

Figure 10. Structures of salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). These three hormones are the main ones acting as signaling molecules in the plant defense regulation. SA (A) is mainly implicated in the defense against biotrophic pathogens, while JA (B) and ET (C) are mainly involved in the response against necrotrophic pathogens. Structures from PubChem.

parts of the plant (Mcdowell and Dangl, 2000). It only takes few minutes to activate local plant defense responses, and systemic defense responses in distant tissues are activated within hours. Nonetheless these responses are resource-intensive, and they cause some collateral damages on host tissues. Thus, plants must limit these responses to the proper place and time (Mcdowell and Dangl, 2000).

Activation of the plant defenses rely on a complex regulatory network of hormones dependently to the kind of pathogen attacking the plant (Jones and Dangl, 2006; Pieterse et al., 2012). Basically, three main hormones act as signaling molecules in plants to regulate their defense against pathogens: salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) (**Figure 10**). Globally, there is a balance between SA, which is efficient against many biotrophs, on one hand, and JA and ET that rather promote defense against necrotrophic pathogens, on the other hand (Glazebrook, 2005; Thomma et al., 2001). The wound response efficient against herbivores is also regulated by the JA signaling pathway (Pieterse et al., 2009). These hormones are involved in the establishment of induced resistance in local and distant tissues of the plant.

The outcome of these signaling pathways is the effectiveness of the plant defense against a broad range of pathogens and its preparation to further infection (Ton et al., 2002). The induced disease resistance is manifested by a less effective disease and a restriction of the colonization by the pathogen, in comparison to plants that are not in this state of induced resistance that we can qualify as a “primed” state.

4.3.1. Salicylic acid: implication in Systemic Acquired Resistance (SAR)

SA is mainly active against biotrophic and hemibiotrophic pathogens such as *Pst*DC3000. It is first a local and then a systemic signal transported via the plant phloem to distant uninfected tissues. This hormone is a key regulator of pathogen-induced systemic acquired resistance (SAR) (Uknes et al., 1992). SAR could also be activated when the plant is attacked by fungi or viruses, not only bacteria.

Following the pathogen detection, some genes implicated in SA biosynthesis and others implicated in the regulation of its biosynthesis are activated in order to allow the hormone accumulation. Signaling downstream of SA is mainly under the control of the

regulatory protein NON-EXPRESSOR OF *PR* GENES1 (NPR1), a transcriptional coactivator of some defense-related genes such as *PATHOGENESIS RELATED 1* (*PR-1*) (**Figure 11**) (Kunkel and Brooks, 2002; Pieterse et al., 2012). Moreover, WRKY transcription factors play an important role in the regulation of SA-dependent responses. For instance, in *A. thaliana*, WRKY70 induces the expression of SA-responsive *PR* genes and concomitantly suppresses the expression of JA-responsive marker gene *PLANT DEFENSIN1.2* (*PDF1.2*) (Koornneef and Pieterse, 2008). For activation of SAR in distant tissues, a long-distance signaling cascade is essential. This requires the lipid-transfer protein DEFECTIVE IN INDUCED RESISTANCE1 (DIR1) that acts as a chaperone for the mobile SAR signal (Maldonado et al., 2002). Methyl ester of SA (MeSA) is one of the long-distance mobile signals involved in the establishment of SAR in distant tissues.

SA potentiates many plant defense responses and induces the activation of some pathogenesis-related genes (*PR* genes) expression also classified as effector genes, even in tissues distant from the infection site, allowing the protection of the plant, notably from further infection (Glazebrook, 2005). Some *PR* genes implicated in SAR encode pathogenesis-related proteins such as glucanases, chitinases, or enzymes involved in the biosynthesis of phytoalexins (Scheel, 1998). These proteins can be found directly in the apoplast, in such a way that they can come in contact with the pathogen during the infection process (Hammerschmidt, 1999; Van Loon, 1997). In *A. thaliana*, some of these *PR* genes such as *PR-1*, *PR-2* and *PR-5* that encode antimicrobial proteins are SAR marker genes (Nawrath and Métraux, 1999; Thomma et al., 2001; Uknes et al., 1992). PR-2 is a β-1,3-glucanases, and PR-5 is a thaumatin-like protein implicated in the mechanism of defense, while the mode of action of PR-1 remains poorly understood. *PR-1* and *PR-5* are often strongly induced and they seem to affect the plant cell membranes (Van Loon, 1997). *PR-2* and *PR-5* genes can also be induced by an SA-independent pathway, independently from *PR-1*, highlighting their importance in the plant immune response (Thomma et al., 2001). Contrary to the induction of phytoalexins or cell wall rigidification by callose deposition that are local reactions, accumulation of pathogenesis-related proteins extends into non-inoculated parts of the plant that, upon challenge, exhibit acquired resistance (Van Loon, 1997).

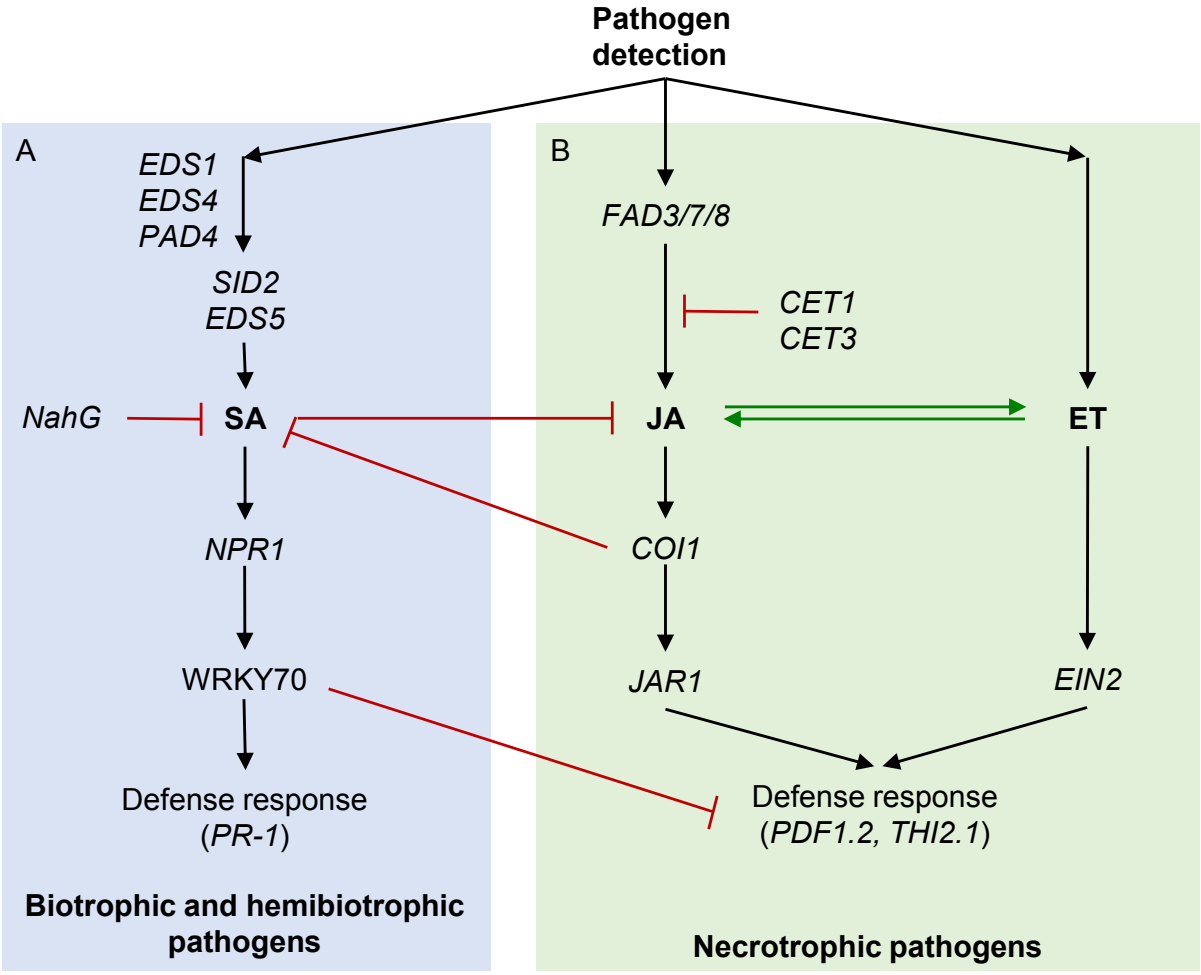

Figure 11. Model of the SA, and JA/ET signaling pathways in *Arabidopsis thaliana*. Following the pathogen detection, SA, JA and ET pathways could be activated depending on the upstream signal. (A) The SA pathway is activated upon detection of a (hemi)biotrophic pathogen. SA biosynthesis requires the activation of *SID2* (*SA INDUCTION DEFICIENT2*) and *EDS5* (*ENHANCED DISEASE SUSCEPTIBILITY5*) and is controlled by *EDS1*, *EDS4* and *PAD4* (*PHYTOALEXIN DEFICIENT4*). The SA-degrading enzyme salicylate hydroxylase (NahG) exerts a retro-control on the SA biosynthesis. SA leads to the activation of *NPR1* (NON-EXPRESSOR OF *PR* GENES1), which is directly implicated in the activation of some defense-related genes such as *PR-1*. Moreover, WRKY70 transcription factor induces the expression of SA-responsive *PR* genes and concomitantly suppresses the expression of JA-responsive marker gene *PLANT DEFENSIN1.2* (*PDF1.2*) (Koornneef and Pieterse, 2008). (B) In contrast, following the detection of necrotrophic pathogens, JA is synthetized thanks to *FATTY ACID DESATURASE3/7/8* (*FAD3/7/8*), and is then perceived by *CORONATINE INSENSITIVE1* (*COI1*) that acts in proteolysis, and *JASMONIC ACID RESISTANT1* (*JAR1*) that can form JA-Isoleucine, the active form of JA. This leads to the activation of defense effectors, including *PDF1.2* and *THIONIN2.1* (*THI2.1*). CONSTITUTIVE EXPRESSOR OF THIONIN1 (CET1) and CET3 act as negative regulators of JA biosynthesis, exerting a last level of JA regulation. ET biosynthesis leads to the activation of *ETHYLENE INSENSITIVE2* (*EIN2*) upstream of the induced defenses characterized by *PDF1.2* and *THI2.1*. There is a connection between both pathways, leading to a regulation of one by another. Positive regulatory interactions between these signaling pathways are indicated by green arrows, antagonistic interactions by red lines. Adapted from Kunkel *et al.*, 2002.

4.3.2. Jasmonic acid and Ethylene: implication in a SA-independent resistance

In addition to the response based on SA for (hemi)-biotrophic pathogens, necrotrophic pathogens such as *Botrytis cinerea* and insects or wounding, for their part, induce a response based on the combination of JA and ET in the plant. In *A. thaliana*, the detection of necrotrophic pathogens is followed by the biosynthesis of JA which leads to the formation of conjugates between JA and several amino acids, including isoleucine (Ile) to form JA-Ile, the active form of JA. This leads to the activation of defense effectors, including *PLANT DEFENSIN1.2* (*PDF1.2*) and *THIONIN2.1* (*THI2.1*), which are also under the control of ET. In response to necrotrophs, *A. thaliana* also induces the biosynthesis of ET, followed by the activation of *ETHYLENE INSENSITIVE2* (*EIN2*) upstream of the induced defenses characterized by the below mentioned *PDF1.2* and *THI2.1* (**Figure 11**) (Glazebrook, 2005; Kunkel and Brooks, 2002).

4.3.3. Interconnection between both pathways

It is important to mention that SA and JA/ET defense pathways are considered as mutually antagonistic, giving rise to the apparition of new mechanisms evolved by bacteria to exploit this to overcome SA-mediated defense (Kunkel and Brooks, 2002). This is part of the armament race previously mentioned. Some pathogens such as *P. syringae* belongs to pathogens taking advantage from this balance. Indeed, this pathogen synthetizes coronatine, a non-host-specific phytotoxin that is not essential for pathogenicity but enhances virulence and symptoms development (Bender et al., 1996; Hauck et al., 2003). This molecule exhibits structural similarity with jasmonic acid-isoleucine (JA-Ile), the active form of JA, supporting the idea that the toxin acts as a molecular mimic of JA-Ile. More precisely, the coronafacic acid moiety of coronatine is structurally and functionally analogous to JA, which is produced by the plant in response to stress; and the coronamic acid moiety is derived from isoleucine (Bender et al., 1996). Thus, coronatine activates the JA signaling response which inhibits the SA-mediated host response normally active against biotrophs, because of the balance between the two pathways. In addition, coronatine has been shown to

prevent stomatal closure usually induced by the plant after the pathogen detection, and thus facilitates bacterial entry into the leaves (Melotto et al., 2006).

Interestingly, basal resistance against *Pst*DC3000 was found to be affected in both NahG plants expressing the bacterial salicylate hydroxylase (*nahG*) gene (SA signaling pathway) and in JA- and ET-response mutants (Pieterse et al. 1998), suggesting that activation of both the SA-dependent pathway and the JA/ET-dependent pathway may act together to enhance the plant protection thanks to the systemic resistance (Ton et al., 2002). Thus, these responses are compatible and additive (van Wees et al., 2000). Coronatine and T3SS effectors also induce numerous ABA-responsive genes in *A. thaliana*, suggesting that diverse mechanisms of defense are solicited to fight against *Pst*DC3000 (Thilmony et al., 2006). Nevertheless, genes contributing to the defense against *Pst*DC3000 appear to be mainly involved in the SA-dependent signaling pathway. This is validated by the fact that plants carrying the NahG transgene responsible for SA degradation are more affected by *Pst*DC3000 infection (Katagiri et al., 2002; Thomma et al., 2001).

4.3.4. Other molecules implicated in the plant systemic resistance

Finally, the classical view of two main signaling pathways requires revisions because additional plant hormones may have an impact on the balance between these two hormones signaling pathways (Jones and Dangl, 2006). Recently, abscisic acid (ABA), auxins, gibberellins, brassinosteroids and cytokinins have emerged as other keys implicated in plant signaling (Kumar, 2014; Pieterse et al., 2012). Particularly, ABA is known for its role in abiotic stress tolerance for a long time, but recent evidences show that it also has a role in biotic stress tolerance (Asselbergh et al., 2008; Ton et al., 2009). Indeed, it seems that the role of ABA is not clearly established since this molecule can act as a positive or a negative regulator of disease resistance to both necrotrophic or biotrophic pathogens by interfering with signaling pathways implicated in biotic stress resistance (Asselbergh et al., 2008). A wide range of mechanisms underlying the role of this hormone in biotic stress have been suggested, considering the interaction with SA- and JA/ET- pathways, suppression of ROS, induction of stomatal closure or stimulation of callose deposition, for instance. Actually, SA or JA/Et

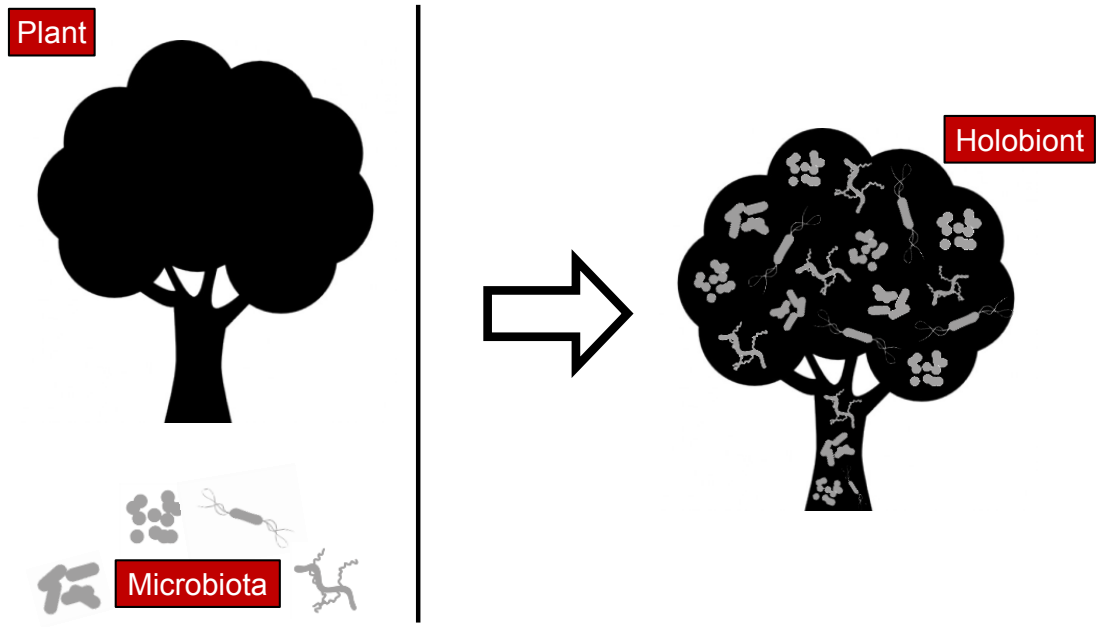

Figure 12. Schematic representation of the holobiont concept. The holobiont concept has been popularized by Rosenberg and Zilber-Rosenberg in 2007 and consists in considering the host and its associated microbiota as an additional organismal level in addition to the ones previously considered independently.

hormonal signalization mainly depend on the nature of the pathogen lifecycle, while ABA induction rather depends on the timing of recognition, the activity of effectors, or the plant tissue which is affected (Ton et al., 2009).

5. Interactions with non-pathogenic micro-organisms

Interactions with pathogenic micro-organisms previously developed are not the more common interactions between plants and micro-organisms. Actually, we should take in consideration mutualist micro-organisms that form a community naturally interacting with the plant.

5.1. The holobiont concept

For a long time, we were used to consider the plant as a single fully-fledged and autonomous organism. In reality, plants are directly and indirectly interacting with millions of micro-organisms both inside and outside their tissues that we did not consider at first sight. These micro-organisms may play a role on the plant physiology and health. That is why plants can no longer be considered as standalone entities and researchers tend to have a more holistic vision nowadays, considering the plant *per se* and its microbiota collectively as an entity, an additional organismal level compared to the ones previously considered independently (Vandenkoornhuyse et al., 2015).

We refer to this additional organismal level as the “holobiont”, a term that was first proposed by Lynn Margulis in 1991 to describe a host and a single symbiont together as a biological entity (Simon et al., 2019). The notion was further extended to the description of a host and all the micro-organisms interacting with it (**Figure 12**). It is also extended to the “hologenome” concept introduced by Ilana Zilber-Rosenberg and Eugene Rosenberg in 2007, represented by the host and microbes genomes (Rosenberg and Zilber-Rosenberg, 2018; Rosenberg et al., 2007). The hologenome concept is based on 4 basic principles according to Rosenberg and Zilber-Rosenberg. The first principle is that all hosts (animals and plants) harbor complex microbiota and are thus considered as holobionts that function generally as a distinct entity. The second principle consists in the consideration of the entire holobiont as a huge distinct

unit among which different partner have specific roles allowing the ensemble to live on earth. Interestingly, the third principle proposes that a fraction of the microbiome genome is considered to be transmitted together with the host genome to the next generation to propagate properties of this holobiont. For instance, even if a large part of the root endosphere microbiota is considered to be reestablished when the plant germinates (van der Heijden and Schlaeppi, 2015), it has been shown that there is a vertical transmission of some bacteria via the seeds in some plant species, among which grass species (Barret et al., 2015; Vannier et al., 2018). Finally, the fourth principle of the hologenome assumes that there is a role of the microbiome in the adaptation and evolution of the holobiont (Rosenberg and Zilber-Rosenberg, 2018). Bacteria from the microbiota are not just associated with their host by chance, but they are required for the plant to grow and survive in different ecosystems (Hardoim et al., 2008).

5.2. Techniques for the study of plant-bacteria interactions

The emergence of the holobiont concept led researchers to study interactions between plants and their associated communities, first by the identification of the bacteria that form the microbiota and then by the study of their effect on plants.

5.2.1. Identification of the bacterial communities interacting with plants

Thanks to the development of the environmental microbial and genomic approaches last decades (Guttman et al., 2014), a precise inventory of the bacterial communities interacting with many plants including *A. thaliana* (Bai et al., 2015; Bodenhausen et al., 2013; Bulgarelli et al., 2012; Horton et al., 2014; Lundberg et al., 2012; Schlaeppi et al., 2014), *Nicotiana tabacum* (Saleem et al., 2016; Santhanam et al., 2014) or *Hordeum vulgare* (Bulgarelli et al., 2015) has been done. These analyses are possible nowadays thanks to culture-independent community profiling methods coupled with metagenomic studies, avoiding the bias induced by non-cultivable bacteria (Guttman et al., 2014). The basis for making an inventory of the communities interacting with a host is commonly the amplification and sequencing of a part of the

gene coding the 16S rRNA which is distinctive to each bacteria (Bai et al., 2015; Bulgarelli et al., 2012, 2015; Lundberg et al., 2012; Saleem et al., 2016; Santhanam et al., 2014; Schlaeppi et al., 2014).

Lundberg *et al*. showed that the soil type and the compartment of the plant are the main drivers of diversity between microbiotas (Lundberg et al., 2012). The main source of bacteria considered as the “start inoculum” of the *A. thaliana* root microbiota is the soil, which harbors a huge diversity of bacteria; while the start inoculum of the leaf microbiota is probably more variable, with bacteria coming from the atmosphere, insects, or soil. However, despite this diversity that can be introduced by environmental conditions, it has been shown that there is also an extensive taxonomic overlap between the microbiota of the leaves and the one of the roots (Bai et al., 2015). In *A. thaliana*, the inventory of the microbiota of plants coming from different soils and at different developmental stages highlighted the existence of a core microbiota that is stable enough to remain the same independently of the soil, ecotype, or developmental stage (Bai et al., 2015; Bulgarelli et al., 2012, 2015; Lundberg et al., 2012; Saleem et al., 2016; Santhanam et al., 2014; Schlaeppi et al., 2014). More generally, the study of microbiotas interacting with *A. thaliana*, but also *Nicotiana tabacum* and *Hordeum vulgare* has highlighted the co-occurrence of four main bacterial phyla that are always found in the associated communities: *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria* (Bai et al., 2015).

5.2.2. Investigation of the effect of some bacteria on the plant phenotype and fitness

Once the interacting communities are identified, it is interesting to study their impact on the plant. For that purpose, Bai *et al*. created synthetic communities (SynComs) by isolating the majority of bacterial species constantly detectable by metabarcoding. It is important to notice that the plant-associated microbiota contains a relatively high cultivable fraction of bacteria (up to 85% of the culture-independent communities), allowing the creation of these SynComs (Bai et al., 2015; Bodenhausen et al., 2013; Burch et al., 2016). Synthetic communities constitute a great tool to assess the potential of a microbiota to colonize a host plant in a gnotobiotic system, meaning a

sterile system inoculated with a specific strain or community. Actually, it is challenging to determine which bacteria or combinations of bacteria are responsible of an effect on the plant phenotype and fitness. Thus, such resources together with the reproducibility of a gnotobiotic system will allow further studies on bacterial communities establishment and functions in laboratory conditions (Herrera Paredes et al., 2018). Such systems are already used in few laboratories, allowing the inoculation of a unique bacteria or synthetic communities to germ-free plants under controlled culture conditions. It is possible to use microboxes or plates containing solid medium allowing the plant growth for up to one month (Herrera Paredes et al., 2018; Innerebner et al., 2011). Another kind of system was developed to keep wheatgrass (*Agropyron cristatum* cv. CDII) in health for at least 70 days. In this case, plants were grown in sterile quartz sand within flow-through glass columns (Henry et al., 2006).

Different parameters can be assessed following the inoculation of bacteria in such systems. For instance, the effect of some bacteria on the host plant in stress conditions such as phosphate starvation has been analyzed. Herrera Paredes *et al*. thus demonstrated that it is possible to influence phosphate accumulation in the plant shoot in a controlled manner (Herrera Paredes et al., 2018). They demonstrated that it is possible to establish a link between the microbiota composition and the host phenotype, highlighting the utility of axenic culture systems for plant-bacteria interactions studies.

5.3. Beneficial bacteria associated with plants

The study of the interactions between plants and bacteria still gains some interest since there is an increasing need to optimize crop culture sustainability and productivity thanks to new methods such as bacterial inoculants (Finkel et al., 2017; Keven Vessey, 2003; Schütz et al., 2018). Indeed, the human population and the food needs are growing even faster, while the different kinds of stresses mentioned at the beginning of the introduction have harmful consequences on crop cultures worldwide. This disturbing situation forces humans to find solutions to improve agricultural conditions in order to protect plant cultures and reduce crop losses (Piasecka et al., 2019; Suzuki et al., 2014; Wang et al., 2003). In an agricultural context in which the aim is to reduce

the use of pesticides and chemical fertilizers, researchers are increasingly thinking about an alternative solution which is the utilization of bacteria that have beneficial, promoting growth effects on the plant, the so-called plant growth promoting bacteria (PGPB). The aim is to use the potential of microbial inoculants as biofertilizers, plant strengtheners, phytostimulators or biopesticides, depending on their mode of action and their impact on the plant.

Some PGPB are found in the rhizosphere and are named plant growth promoting rhizobacteria (PGPR). Relationships between PGPB and their host plant can be considered on different levels of complexity. In rhizospheric relationships, the PGPB remains outside of the plant, they bind on root or seed surface. In endophytic relationships that are less known, PGPB enter the tissues inside the plant, or the apoplast, and in the case of nitrogen fixation, some are intracellular. Finally, phyllospheric PGBP binds to leaf or stem surface (Glick, 2014; Keven Vessey, 2003). The positive effect of PGPB can be direct, by promoting the plant growth in absence of pathogens; but in can also be indirect, by protecting the plant against pathogens (Lugtenberg and Kamilova, 2009).

The use of PGPB that have powerful capacities to help the plant to grow and resist pathogens and their manipulation as biofertilizers or biocontrol agents is actually a really old concept, already used 300 before Christ. Even if bacteria were not already described at this time, the mixing of different soils, and thus different microbiotas, was a way to remedy troubles of culture (Keven Vessey, 2003). PGPB have already been used for years as single strain inoculants, but now some laboratories tend to innovate new biocontrol strategies involving microbial communities or strain mixtures (Dessaux et al., 2016). Indeed, the first commercialized bioinoculant was patented in 1896 (Finkel et al., 2017) and there are currently more than 190 products classified as “microbial inoculants” according to the Organic Materials Review Institute (OMRI). This number is increasing every year, showing a growing interest in the use of bioinoculants worldwide, with a market growth rate of 10% per year, because of its environmental friendly character (Berg, 2009; Schütz et al., 2018). Interestingly, biofertilizers showed an increased efficiency in dry climates, where it is typically more difficult for the plant to survive as it has been shown with a diminution of productivity of the cultures of up to 30% (Rubin et al., 2017; Schütz et al., 2018). This is in accordance with the stress gradient hypothesis which suggests that inter-specific interactions shifts from

competitive to facilitative under increasing abiotic stress (Bertness and Callaway, 1994). Interestingly, some new research projects also tend to focus on how to manipulate the microbiota to reduce postharvest food loss instead of increase food production in order to ensure food supply (Buchholz et al., 2018). Nowadays, the advantage is that we are capable to study more in details the microbiota thanks to laboratory technologies, allowing deciphering more precisely which bacteria have plant growth promoting skills or protective traits.

5.3.1. Direct beneficial effects on the plant

First of all, PGPB can have direct effects on the plant by promoting nutrient availability for instance, including improved solubilization of phosphorus or iron, or nitrogen fixation. Some other PGPB can modulate the plant growth via production of hormones and/or inhibition of the synthesis of the plants ones (Belimov et al., 2015; Dessaux et al., 2016; Olanrewaju et al., 2017).

The most studied PGPR are nitrogen-fixing (N_2-fixing) bacteria such as *Rhizobium*, *Bradyrhizobium* or *Frankia*. Nitrogen is a nutriment essential for the growth of all living organisms. However, a large amount of nitrogen is gaseous, a form in which it is not suitable for plant assimilation. Nitrogen-fixing bacteria form nodules on roots of leguminous plants, where they convert atmospheric nitrogen (N_2) into ammonia (NH_3) or amino acids, a source of nitrogen that can be assessed by the host plant. This is possible thanks to the synthesis of a nitrogenase (Glick, 2012; Olanrewaju et al., 2017; van Rhijn and Vanderleyden, 1995). Moreover, there are bacteria with positive effects on the plant root growth and morphology that are also crucial for the uptake of a variety of nutrients in the soil (Keven Vessey, 2003).

Interestingly, some PGPR are also capable to synthetize hormones analogous to plant hormones like auxins, gibberellins and cytokinins, and thus affect plant growth and development (Hardoim et al., 2008). For instance, the main auxin phytohormone, indole-3-acetic acid (IAA), is synthetized by several bacteria, among which some nitrogen fixators (Spaepen et al., 2007). In plants, this hormone is implicated in diverse processes including cell enlargement and division, tissue differentiation, and responses to light. By producing IAA, PGPR have the capacity to enhance root

proliferation, like *Azospirillum brasilense* or *Pseudomonas putida* do. This increased root system enhances nutrients uptakes and root exudation, which in turn increases the colonization by bacteria from the soil (Bashan et al., 2004; Dobbelaere et al., 1999).

Some PGPB such as *Pseudomonas putida* synthetize 1-aminocyclopropane-1-carboxylate (ACC) deaminase to modulate the plant ethylene levels. Actually, ACC is the precursor of ethylene, a phytohormone implicated in the modulation of plant growth and development, and in the response to a wide range of stresses as previously mentioned in the introduction. Regarding the plant response to stressful conditions, ethylene levels increase leading to a situation called “stress ethylene”. This is characterized by two peaks of ethylene synthesis. The first one consumes the existing pool of ACC in stressed tissues. The second ethylene peak which is much important occurs following the synthesis of additional ACC by the plant in response to stress and has generally consequences on the plant growth and health. ACC deaminase of PGPB leads to the cleavage of ACC into ammonia and α-ketobutyrate, and thus decreases plant ethylene levels. This activity can prevent damages caused by high levels of ethylene in the plant (Glick, 2014) and could lead to plant growth modulation.

More recently, it has been shown that some bacteria are able to synthetize other molecules that can have an impact on the plant. For example, *Microbacterium imperial* Rz19M10, *Kocuria erythromyxa* Rt5M10 and *Terribacillus saccharophilus* Rt17M10 are able to induce the biosynthesis of secondary metabolites like terpenes in *Vitis vinifera* cv. Malbec. This terpenes biosynthesis results in the increment of antioxidant capacity of the tissues and a better resistance to pathogens such as *Botrytis cinerea* (Salomon et al., 2016), highlighting their implication in the plant health.

5.3.2. Indirect beneficial effects on the plant

Indirect effects of PGPB that are also valuable for the plant are due to a competition between bacteria. Some PBPB can act as biocontrol agents by the production of antibacterial, antifungal or nematicide compounds, allowing a first line of defense against pathogens.

The main mechanism used by PGPB to act against pathogens is the production of antibiotic compounds. In this case, some bacteria such as certain *Pseudomonas*

strains possess a biocontrol activity by producing well-characterized antibiotics such as phenazines or pyrrolnitrin, for instance (Haas and Keel, 2003). This is also the case of *Streptomyces* spp. or *Bacillus* spp. (Haas and Keel, 2003). Some others produce lytic enzymes such as β-1,3 glucanases or proteases that are efficient against a range of pathogenic fungi including *Botrytis cinerea* (Glick, 2012).

In recent studies, experiments on bacteria interacting with *A. thaliana* led to the observation that some strains such as *Sphingomonas melonis* sp. Fr1 confer protection against the phytopathogen *Pst*DC3000 (Innerebner et al., 2011; Vogel et al., 2016). The metabolic profiles of axenic leaves or leaves inoculated with *Sphingomonas melonis* sp. FR1, *Methylobacterium extorquens* PA1 or *Pst*DC3000 have also been studied. This revealed that *Sphingomonas melonis* sp. Fr1 occupied a niche that overlaps the one of *Pst*DC3000, potentially leading to a competition for nutrients. This could at least partially explain the protective effect of *Sphingomonas melonis* sp. Fr1 against *Pst*DC3000 (Ryffel et al., 2016).

Additionally, PGPB can stimulate the plant defense machinery by induction of the induced systemic resistance (ISR) (Dessaux et al., 2016; Glick, 2012; Olanrewaju et al., 2017). In this situation, bacteria interacting with the roots are perceived like elicitors and activate the synthesis of secondary metabolites and pathogen related proteins by the plant (Heil, 2002; Salomon et al., 2016). This process induces in the plant a state of enhanced defensive capacity to resist future pathogen attacks. A wide variety of root-associated mutualist bacteria, including *Pseudomonas*, *Bacillus*, *Trichoderma* and mycorrhiza species prime the plant immune system without activating costly defenses (Glick, 2012; van Loon et al., 1998; Pieterse et al., 2014). ISR has initially been demonstrated in plants colonized by *Pseudomonas fluorescens* strain WCS417r that was shown to protect the plant against the fungal pathogen *Fusarium oxysporum*. This induced resistance was initially thought to be SAR, but it was not correlated with accumulation of PR proteins that are characteristic of SAR (Hammerschmidt, 1999; Hoffland et al., 1995; Van Peer and Schippers, 1992). Moreover, transgenic NahG *A. thaliana* that do not accumulate SA exhibit an enhanced protection against *Pst*DC3000 mediated by *Pseudomonas fluorescens* WCS417r, supporting that ISR is mediated by a SA-independent signalization pathway (Pieterse et al., 1996, 2000). This defense mechanism involves JA and ET signaling to induce the plant defenses since *A. thaliana* mutants impaired in JA (*jar1* for instance) and ET (*etr1* for instance)

were shown to be defective in ISR induced by *Pseudomonas fluorescens* WCS417r (Pieterse et al., 1998; Thomma et al., 2001). ISR is based on an enhanced sensitivity to JA/ET rather than on an increased biosynthesis (Pieterse et al., 2000, 2014). As another example, *Pseudomonas fluorescens* S97 is able to limit the infection of bean after inoculation to the seeds, in comparison to non-inoculated seeds (Alström, 1991). Same observations have been made for other PGPR and other plant species (Pieterse et al., 2014 for review).

Basically, ISR is first induced at a local level and requires *MYB72* transcription factor gene, which has been identified as one of the significantly induced genes in *A. thaliana* following its colonization by the PGPR *Pseudomonas fluorescens* WSC417r (Verhagen et al., 2004). ISR is then extended to a systemic level and requires NPR1 which is also involved in SAR as co-activator of some defense proteins, indicating that it differentially regulates defense responses in accordance with the signals that are perceived by the plant, but its functions remain poorly understood (Pieterse et al., 1998). NPR1 co-activates some transcription factors that give the plant the capacity to react in an accelerated defense response upon perception of pathogens. Among them, AP2/ERF are notably abundant and implicated in the regulation of JA and ET dependent defenses. In addition, MYC2 is a key transcriptional regulator of JA-dependent defenses since mutants impaired in MYC2 are unable to mediate ISR (Memelink, 2009). Together, these signaling events lead to the priming of callose and activation of JA/ET-dependent defense genes characteristic of the ISR such as *VSP* (*VEGETATIVE STORAGE PROTEIN*), *PDF1.2* and *HEL* (*HEVEIN-LIKE PROTEIN*) (Verhagen et al., 2004; van Wees et al., 1999).

5.4. How does the plant select the microbiota?

Since plants have a complex immune system, they must be able to recognize the beneficial micro-organisms from the pathogenic ones in order to deal with them rather than killing them. For instance, *A. thaliana* can presumably discriminate non-pathogenic micro-organisms from the pathogenic ones, allowing a variable response depending on the impact of the bacteria on the plant (Finkel et al., 2017; Zamioudis and Pieterse, 2012).

Interactions between plants and beneficial bacteria are partially allowed by the bacteria themselves. It seems that they have evolved the ability to escape the plant defense mechanisms by modifying their MAMP epitope, or inhibiting the biosynthesis of their MAMP-containing molecules, for instance (Hacquard et al., 2017). Plant-associated bacteria such as *Xanthomonas* species, for which there is no sequence information for flagellin, one of the best-known MAMP, do not induce rapid defense response in tomato cells, for example (Felix et al., 1999). In some other situations, commensal bacteria like *Pseudomonas protegens* Pf-5 produce elevated levels of cyclic-di-GMP (bis-(3'-5')-cyclic di-guanosine monophosphate) which inhibits the flagellin synthesis. Hence, it helps the bacteria to evade the recognition by FLS2 and thus, the plant defense mechanisms (Pfeilmeier et al., 2016).

Sometimes, some members of the plant microbiota still activate the first line of the plant immunity, which is the PTI. But this is actually needed for the protective activity mediated by commensals. For instance, *Methylobacterium extorquens* PA1 does not induce a significant transcriptional response from *A. thaliana*, while *Sphingomonas melonis* sp. FR1 activates the expression of genes that are implicated in the defense against pathogens and thus confers resistance against the pathogen *Pst*DC3000 (Innerebner et al., 2011; Vogel et al., 2016). In contrast, the protection against the pathogen conferred by this protective strain is clearly reduced in the pattern-recognition co-receptor mutant *bak1/bkk1*, in which the formation of some PRR complexes is impaired (Roux et al., 2011; Vogel et al., 2016). This indicates that the PTI is necessary for the protective activity conferred by commensal bacteria such as *Sphingomonas melonis* sp. Fr1. This may be considered as a mechanism of defense priming in which the plant is in a state of ISR (Finkel et al., 2017).

The accommodation of the associated microbiota is also affected by the exchange of some molecules between plant and bacteria. This is particularly well documented in the case of nitrogen fixation. *Medicago sativa* L. seeds and roots release molecules such as flavonoids that are implicated in the selection of *Rhizobium meliloti*. Actually, many flavonoids notably induce nodulation genes in *Rhizobium meliloti* (Hartwig et al., 1991). We can also mention isoprenoids as molecules presumably impacting the relationship between plants and their microbiota. As detailed in the following part of the introduction, like plants, bacteria are able to synthetize isoprenoids that may have an impact on their interaction with the plant. Among these isoprenoids, bacteria have the

ability to synthetize hopanoids that resemble sterols of plants, and they are found in diverse bacteria where they play a similar role (Belin et al., 2018; Kannenberg and Poralla, 1999). Interestingly, *Bradyrhizobium*, a symbiont of *Aeschynomene* legumes synthetizes LPS in which lipid A is bearing a hopanoid. This hopanoid attached to the lipid A appears to be important for the stability and rigidity of the outer membrane, but also for the resistance to some stressful conditions and the survival of the bacteria in the host plant (Silipo et al., 2014). Thus, bacterial isoprenoids may have a role in the interactions with the plant (Belin et al., 2018). Caryolanes are another kind of isoprenoids that can be produced by bacteria, especially *Streptomyces* sp. JMRC:ST027706 which is an endophyte of the mangrove plant *Bruguiera gymnorrhiza*. These caryolanes may be important for the interaction and communication with the host plant (Ding et al., 2015).

In the laboratory, we decided to study the role of isoprenoids in the interactions between plants and bacteria.

II. Isoprenoid diversity and functions

1. Generalities about isoprenoids

Isoprenoids, also called terpenoids, represent a large class of molecules that are essential for all living organisms, from animals to algae, including plants and bacteria. The term terpenoid originates from the word turpentine (“terpentin” in German), since some of the first terpenoids described were isolated from turpentine. Isoprenoids are known as the group of metabolites that are functionally and structurally the most diverse, with more than 55 000 metabolites identified among all living organisms, and they reach their greatest structural and functional diversity in plants (Thulasiram et al., 2007). Plant isoprenoids play a role in a wide variety of essential biological processes such as respiration (ubiquinones), photosynthesis (chlorophylls and carotenoids), or cell division or elongation (sterols). Because of their essential nature, they are categorized into what is commonly called primary metabolism. However, they are also

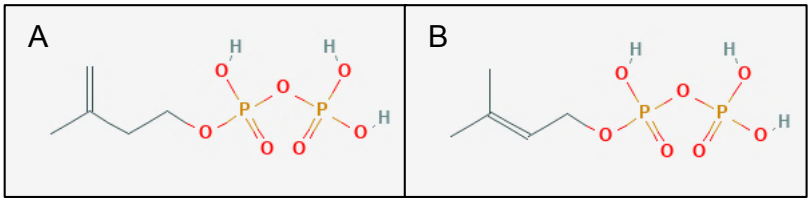

Figure 13. Five-carbon units at the basis of all isoprenoid biosynthesis. Isoprene units are derived from (A) isopentenyl diphosphate (IPP) or its isomer (B) dimethylallyl diphosphate (DMAPP). Structures from PubChem.

implicated in secondary metabolism by helping the organism to adapt to the environment and interact with other organisms. For instance, some isoprenoids participate in the plant defense against pathogens (phytoalexins) and they are also essential to attract pollinators and seed-dispersing animals (carotenes) (Singh and Sharma, 2015). In short, they are recognized for their diverse biological activities and properties, leading humans to exploit them since ancient time, and more recently in industry and agriculture, for the production of drugs, flavors, pigments or fragrances (Bohlmann and Keeling, 2008). As all living organisms, bacteria need isoprenoids and they are capable to synthetize them, even if they exhibit a lower diversity than in other organisms (Rohmer, 2007). These molecules are essential for the growth and the development of bacteria. Indeed, they play a crucial role in cell wall and membrane biosynthesis (hopanoids and bactoprenol), but also in electron transport (ubiquinone and menaquinone), or light energy to chemical energy conversion (chlorophylls, bacteriochlorophylls, carotenoids and rhodopsins) and some other processes like protein synthesis (isopentenyl tRNA) (Pérez-Gil and Rodríguez-Concepción, 2013; Rodríguez-Concepción and Boronat, 2012).

All isoprenoids are derived from a same structural basis which is a five-carbon unit called isopentenyl diphosphate (IPP), or its isomer dimethylallyl diphosphate (DMAPP) (**Figure 13**). The structure of isoprenoids leads to their classification and nomenclature described by the International Union of Pure and Applied Chemistry (IUPAC). They are classified according to their number of isoprene units: C_{10} are monoterpenes (e.g. menthol), C_{15} are sesquiterpenes (e.g. geosmin), C_{20} are diterpenes (e.g. side chain of chlorophylls), C_{30} are triterpenes (e.g. squalene), C_{40} are tetraterpenes (e.g. carotenoids), C_{5n} are polyterpenes. Based on these possibilities of assemblage, there are a huge variety of isoprenoids known today that assume a wide range of functions.

2. Overview of isoprenoid functions

2.1. A great example of universal compounds: sterols

A well-known example of isoprenoid that is common to mammals, fungi and plants but also bacteria, and which is classified both as a primary and a secondary metabolite

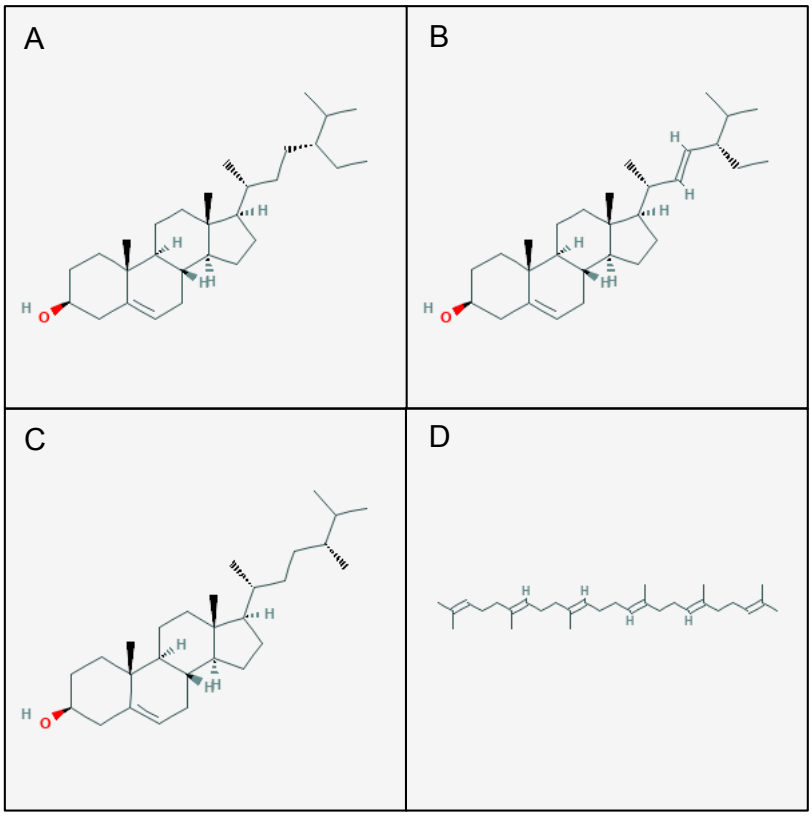

Figure 14. Structures of some plant sterols. Sterols lead to the formation of diverse molecules of diverse functions in all living organisms, including plants. β-sitosterol (A), stigmasterol (B) and campesterol (C) are the major membrane sterols of higher plants. They are all derived from squalene (D). Structures from PubChem.

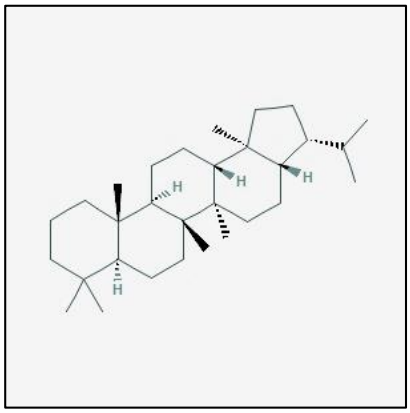

Figure 15. Structure of hopane, a bacterial sterol. Hopane is a membrane lipid synthetized in bacteria which is implicated in the membrane fluidity and stability. Structure from PubChem.

is sterol. Indeed, sterols are considered as primary metabolites since they are essential membrane constituents, as in the case of cholesterol in animals or ergosterol in fungi. But they are also referred as secondary metabolites since they are precursors for the production of other molecules such as the brassinosteroid hormones in plants (Clouse, 2011), or estradiol, progesterone and testosterone implicated in mammalian development and reproduction, for instance.

Even if sterols, and more generally isoprenoids, are essential in diverse physiological processes, some organisms are not capable to synthetize them. For instance, some invertebrate organisms such as *Caenorhabditis elegans* need cholesterol for their growth and development, despite their inability to synthetize it. That is why they need an external uptake in order to synthetize ecdysteroids, molecules implicated in larval development and reproduction (Wollam and Antebi, 2011).

In plants, sterols are essential since they are inserted in phospholipid bilayers forming plant membranes. We can mention β-sitosterol (**Figure 14, A**), stigmasterol (**Figure 14, B**) and campesterol (**Figure 14, C**) that are the major membrane sterol in higher plants (Valitova et al., 2016). They are all derived from the same precursor which is squalene (**Figure 14,D**). They regulate the membrane fluidity and permeability, by interaction with fatty acyl chains of phospholipids and proteins. Moreover, they can also participate in the control of some metabolic processes that happen at the membrane location, notably in the cell proliferation process (Hartmann, 1998). Plant sterols exhibit a huge complexity and diversity, with more than 200 compounds (Hartmann, 1998).

Finally, to continue with the example of molecules derived from sterols, we can mention that bacteria produce hopanoids (**Figure 15**) to assume the same role as membrane lipids in the regulation of membrane fluidity and stability (Belin et al., 2018; Kannenberg and Poralla, 1999; Sáenz et al., 2015).

2.2. Chlorophylls and carotenoids

In land plants, photosynthesis is a key mechanism allowing the conversion of light energy into chemical energy necessary for plant activities. This mechanism occurring mainly in leaves, in chloroplasts, requires chlorophylls such as chlorophyll A

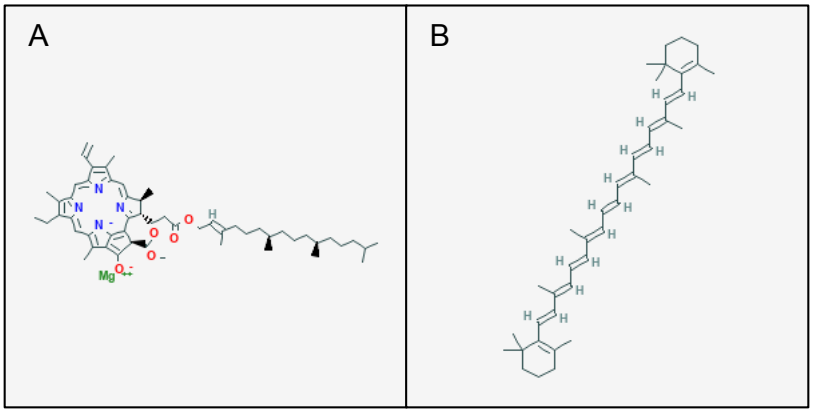

Figure 16. Structure of isoprenoids implicated in the photosynthesis. The phytol chain of chlorophyll A (A) and β-carotene (B) are implicated in photosynthesis. Structures from PubChem.

(**Figure 16, A**) and carotenoids like β-carotene (**Figure 16, B**). Basically, chlorophylls are essential pigments for all phototrophic organisms, allowing them to absorb blue and red light, while carotenoids absorb only blue light and are implicated in photoprotection by avoiding the formation of reactive oxygen species (Blankenship, 2010; Johnson, 2016). This is a great example of isoprenoid function that is required for the plants to live.

2.3. Hormones

Five of the major plant hormones are derived from isoprenoids: brassinosteroids cytokinins, gibberellins, abscisic acid (ABA) and strigolactones. All of these hormones play a role in the regulation of the plant growth (Santner et al., 2009), but they also participate in the regulation of other aspects of the plant life, such as responses to stresses.

Brassinosteroids are involved in plant growth regulation, cell division and elongation. They also act in some other plant life processes such as seed germination, flowering time, maturation, resistance to stress and senescence (Bajguz, 2007).

Cytokinins are able to promote plant cell division, they are implicated in seed germination, leaf senescence, but they also play a role in the formation of nitrogen-fixing nodules in plant-microbe interactions and participate in the plant defense (Albrecht and Argueso, 2017; Frugier et al., 2008; Santner et al., 2009).

Gibberellins were first isolated from a fungal rice pathogen, *Gibberella fujikuroi* because of the resulting excessive stem elongation. More than a hundred gibberellins were already identified from plants and are implicated in diverse plant growth and development processes like seed germination, organ elongation, leaf expansion and flowering time (Yamaguchi, 2008).

Abscisic acid exhibits a dual role in the development of the seeds. In early development, ABA prevents seed abortion and promotes embryo growth, while later, it promotes seed maturation. This molecule is necessary to induce seed dormancy during late embryogenesis and to maintain it during imbibition. Moreover, ABA is also implicated in drought response and in response to other stresses as mentioned in the

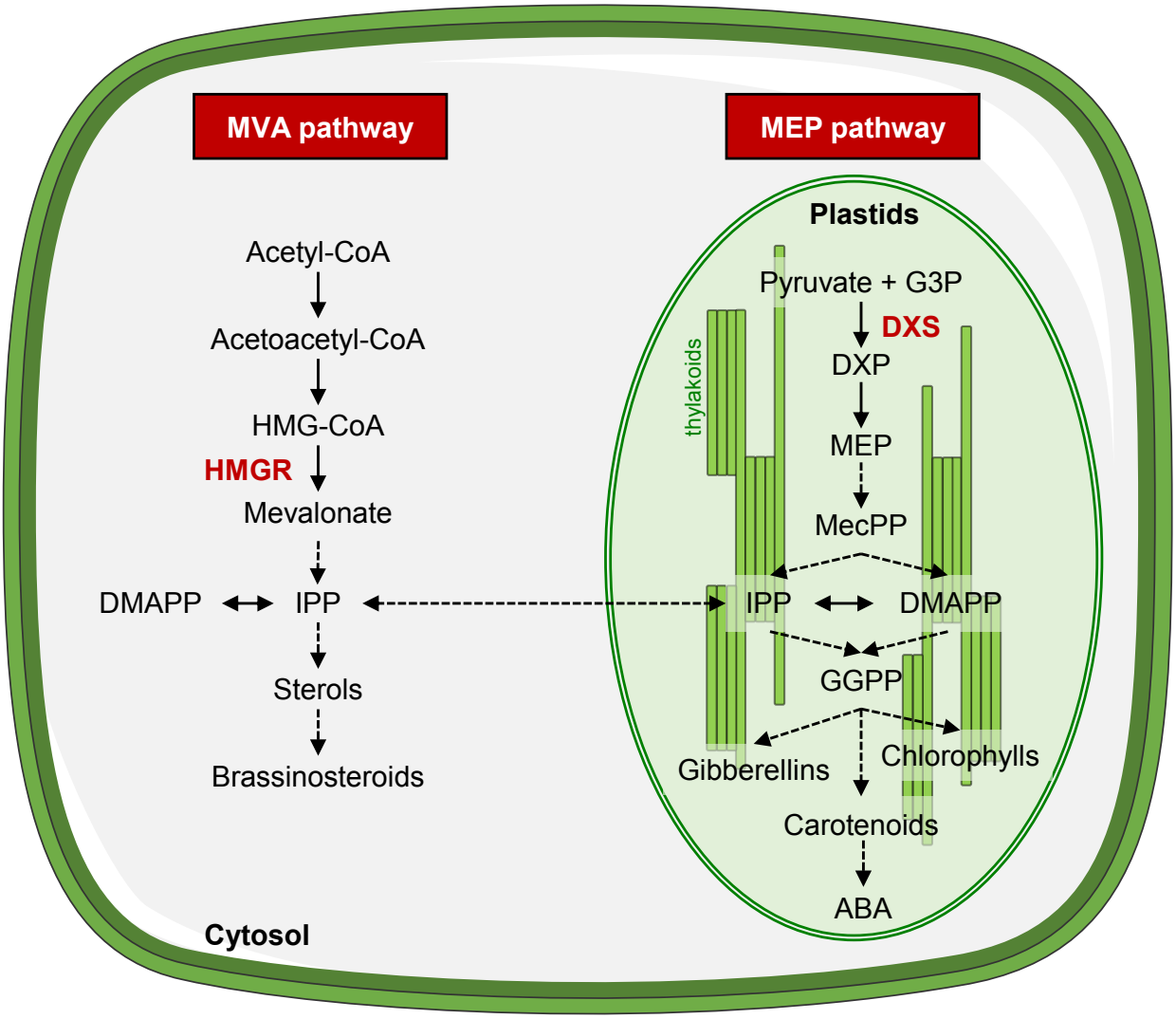

Figure 17. Simplified isoprenoid biosynthesis pathways in *Arabidopsis thaliana*. The mevalonate (MVA) pathway occurs in the cytosol and requires the 3-hydroxyl-3-methylglutaryl CoA reductase (HMGR) as a key enzyme to obtain mevalonate. The 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway occurs specifically in plastids and requires the 1-deoxy-D-xylulose 5 phosphate synthase (DXS) as a key enzyme to form 1-deoxy-D-xylulose 5 phosphate (DXP). Both biosynthesis pathways lead to the formation of isopentenyl diphosphate (IPP), or its isoform dimethylallyl diphosphate (DMAPP), precursors of all isoprenoids. Full arrows indicate single steps, and dashed arrows indicate multiple steps. G3P: glyceraldehyde 3-phosphate; MEcPP: 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate; GGPP: geranylgeranyl diphosphate. Adapted from Claire Villette's thesis.

paragraph 4.3.4 in the first part of the introduction (Nambara and Marion-Poll, 2005; Santner et al., 2009).

For their part, strigolactones seem to be involved in shoot branching inhibition, like auxins. They were previously identified as communication chemicals found in root exudates, implicated in interactions with parasitic weeds and symbiotic arbuscular mycorrhizal fungi (Umehara et al., 2008). They may have additional functions by inducing seed germination.

2.4. Other functions

Among the huge diversity of isoprenoids, some of them have been valorized by humans for pharmacological or economic reasons. We can mention menthol, for instance, which is a monoterpenoid produced from peppermint and used in medicine, particularly as a local anesthetic. Artemisinin is a sesquiterpenoid coming from annual wormwood, *Artemisia annua L.* that is employed as an anti-malarial drug and which is efficient against parasitic protozoa (Loo et al., 2017). Another unavoidable example is paclitaxel, or Taxol, a diterpenoid-derived anti-cancer drug coming from the bark of the Pacific yew tree, *Taxus brevifolia* (Bohlmann and Keeling, 2008).

3. Biosynthesis of IPP and DMAPP, precursors of all isoprenoids

3.1. Two biosynthesis pathways for IPP and DMAPP

To synthetize IPP and DMAPP that are the 2 precursors required for the biosynthesis of all isoprenoids, two distinct biosynthesis pathways are known. Animals, fungi or archaea synthetize their isoprenoids via the mevalonate (MVA) pathway, while algae exhibit the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway. Higher plants exhibit the particularity to use these two pathways to synthetize IPP and DMAPP (**Figure 17**). Indeed, they maintained the “classical” eukaryotic MVA pathway that occurs in the cytosol, and acquired the later described “alternative” MEP pathway occurring in plastids (Rohmer, 1999, 2007). Concerning bacteria, most of them synthetize isoprenoids only via the MEP pathway. However, some bacteria, including

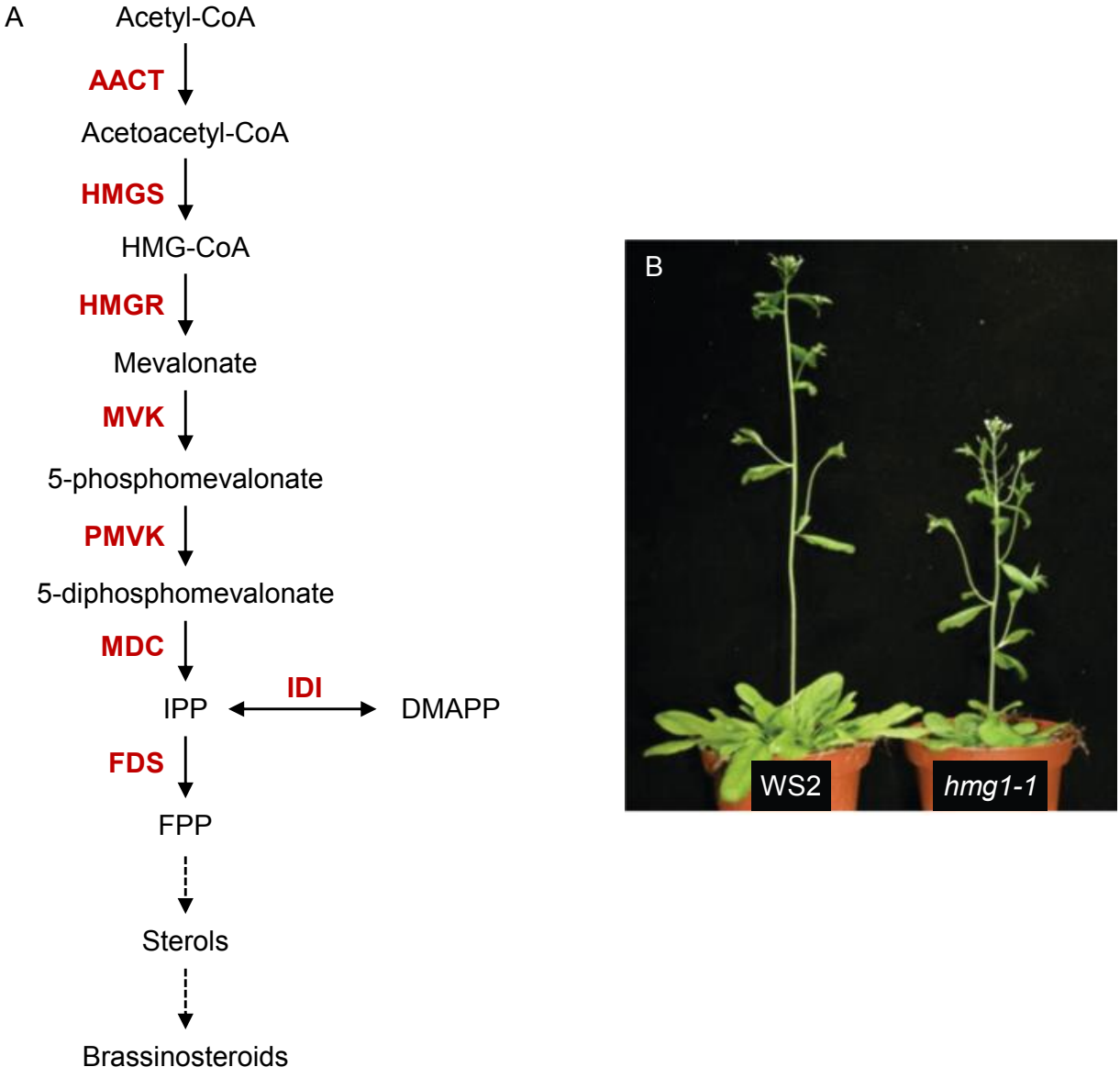

Figure 18. The mevalonate (MVA) biosynthesis pathway and *A. thaliana hmg1-1* mutant. (A) The MVA pathway occurs in the cytosol and leads to the formation of isopentenyl diphosphate (IPP) who can be isomerized in dimethylallyl diphosphate (DMAPP). Full arrows indicate single steps, dashed arrows indicate multiple steps. AACT: acetyl-coA C-acetyltransferase; HMGS: 3-hydroxy-3-methylglutaryl-CoA synthase; HMG-CoA: 3-hydroxy-3-methylglutaryl-CoA; HMGR: 3-hydroxy-3-methylglutaryl-CoA reductase; MVK: mevalonate kinase; PMVK: phosphomevalonate kinase; MDC: mevalonate diphosphate decarboxylase; IPP: isopentenyl diphosphate; DMAPP: dimethylallyl diphosphate; IDI: isopentenyl diphosphate:dimethylallyl diphosphate isomerase. FDS: farnesyl diphosphate synthase; FPP: farnesyl diphosphate. (B) *hmg1-1* is affected in the MVA pathway by a mutation in the *HMGR1* gene. The plant is characterized by a dwarf phenotype (pictures by Claire Villette, adapted from Heintz et al., 2012).

Borrelia burgdorferi and *Staphylococcus aureus* use the MVA pathway instead of the MEP pathway. Some exceptions such as *Listeria monocytogenes* and some *Streptomyces* have been confirmed to possess the two full pathways. For instance, some *Streptomyces* strains have the capacity to use the additional MVA pathway, particularly to synthetize antibiotics and other secondary metabolites. Finally, few bacteria including parasitic *Rickettsia* for instance, lack these biosynthesis pathways, probably because they are obligatory intracellular parasites and obtain their isoprenoids from their host (Kuzuyama and Seto, 2003; Pérez-Gil and Rodríguez-Concepción, 2013).

We can notice that multiple studies using inhibitors of one of the biosynthesis pathways, mutants, or labelled precursors in feeding experiments highlighted that some exchanges of IPP and other prenyl diphosphates are possible between the two biosynthesis pathways, and thus between the cytosol and plastids (Flores-Pérez et al., 2010; Hemmerlin et al., 2003; Pulido et al., 2012). However, these exchanges represent only small amounts that cannot allow the complete compensation of one pathway by the other (Pulido et al., 2012; Rodríguez-Concepción and Boronat, 2015). Moreover, the process of exchange between the different compartments it is not fully understood (Flores-Pérez et al., 2010).

3.2. The MVA biosynthesis pathway

3.2.1. Description of the biosynthesis pathway

The mevalonate (MVA) pathway (**Figure 18, A**) allows the synthesis of cytosolic and mitochondrial precursors, thanks to enzymes found in the endoplasmic reticulum, the peroxisome and the cytosol (Lange et al., 2002). This pathway starts with the condensation of two molecules of acetyl-coenzyme A (acetyl-CoA) by acetyl-CoA C-acetyltransferase (AACT) to form acetoacetyl-CoA. Acetoacetyl-CoA is then converted to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) by 3-hydroxy-3-methylglutaryl-CoA synthase (HMGS). The conversion of HMG-CoA to mevalonate (MVA) is a key step in this pathway and this reduction is done by the 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR). A double mutant for *HGM1* and *HMG2* genes in *Arabidopsis thaliana* is lethal (Suzuki et al., 2009), indicating the essential role of

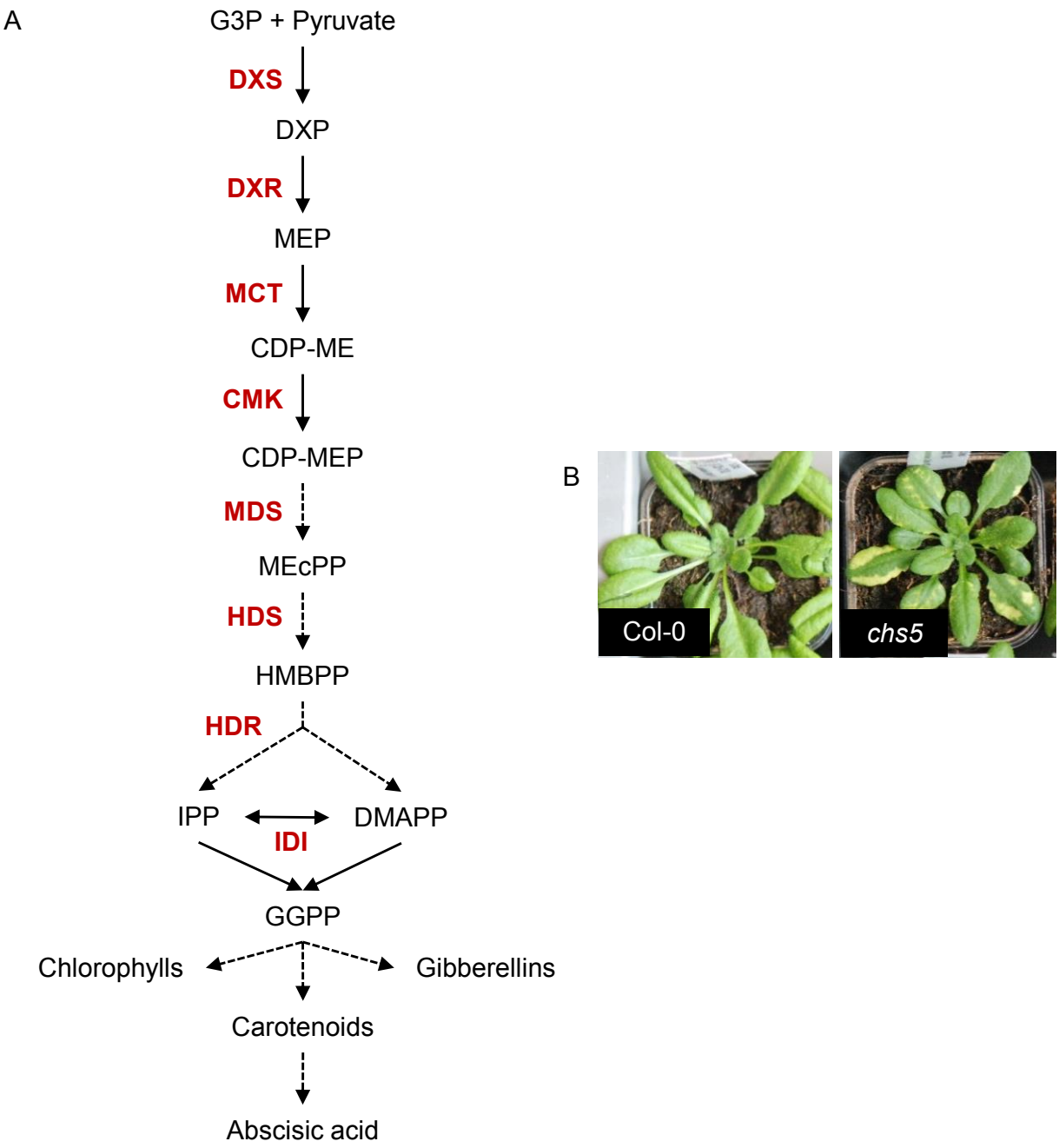

Figure 19. The methyl-D-erythritol 4-phosphate (MEP) biosynthesis pathway and *A. thaliana chs5* mutant. The MEP pathway occurs in plastids and leads to the formation of isopentenyl diphosphate (IPP) who can be isomerized in dimethylallyl diphosphate (DMAPP). G3P: D-glyceraldehyde 3-phosphate; DXS: 1-deoxy-D-xylulose 5-phosphate synthase; DXP: 1-deoxy-D-xylulose 5-phosphate; DXR: 1-deoxy-D-xylulose 5-phosphate reductoisomerase; MEP: methyl-D-erythritol 4-phosphate; MCT: MEP cytidyltransferase; CDP-ME: 4-(cytidine 5'-diphospho)-2-C-methylerythritol; CMK: 4-(cytidine 5'-diphospho)-2-Cmethylerythritol kinase; CDP-MEP: 2-phospho-4-(cytidine 5-diphospho)- 2-C-methyl-D-erythritol; MDS: 2-C-methylerythritol-2,4-cyclodiphosphate synthase; MEcPP: 2-C-methylerythritol 2,4-cyclodiphosphate; HDS: 4-hydroxy-3-methylbut-2-enyl diphosphate synthase; HMBPP: 4-hydroxy-3-methylbut-2-enyldiphosphate; HDR: 4-hydroxy-3-methylbut-2-enyl diphosphate reductase; IPP: isopentenyl diphosphate; DMAPP: dimethylallyl diphosphate. (B) *chs5* is affected in the MEP pathway by a mutation in the *DXS1* gene. The plant is characterized by a chlorotic phenotype.

these enzyme. Then, two successive phosphorylation reactions are catalyzed by mevalonate kinase (MVK) and phosphomevalonate kinase (PMVK) to obtain respectively 5-phosphomevalonate and then 5-diphosphomevalonate. The final obtention of isopentenyl diphosphate (IPP) is catalyzed by a mevalonate diphosphate decarboxylase (MDC). IPP can be further isomerized in dimethylallyl diphosphate (DMAPP) thanks to an isopentenyl diphosphate:dimethylallyl diphosphate isomerase (IDI). Afterward, a subsequent addition of IPP leads to more specialized branches of the isoprenoid biosynthesis. An addition of two IPPs on a DMAPP leads to the formation of farnesyl diphosphate (FPP) by a farnesyl diphosphate synthase (FDS). Two molecules of FPP can condense together by squalene synthase (SQS) to form squalene (**Figure 14, D**), which is the precursor of sterols that are major constituent of membranes, but also brassinosteroids that are implicated in cellular elongation and protection against some abiotic stresses.

3.2.2. *hmg1-1* mutant

In *A. thaliana*, the simple mutant *hmg1-1* is viable and exhibits a growth delay characterized by its small size compared to the wild-type, as well as a low seed production associated with a reduced size of the siliques (Suzuki et al., 2004) (**Figure 18, B**). This mutant is carrying a T-DNA insertion in the first exon of the gene coding for the 3-hydroxy-3-methylglutaryl coenzyme A reductase 1 (*HMG1*, At1g76490). The *HMG1* gene expression is very low in *hmg1-1* mutants, leading to a defect in plant growth and fertility, associated with a decrease of metabolites downstream of the MVA pathway (Heintz et al., 2012; Suzuki et al., 2004).

3.3. The MEP biosynthesis pathway

3.3.1. Description of the biosynthesis pathway

In addition to the MVA pathway, there is a more recently discovered pathway for isoprenoid biosynthesis which is called the non-mevalonate pathway, or the methyl-D-erythritol 4-phosphate (MEP) pathway (**Figure 19, A**), which allows the synthesis of

plastidial isoprenoids. It starts with the condensation of pyruvate and D-glyceraldehyde 3-phosphate (G3P), catalyzed by a 1-deoxy-D-xylulose 5-phosphate synthase (DXS), to form 1-deoxy-D-xylulose 5-phosphate (DXP). DXP is then reduced to methyl-D-erythritol 4-phosphate (MEP) by a 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR). These steps are key steps in the formation of IPP and DMAPP in the MEP pathway. Afterward, MEP is then converted to 4-(cytidine 5'-diphospho)-2-C-methylerythritol (CDP-ME) by conjugation with cytidine diphosphate by a MEP cytidyltransferase (MCT), and further phosphorylated by 4-(cytidine 5'-diphospho)-2-Cmethylerythritol kinase (CMK), a member of the same family of metabolite kinases as MVK and PMVK from the MVA pathway. This allows the production of 2-phospho-4-(cytidine 5-diphospho)- 2-C-methyl-D-erythritol (CDP-MEP), which is then converted to 2-C-methylerythritol 2,4-cyclodiphosphate (MEcPP) by 2-C-methylerythritol-2,4-cyclodiphosphate synthase (MDS). The two final steps of this pathway to form isoprenoid precursors are catalyzed by 4-hydroxy-3-methylbut-2-enyl diphosphate synthase (HDS) and reductase (HDR) (Lange et al., 2002; Phillips et al., 2008) to form respectively 4-hydroxy-3-methylbut-2-enyldiphosphate (HMBPP) and a mixture of IPP and DMAPP. This pathway leads to the formation of isoprenoids such as chlorophylls and carotenoids implicated in the photosynthesis, or hormones like gibberellins or abscisic acid that is involved in the plant defense. Because of the essential character of many isoprenoids synthetized by this route for plant development and survival, the complete blockage of any step of this MEP pathway is lethal

3.3.2. *chs5* mutant

In *A. thaliana*, the *chs5* mutant on which I focused during my thesis can grow but is characterized by an albino phenotype due to the halt of chloroplasts development at early stages (Phillips et al., 2008). This mutant was originally isolated from a genetic screen for chilling sensitive mutants that display a normal wild-type phenotype at 22°C and a chlorotic phenotype at lower temperatures (15°C) (Hugly and Somerville, 1992; Schneider et al., 1995; Wright et al., 2014). The *chs5* mutant presents a missense mutation in the exon 8 of the gene coding for the 1-deoxy-D-xylulose 5-phosphate synthase (*DXS1*, At4g15560). This change of GAC to AAC results in the modification of an aspartic acid (D) to an asparagine (N) residue at position 627 (D627N) of the

encoded protein. This mutation is responsible for a chlorotic phenotype (**Figure 19, B**) due to a defect in plastidial 1-deoxy-D-xylulose 5-phosphate (DXP) biosynthesis (Araki et al., 2000; Wright et al., 2014).

4. Could the microbiota play a role in the plant isoprenoid biosynthesis?

Over the past ten years, some studies started to highlight a correlation between the plant isoprenoid status and the microbiota associated with some plants. The microbiota may have an impact on the plant isoprenoid biosynthesis, and indirectly on the plant mechanisms of defense, conferring a real advantage in the field.

For instance, *Vetiveria zizanioides* (L.) Nash, a grass cultivated for its essential oil production in its roots, exhibits differences of isoprenoid production depending on its associated microbiota. Actually, some of its associated bacteria are able to metabolize sesquiterpenes present in the oil and release compounds typically found in commercial Vetiver oils; but some bacteria are also capable to induce gene expression of a sesquiterpene synthase. This suggests that some bacteria from the Vetiver microbiota may play a crucial role in essential oil biosynthesis, opening some possibilities to control the Vetiver essential oil composition (Del Giudice et al., 2008).

Same kind of observations was done more recently on other plants. The grapevine *Vitis vinifera* L. cv. Malbec exhibits an increased production of some isoprenoids such as α-pinene when inoculated with some specific bacteria. Particularly, inoculation with PGPR such as *Microbacterium imperial RZ19M10*, *Kocuria erythromyxa Rt5M10 and Terribacillus saccharophilus Rt17M10* previously isolated from grapevine roots and rhizosphere stimulates plant growth and biosynthesis of secondary metabolites playing a role in plant defense (Salomon et al., 2016).

Such differences of isoprenoid status in plants were also observed in flowers and leaves of *Sambucus nigra* depending on the presence or absence of microbial communities. Following the elimination of bacteria from the microbiota, a decrease in the concentration of some compounds has been observed, among which isoprenoids that play a key role in pollination, suggesting once again an impact of the plant microbiota on the isoprenoid biosynthesis (Gargallo-Garriga et al., 2016; Peñuelas et al., 2015).

Finally, bacterial endophytes of *Withania somnifera*, also called Indian ginseng, enhanced the withanolide content of the plants by modulating its biosynthesis in leaves and roots. Withanolides are terpenoids of pharmaceutical interest (potential neurological, immunological and anti-stress agent). Thus, the use of bacteria in the control of isoprenoid biosynthesis could be a great tool (Pandey et al., 2018).

III. Objectives of the thesis

Since a plant and its associated microbiota tend to be considered as a single entity referred as the holobiont, it becomes consistent to study the interactions between these organisms instead of studying each one independently. Few studies have previously suggested that isoprenoids may be important for the interactions between plants and micro-organism. Some others revealed that the microbiota may influence the plant isoprenoid biosynthesis.

My thesis project focused on the interactions between the model plant *A. thaliana* and bacteria in the context of isoprenoid biosynthesis. My thesis objectives could be defined by two main axes:

- Are plant isoprenoids implicated in plant-bacteria interactions in general? And are they implicated in the particular plant-pathogens interactions?
- Do some bacteria whose presence in the plant microbiota is dependent on the isoprenoid content have an impact on the plant physiology and resistance against pathogens?

To answer the first question and thus investigate the role of plant isoprenoids in the interaction between the plant and its microbiota, the first step was to establish an inventory of the communities interacting with wild-type *A. thaliana* and mutants affected in isoprenoid biosynthesis. This has been done for Col-0 and *chs5* mutant which was described in paragraph II.3.2.2, on one hand, and WS2 and *hmg1-1* mutant which was described in paragraph II.3.3.2, on another hand, in order to study both biosynthesis

pathways. Furthermore, in nature, plants are also subject to different stresses among which pathogens enter in consideration. In order to determine if isoprenoids may play a role in the protection of the plant against pathogenic bacteria, a comparison of the holoxenic wild-type and mutant plants susceptibility to *Pst*DC3000, a well-known phytopathogen, has been done. The work made on these two main axes is synthetized in the first chapter of my thesis.

The second question of my project was intended to elucidate whether some bacteria from the microbiota whose presence was influenced by the isoprenoid status of the plant may have an impact on the plant physiology and resistance against pathogens. For that purpose, the strategy was to isolate bacteria interacting with *A. thaliana* phyllosphere, roots or rhizosphere, but also bacteria present in the soil, to create a strain collection. Then, their 16S rRNA gene sequence would be compared with those of the communities interacting with wild-type and mutants. Hence, some bacteria from our strain collection that are differentially abundant between wild-type and mutants could be inoculated to axenic plants in order to determine their effect on the plant physiology, and susceptibility to *Pst*DC3000. Results relative to that question are presented in the second chapter of my thesis.

Chapter 1
Do isoprenoids influence the interactions between plants and micro-organisms?

I. Introduction

The first axis of my thesis consisted in investigating whether isoprenoids may influence the interactions between plants and micro-organisms. This problematic could be studied at two different levels: do isoprenoids impact plant-bacteria interactions in general? Do they also play a role in the specific interaction of the plant with pathogens? To answer these questions, we worked with *A. thaliana* wild-types and mutants affected in both isoprenoid biosynthesis pathways. As a reminder, *chs5* mutant is altered in the biosynthesis of isoprenoids that require the plastidial 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway, while *hmg1-1* mutant is altered in the cytosolic mevalonate (MVA) isoprenoid biosynthesis pathway.

To decipher whether plant isoprenoids may influence the interactions between plants and bacteria in general, an inventory of the communities interacting with wild-types and mutants was performed. This has been proceeded for the microbiota of Col-0 and *chs5* on one hand, and WS2 and *hmg1-1* on the other hand and should allow us to determine if isoprenoids from one biosynthesis pathway or the other may play a role in the establishment of the plant associated microbiota.

Furthermore, plants are not only interacting with their naturally associated microbiota in the environment. They are also exposed to a wide range of stresses such as pathogens. Therefore, once we had a look on the impact of isoprenoids on the naturally associated communities, it seemed interesting to determine if they could also play a role in the interactions with pathogenic micro-organisms. As stated before, *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*DC3000) has been widely used to study the interactions between plants and pathogens, and notably to establish the model of the plant immunity (Jones and Dangl, 2006). We considered that it could be a great tool to study the sensitivity of wild-types and mutants, and hence to determine if isoprenoids may play a role in the plant interactions with pathogens.

I first optimized the infection protocol, and I particularly tried to find an easy and quick way to proceed bacterial numerations. I compared the effects of slightly variable quantity of pathogen inoculated to the plants in three experiments. I also tried to setup a method to quantify the size and the severity of the lesions caused by *Pst*DC3000,

however, given the difficulty to delimit these lesions, I finally focused on the pathogen numerations. For that purpose, I first used different culture media, and I tried to find an alternative to the plating process by using *Pst*DC3000 GFPuv or *Pst*DC3000 lux strains that were supposed to be easily detectable by a microplate reader. Finally, the optimal conditions for the infection experiments in our laboratory were the use of the classic *Pst*DC3000 strain with a needle-less syringe infiltration, followed 6 days later by plating of the grinded leaves extracts at different dilutions. I performed a last infection experiment on a large batch of plants in order to accumulate material for pathogen quantification on the one hand, and for key genes and metabolites analyses on the other hand. I decided to plate 20 µL droplets on NYGB medium supplemented with rifampicin to reduce the number of plates needed and the time of manipulation. Key metabolite analyses and pathogen quantification were proceeded 6 days post infection (6 dpi). Expression of defense key genes called *pathogenesis related* (*PR*) genes, known to be induced upon infection by (hemi)-biotrophic pathogens such as *Pst*DC3000 (Thomma et al., 2001), was monitored by RT-qPCR at 1 dpi, 3 dpi and 6 dpi.

This first chapter describes the optimization of the infection protocol by the use of different pathogen detection methods, and some complementary data obtained for these infection experiments are exposed. Finally, the work related to the inventory of the communities that naturally interact with wild-types and mutants, as well as the study of the plant interaction with *Pst*DC3000 are synthesized in a publication (Groh *et al.*, in writing) that will be submitted soon.

II. Optimization and preliminary results

1. Test of different techniques for infection measurements

In the laboratory, since the beginning of the project, we proceeded several infection experiments on wild-type and mutants in order to accumulate data to proceed robust statistics. However, since we changed our proteose peptone supplier (Sigma-Aldrich

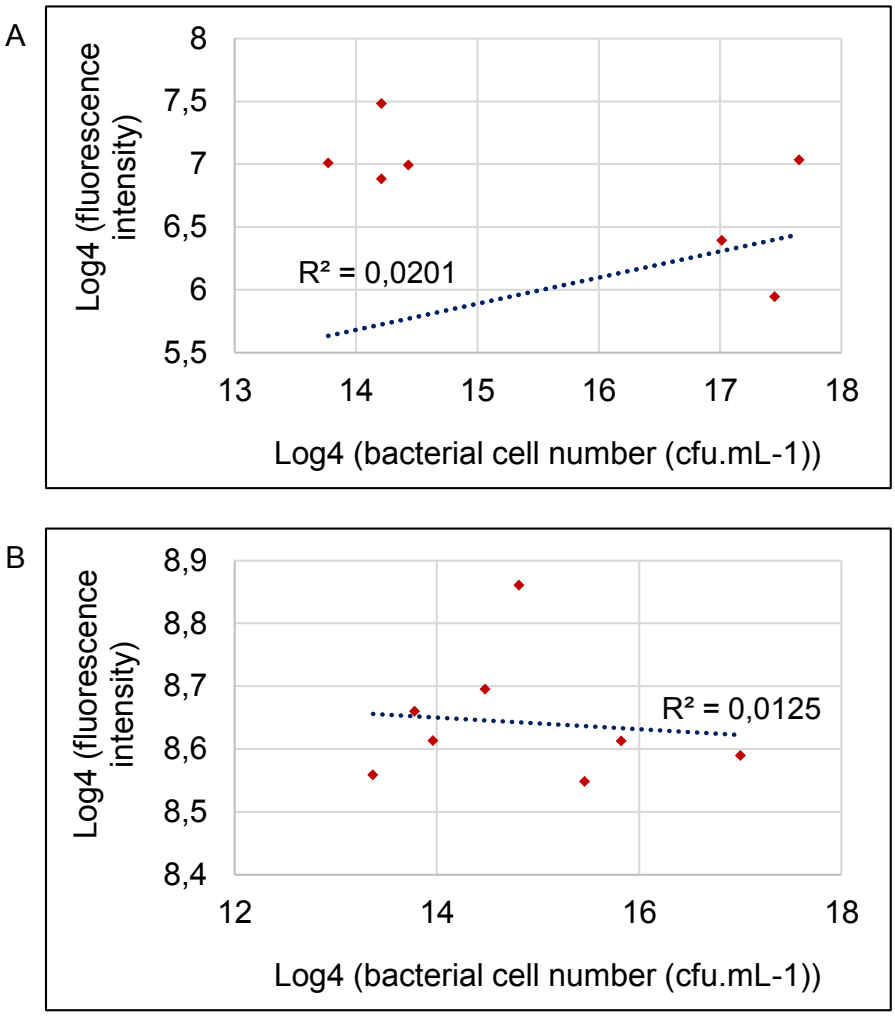

Figure 20. Test of correlation between fluorescence intensity and numerations of *Pst*DC3000 GFPuv. Fluorescence intensity was measured with FLUOstar Omega spectrometer (BMG Labtech). Leaves were grinded in KB medium (A), or in PBS 1X (B) before measurements. Fluorescence intensity measurements were compared with the classic numeration experiments after plating.

instead of Difco), we encountered troubles using KB+r medium for *Pst*DC3000 culture. Moreover, the classic method of numerations by serial dilutions and plating is time-consuming and requires lots of plates. Therefore, we tried to find an alternative approach.

1.1. *Pst*DC3000 GFPuv strain

In order to optimize the infection experiments and to facilitate quantification of the pathogen after infection, we tried to use a *Pst*DC3000 GFPuv strain (Wang et al., 2007). We transformed a *Pst*DC3000 strain with pDSK-GFPuv. This plasmid is a stable and broad-host-range vector that encodes the green fluorescent protein variant GFPuv under the control of the constitutive promoter *psbA* and an efficient ribosome binding site (RBS), allowing a strong expression in bacteria. Thus, the *GFP* could provide the capacity to quantify and follow the bacteria through the process of infection, colonization, multiplication and movement in the infected plants. This strain should have allowed us to directly measure the fluorescence intensity without needing to plate the samples on solid medium and further numerate the colony forming units after two days of incubation at 28°C. I infected plants with *Pst*DC3000 GFPuv, and I collected the infected leaf discs at 6 dpi. The first time, I grinded leaf discs in KB medium as it is done in the usual protocol preceding plating on agar medium, but I did not manage to obtain a correlation between the fluorescence intensity and the numerations (**Figure 20, A**). Thus, I tried to grind leaf discs in phosphate buffered saline (PBS) in attempt to eliminate an eventual background noise, but once again, I did not obtain a correlation between the fluorescence intensity and the numerations (**Figure 20, B**). Regardless of the medium used for leaves grinding, the determination index R^2 clearly indicates that there is no correlation between the fluorescence intensity and the numerations on agar medium. Moreover, samples that did not contain the GFPuv strain also exhibited fluorescence, indicating the presence of a background signal, whether using KB medium or PBS.

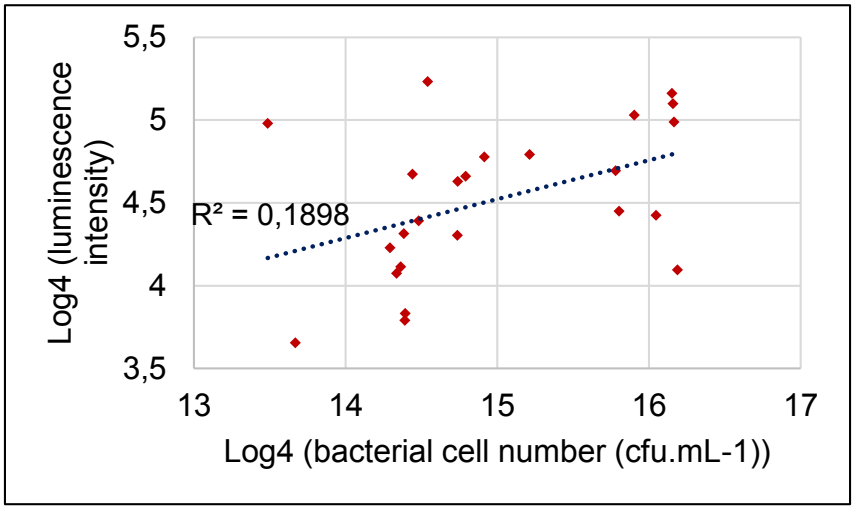

Figure 21. Test of correlation between luminescence intensity and numerations of *Pst*DC3000 lux. Luminescence intensity was measured with FLUOstar Omega spectrometer (BMG Labtech). Luminescence intensity measurements were compared with the classic numeration experiments after plating.

Table 1. Average size and number of leaves of *Arabidopsis thaliana* Col-0 and *chs5* before infection by *Pst*DC3000. Infection experiments 1. 2 and 3 were conducted independently in different culture conditions and at different times, on plants that are approximately the same growing stage.

	Col-0		***chs5***	
	rosette diameter	number of leaves	rosette diameter	number of leaves
Exp 1	7.2 cm	19	6.8 cm	18
Exp 2	8.1 cm	18.9	7.2 cm	18.1
Exp 3	7.6 cm	20.4	6.6 cm	19.1

1.2. *Pst*DC3000 lux strain

Since the *Pst*DC3000 GFPuv strain did not allow us to optimize our infection experiments, we tried to use a luminescent strain *Pst*DC3000 lux (Fan et al., 2007). This strain is bioluminescent thanks to the insertion of the *luxCDABE* operon from *Photorhabdus luminescens* into its chromosome, under the control of a constitutive promoter. As for the GFPuv strain, the lux strain was designed for direct quantification of the bacterial growth without serial dilutions and plating on agar medium. I tried to measure the luminescence both on leaf discs and on grinded material. However, as for the GFPuv strain, I was not able to find ant correlation between the luminescence and the pathogen numerations (**Figure 21**). Nevertheless, this method is commonly used in the laboratory of Pr. Julia Vorholt who provided us the strain (Vogel et al., 2012). Since we do not use the same device for the luminescence detection, we suppose that we are not in the adapted conditions for the use of this method to quantitatively measure the luminescence. Thus, I decided to proceed the further infection experiments with the classic *Pst*DC3000 strain and to proceed numerations by plating several dilutions of the grinded leaves extract.

1.3. Plating method for numerations

Since serial dilutions and plating steps on agar medium are necessary, I tried to find a way to use less agar plates and to count more easily and quickly the bacteria after 2 days of incubation at 28°C. Thus, I decided to plate 3 spots of 20 µL for each of the appropriate dilution on one plate per plant, as recommended in a previous paper (Liu et al., 2015). I took care to plate each spot of each dilution far enough, so they do not overlap, on plates that were dried overnight at room temperature. Moreover, it was important to count colonies early enough, before they overgrow, in order to avoid possible overlaps. It generally corresponded to 5 to 70 colonies per spot, at a maximum of 48h after incubation at 28°C. This method was used for the last infection experiment proceeded in the laboratory.

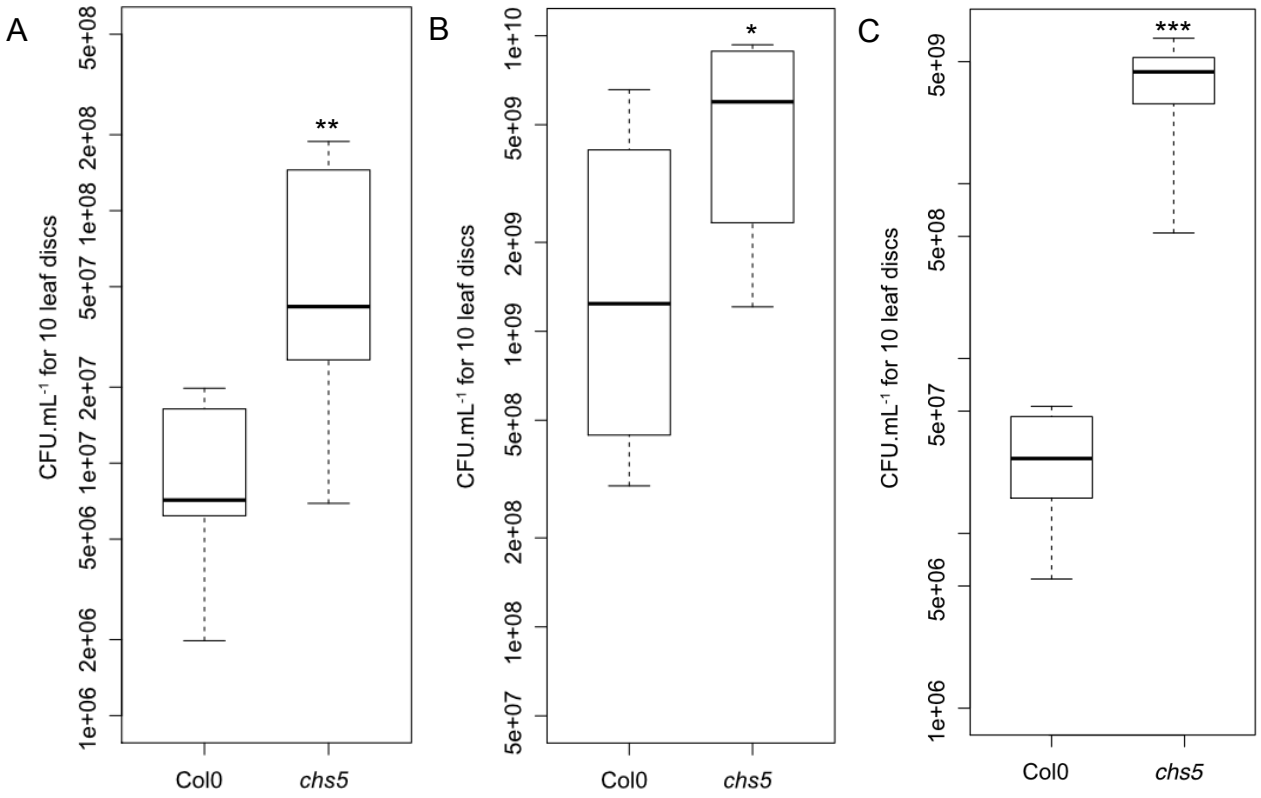

Figure 22. Pathogen quantification upon infection of *Arabidopsis thaliana* by *Pseudomonas syringae* pv. *tomato* DC3000. Pathogen numerations by plating were done for both Col-0 and *chs5* at 6dpi (A) First Infection was proceeded in summer 2017. N=8 for Col-0 and N=10 for *chs5*. There is an increased sensitivity of *chs5* compared to Col-0. (**P < 0.05 Student's t test). (B) Infection 2 was proceeded in winter 2018-2019. N=12. Despite more variability between the samples. there is also an increased sensitivity of *chs5* compared to Col-0. (*P < 0.1 Student's t test). (C) Last infection was proceeded in spring 2019. N=12. There is an increased sensitivity of *chs5* compared to Col-0. (***P < 0.01 Student's t test).

Figure 23. Visible lesions on infected leaves of Col-0 and *chs5* at 6dpi. Pictures were taken 6 days following the infection by *Pst*DC3000. Col-0 infected leaves (A) were less drastically affected than *chs5* infected leaves (B). We often saw that lesions on *chs5* were necrotic-like only 4dpi while it took more time on Col-0 (6dpi).

2. Infection of Arabidopsis thaliana Col-0 and chs5 by *Pseudomonas syringae* pv. *tomato* DC3000

The draft paper presented below refers to the first infection experiment that I made in the laboratory, in the same batch of plants as those used for the inventory of the communities. I repeated the infection experiments independently, in different culture chambers but in the same conditions. I always highlighted a more or less important difference of sensitivity between Col-0 and *chs5*, which led me, in the last experiment, to deepen these results. Thus, I performed the infection on enough plants to analyze gene expression and some key metabolites in addition to the pathogen quantification, as exposed in the paper below. I paid attention to infect plants that were approximately the same size and the same number of leaves for each experiment, as indicated in the **table 1.** Pathogen quantifications from experiment 1 are shown in **figure 22, A**, and in the paper. They were obtained by the classic plating method corresponding to one dilution per plate, in duplicates. Those from experiment 2 are shown in **figure 22, B**, and were obtained by the same method. Results from our last experiment (experiment 3) are shown in **figure 22, C** and in the paper. The pathogen numerations for this last experiment were proceeded using the droplets method mentioned in the paragraph II.1.3. *chs5* mutants have always been shown to be more sensitive to *Pst*DC3000 than Col-0 since there is 10-fold (experiment 1 and 2) to 100-fold (experiment 3) more pathogen in the mutant leaves than in the wild-type leaves. Moreover, it was difficult to quantify the importance of the lesions on the infected leaves, but we also saw that the *chs5* leaves often appeared to be more affected than those of Col-0 (**Figure 23**). The chlorotic lesions appeared already 2 days post infection, but the necrotic-like phenotype appeared after 4 days in the mutant, which was quicker than in the wild-type for which the lesions were necrotic-like only 6 days post infection.

The bacterial titer of the infection suspension for experiment 1 was 2,59 x 10^5 ufc.mL^{-1}, for experiment 2 it was 1,87 x 10^5 ufc.mL^{-1}, and for experiment 3, the pathogen titer was lower with 3.84 x 10^4 ufc.mL^{-1}. This difference of concentration between the infection suspensions does not explain the higher pathogen quantification in experiment 3. However, for this last experiment, we cultivated *Pst*DC3000 in NYGB supplemented with rifampicin (NYGB+r) instead of KB supplemented with rifampicin (KB+r) as we did for experiments 1 and 2. We chose this option since we encountered

difficulties of bacterial growth in KB medium after we changed our peptone supplier. There could be an impact of the culture medium on the bacteria physiology, involving a more or less significant multiplication efficiency for instance. But there is also a higher amount of pathogen counted in experiment 2 compared to experiment 1, despite a similar concentration of the infection suspension. Thus, we can suppose that the pathogen does not multiply the same way depending on the growth culture chambers, even if the culture conditions are the same. In addition, in the last experiment, we plated 20 µL droplets compared to the usual method consisting in plating 100µL per plate, which can also induce a bias in the numerations.

III. Manuscript

To be submitted before the thesis defense.

Microbiota profiling and analysis of pathogen infection in *Arabidopsis thaliana* reveals the influence of isoprenoids upon root and leaf colonization by specific bacteria

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Summary

Isoprenoids, also called terpenoids, are an important class of metabolites involved in cellular division, photosynthesis, respiration or immune response. Recent studies suggest a possible role of these metabolites in plant-microbe interactions. In this study, we analyzed the implication of isoprenoids in the selection of the microbiota by *Arabidopsis thaliana.* Two mutants exhibiting a deficiency in the production of isoprenoids were studied. The *chs-5* mutant carries a weak allele of the gene encoding the enzyme DXS1 (1-deoxy-D-xylulose 5-phosphate synthase), a key component of the plastidial MEP (Methylerythritol phosphate) pathway. The *hmg1-1* mutant carries a knock-out allele of the gene encoding the enzyme HMGR1 (3-hydroxy-3-methylglutaryl-coenzyme A reductase), an important enzyme of the cytosolic MVA (Mevalonate) pathway. The community structures associated with WT or mutants were globally similar but the relative abundance of some OTUs (Operational taxonomic units) were significantly different, suggesting a possible implication of isoprenoids in the selection of a fraction of the associated microbiota. In addition, we demonstrated that plants affected in the MEP biosynthesis pathway were more susceptible to the phytopathogen *Pseudomonas syringae* pv. *tomato* DC3000 than WT. Taken together, these results suggest an implication of isoprenoids in the interaction of the plant with specific bacteria.

Keywords: *Arabidopsis thaliana*, isoprenoids, microbiota profiling, plant-bacteria interactions, *Pseudomonas syringae* pv. *tomato* DC3000.

Introduction

Plants serve as host to different microorganisms which interact with their host and/or with each other (Hassani et al., 2018; Uroz et al., 2019). Plants and their associated microorganisms

in nature, called phytobiome, can be considered as meta-organisms or holobionts (Berg et al., 2014; Bulgarelli et al., 2013; Lebeis, 2015; Simon et al., 2019; Vandenkoornhuyse et al., 2015). Thanks to the recent development of the environmental microbial and genomic approaches, a precise inventory of the bacterial communities interacting with *Arabidopsis thaliana* has been done, and many bacteria associated with this plants have been isolated (Bai et al., 2015; Guttman et al., 2014; Lundberg et al., 2012; Schlaeppi et al., 2014). A large part of this bacterial community is found in the soil in the vicinity of roots, in the rhizosphere, on the surface (epiphytes) or within (endophytes) the plant roots or aerial part (stems, flowers, leaves, called phyllosphere) (Bulgarelli et al., 2013; Gaiero et al., 2013; Hardoim et al., 2015; Lebeis, 2015; Vorholt, 2012). Recent studies aim to decipher the role of such microorganisms on the plant fitness (Bulgarelli et al., 2013; Lebeis, 2015; Vandenkoornhuyse et al., 2015). The microbial community associated to plants may have positive impact by promoting growth, nutrient acquisition, defense against abiotic and biotic stresses (Bulgarelli et al., 2013; Gaiero et al., 2013; Hardoim et al., 2015; Müller et al., 2016; Vacheron et al., 2013; Vogel et al., 2012).

Some of these microorganisms play an important role to prevent plant colonization by pathogens. Indeed, during their whole lifecycle, plants are confronted to a wide range of stresses, among which pathogen attacks (Hahlbrock et al., 2003). *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*DC3000) is probably one of the most studied bacteria in the context of plant-microbe interactions (Baltrus et al., 2017; Katagiri et al., 2002). Notably, this bacterium has played an important role in the establishment of the zigzag model of plant immunity (Dangl et al., 2013; Jones and Dangl, 2006). *Pst*DC3000 is a Gram-negative rod-shaped bacteria responsible for bacterial speck of tomato (Katagiri et al., 2002). This hemi-biotrophic pathogen uses stomata or wounds to enter the plant leaves, where it multiplies in the apoplast. After multiplication, necrotic-like lesions surrounded by chlorotic halos are visible on the infected leaves. *Pst*DC3000 is also virulent against *A. thaliana*, which makes its use common in laboratories (Baltrus et al., 2017; Katagiri et al., 2002). Basically, infection by hemi-biotrophic pathogens such as *Pst*DC3000 often leads to the induction of systemic acquired resistance (SAR). In this mechanism, the phytohormone salicylic acid plays a key role by activating the plant defense (Katagiri et al., 2002; Thomma et al., 2001). Indeed, salicylic acid notably potentiates the activation of the plants pathogenesis-related (PR) genes, that act against the pathogen effectors. In *A. thaliana*, some of these *PR* genes, *PR-1*, *PR-2* and *PR-5* are SAR marker genes and thus, they are induced upon infection *Pst*DC3000 (Nawrath and Métraux, 1999; Thomma et al., 2001).

One of the most interesting question in plant-bacteria interaction field is to decipher how the plant could distinguish between pathogenic, commensal or mutualistic micro-organisms (Bulgarelli et al., 2013). Several recent studies suggest that the plant immune system, plant hormones or secondary metabolites such as flavonoids may play a role in the selection of specific microorganisms by the plant (Cotton et al., 2019; Hu et al., 2018; Jones et al., 2019; Lebeis, 2015; Voges et al., 2019). Interestingly, few studies suggest that isoprenoids could play a role in bacterial selection by plants (Burdon et al., 2018; Wang et al., 2012a; Zahid et al., 2017). Isoprenoids, also called terpenoids, are an important class of metabolites with more than 50 000 different molecules already identified in living organisms (Thulasiram et al., 2007). These metabolites are involved in diverse biological processes in plants such as membrane function or growth (sterols, brassinosteroids, gibberellins), photosynthesis (chlorophylls and carotenoids), respiration (ubiquinones), or stress response (abscisic acid) (Rodríguez-Concepción and Boronat, 2015; Singh and Sharma, 2015). Some isoprenoids have specific non fundamental functions and are considered as "specialized" metabolites (previously called "secondary" metabolites). For example, they could play a role in pollinators attraction or in protection against herbivores and pathogens (Rodríguez-Concepción and Boronat, 2015; Yazaki et al., 2017). These compounds are synthetized from two precursors: isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). In plants, two pathways are required for the biosynthesis of these precursors. The mevalonate pathway (MVA) occurs in the cytosol and implicates several enzymes among which the 3-hydroxyl-3-methylglutaryl CoA reductase (HMGR). The 2-C-methyl-D-erythritol 4-phosphate pathway (MEP) occurs in plastids and requires the 1-deoxy-D-xylulose 5 phosphate synthase (DXS). Both pathways lead to the formation of IPP or DMAPP (Figure 1). It was demonstrated that one pathway could not compensate the defect of the other one (Rodríguez-Concepción and Boronat, 2015).

The aim of our work was to study the implication of isoprenoids in the selection of the microbiota by *A. thaliana*. For that purpose, two mutants affected in the biosynthesis of IPP and DMAPP were chosen. The first mutant, *chs5*, (*chilling sensitive 5*, Col-0 genetic background), carries a missense mutation of the gene encoding the enzyme DXS1 (1-deoxy-D-xylulose 5-phosphate synthase 1), changing an aspartic acid (D) to an asparagine (N) residue at position 627 (D627N) in this key component of the plastidial MEP pathway (Araki et al., 2000; Schneider et al., 1995; Wright et al., 2014). This mutation causes a chlorotic phenotype due to a decrease of plastidial isoprenoid biosynthesis (Suzuki et al., 2004; Wright et al., 2014). The second mutant, *hmg1-1*, carries a T-DNA insertion in the first exon of the gene encoding the

enzyme HMGR1 (3-hydroxy-3-methylglutaryl-coenzyme A reductase), a key enzyme of the MVA pathway (Heintz et al., 2012; Suzuki et al., 2004). Because of a decrease in *HMG1* gene expression in this mutant as compared to the WT, the *hmg1-1* mutant showed a decrease of isoprenoids content downstream of squalene, sterols and terpenoids and therefore a defect in growth and fertility (Heintz et al., 2012; Suzuki et al., 2004). To test if isoprenoids are involved in plant-pathogens interactions, we compared the infection capacity of *Pst*DC3000 in mutants and WT plants. In parallel, to decipher if the plant isoprenoids are involved in the plant-microbiota interactions in general, the communities interacting with the WT and the mutants were compared using a 16S rRNA gene sequencing-based approach. The community structures associated with WT or mutants were globally similar but the relative abundance of some OTUs (Operational Taxonomic Units) were significantly different, suggesting a possible implication of isoprenoids in the selection of a fraction of the associated microbiota.

Material and Methods

Plant material and growth conditions

A. thaliana wild-type ecotype Colombia (Col-0) or Wassilewskija (WS2) or derived mutants *chs5* (Col-0 genetic background), carrying a point mutation in the gene encoding the 1-deoxy-D-xylulose 5-phosphate synthase 1 (DXS1) (Araki et al., 2000; Schneider et al., 1995; Wright et al., 2014), and *hmg1-1* (WS2 genetic background) carrying a transposon insertion in the gene encoding the 3-hydroxy-3-méthylglutarylcoenzyme A reductase 1 (HMGR1) (Heintz et al., 2012; Suzuki et al., 2004) were grown in soil, in growing chambers, for 6 weeks under 12-h photoperiod (6 Lumilux tubes T5, Osram) and temperatures were set at 21°C during the light phase and 18°C during the dark phase. Relative humidity was set to 75%, and light level was set at 250 µE. All the seeds were kept at -20°C for 48h before sowing.

Microbiota profiling

After 6 to 8 weeks, *A. thaliana* plants were extracted from soil, shaken, and roots were shortly washed in sterile distilled water to remove soil (Supplementary figure 1). Plants were cut in order to separate phyllosphere and roots/rhizosphere. Rhizosphere was separated from roots by scrapping using a sterile scrapor. The inner root or leaf tissues and surface were not discriminated, and we refer these samples as “root” and “phyllosphere” microbiota,

respectively. Samples were crushed using a mortar and pestle and frozen at -20°C until DNA extraction. DNA was extracted from frozen rhizosphere (0.25 g), on one hand, or from powder obtained from phyllosphere and roots (50 mg) on the other hand, using the PowerSoil DNA Isolation Kit and PlantDNA Isolation kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA), respectively, according to the manufacturer's instructions. The DNA concentration and quality were estimated by measuring the OD at 260nm and 280nm.

Libraries were constructed according the 16S Metagenomic Sequencing Library Preparation protocol (Illumina Part # 15044223 Rev. B) except some modifications mentioned below. Briefly, 16S RNA encoding gene were amplified in duplicate from the extracted DNA using the primer listed in supplementary table 1 that target the bacterial/archaeal 16 S rRNA gene variable region 5-6. Primers (Supplementary figure 2, Supplementary table 1) used for this first PCR were composed of (from 5' to 3' ends): 1)- the Illumina overhang sequence (containing Read 1 and Read 2 specific sequences) described in the Illumina 16S protocol, 2)- two 16S V5-V6 gene-specific sequences 3)- a 0 to 7pb heterogeneity spacer to increase the nucleotide diversity for sequencing, as described in Fadrosh *et al*. (Fadrosh et al., 2014). This first amplification (PCR1, 25 µl) was performed by mixing 25 ng genomic DNA, the KAPA HiFi HotStart ReadyMix PCR Kit (12.5 µl) (Kapabiosystems, Boston, United States) and primers (5µl at 1 µM) and using the following program: initial denaturation at 95 °C for 3 min; 25 cycles of 95 °C for 30 sec, 55 °C for 30 sec and 72 °C for 30 sec; final elongation at 72°C for 5 min. PCR products were analyzed on 1% agarose gel to verify the success of amplification and duplicate amplified samples were pooled and purified using AMPure XP beads (Agencourt, Beckman-Coulter). The quantity and quality of the amplicons were controlled with the Bioanalyzer (Agilent). A second amplification (PCR2) was performed using the Nextera XT primers (Illumina) containing the full-length P5 and P7 sequences. Amplicons were purified using AMPure XP beads (Agencourt, Beckman-Coulter). Their size was controlled with the Bioanalyzer (Agilent). These libraries were normalized, pooled together and 5% PhiX v3 (Illumina) was added. Sequencing was performed as paired end of 300 base pairs reads (2X300) on a Miseq platform.

The bioinformatics processing was performed using the FROG pipeline under Galaxy environments (Escudié et al., 2018). Shortly, it included a pre-processing of the sequencing read data with "FLASH" (suppress PCR duplicates, too long or too short reads). The quality sequences were clustered to Operational Taxonomic Units (OTUs, >97% sequence similarity,

minimal coverage of 5 sequences) with “Swarm”. Chimeric OTU sequences were removed using “VSEARCH”. Filtering was performed to keep sequences present at least in 3 samples and suppress contaminants (phiX). Taxonomic assignments were done using multi-affiliation output with the Silva, Midas and Greengenes databases.

OTUs classified as mitochondrial or Cyanobacteria/chloroplasts sequences were removed. Data from WT and mutants were compared using the Phyloseq pipeline (McMurdie and Holmes, 2013).

Infection of *A. thaliana* by *Pseudomonas syringae* pv. *tomato* DC3000

The phytopathogen *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*DC3000) was grown on solid King' B (KB) medium (20 $g.L^{-1}$ proteose peptone (Conda); 1.5 $g.L^{-1}$ K_2HPO_4 (Merck); 15 $g.L^{-1}$ glycerol (VWR); 1.5 $g.L^{-1}$ $MgSO_4.7H_2O$ (Merck); 15 $g.L^{-1}$ agar (Sigma-Aldrich)), supplemented with 50 $\mu g.mL^{-1}$ rifampicin (Sigma) (KB+r), at 28°C for 2 days. Bacteria were transferred onto liquid KB+r medium and grown with shaking at 28°C until exponential growing phase. The bacterial culture was centrifuged at 2500 g for 10 min to recover bacteria. They were washed twice and resuspended in 10 mM $MgCl_2$. OD_{600nm} was adjusted to obtain 1 x 10^5 colony forming units per milliliter ($cfu.mL^{-1}$). Using a needle-less syringe, 10 leaves of each plant were pressure infiltrated with either 1 x 10^5 $cfu.mL^{-1}$ of *Pst*DC3000, or mock infiltrated with sterile 10 mM $MgCl_2$ (Merck) (adapted from Katagiri et al., 2002). For each genotype, infiltrated leaves from 12 infected plants that were distributed in 2 independent boxes and 2 mock-treated plants of *A. thaliana* were harvested 6 days post infection (dpi). For each plant, 10 leaf discs were crushed in KB+r. After serial dilutions, samples were plated onto KB+r agar plates and incubated at 28°C for 2 days and numerated.

Quantitative PCR analyses of *PR-1*, *PR-2* and *PR-5* expression

Infiltrated leaves from 9 infected plants and 9 mock-treated plants of Col-0 and *chs5* were harvested before infection by *Pst*DC3000 and at 1, 3 and 6 dpi, and grouped in bulks of 3 plants per condition. Plant material was crushed in liquid nitrogen and stored at -80°C until extractions. 1 mL of TRIzol reagent (Molecular Research Center) was added to approximately 60 mg of grinded material in 2 mL tubes containing glass beads before grinding 2 x 30 sec with Precellys®. Samples were kept at room temperature for 5 min before adding 200 µL of chloroform (Sigma-Aldrich) and 15 sec agitation. Samples were kept at room temperature for

2 min 30 before centrifugation at 4°C, 12 000 g for 15 min. 400 µL of supernatant were collected and 333 µL of isopropanol (Sigma-Aldrich) were added to the supernatant. Samples were kept at room temperature for 10 min before centrifugation at 4°C, 12 000 g for 20 min. The supernatant was poured off and 1 mL of 80% ethanol (Sigma-Aldrich) was added to the samples. Samples were centrifuged at 4°C, 12 000 g for 5 min and the supernatant was poured off. 1 mL of 100% ethanol was added to the samples before centrifugation at 4°C, 12 000 g for 5 min and elimination of the supernatant. Pellets were air-dried before addition of 50 µL of milli-Q H_2O and incubation at 4°C for 30 min. Samples were then vortexed and incubated at 50°C for 5 min, twice. RNAs were finally stored at -20°C until reverse transcription. A DNAse treatment was carried out on the RNAs before reverse transcription. For that, 1 µg of RNA was resuspended in H_2O supplemented with 10 µL of DNAse mix: 0.1 µL RNAse OUT (Promega); 6 µL H_2O; 2 µL DNAse 10X buffer (Promega); 2 µL DNAse (Promega). The mix was incubated at 37°C for 45 min. Then the reaction was stopped by addition of 1 µL of Stop DNAse (EGTA, 20 mM, Promega) and incubation at 65°C for 10 min. After 5 min of incubation on ice, samples were supplemented with 20 µL of RT mix: 6 µL H_2O; 8 µL 5X SuperScript IV buffer (Invitrogen), 2 µL 0.1 M DTT (Invitrogen); 2 µL 10 mM dNTPs (ThermoFisher Scientific); 2 µL 40 µM smart-Oligo-dT (ThermoFisher Scientific), and 0.5 µL of 200 $\mu g.\mu L^{-1}$ SuperScript IV (Invitrogen). Samples were incubated at 50°C for 10 min and at 80°C for 10 min. cDNAs were diluted by addition of 40 µL H_2O. Real-time PCR was performed on 10 ng of cDNA. The reaction mix contained 1 µL of cDNA, 5 µL of SYBR® Green (Roche), 2 µL of H_2O, and the couple of primers (2.5 µM) for each gene. Primers used for the qPCR are listed in supplementary table 1. The housekeeping genes *ACT2* (At3g18780) and *GADPH* (At1g13440) (Czechowski et al., 2005) were used as internal references and their constitutive expression was tested on the studied material. Primers targeting *PR-1* (Atg14160.1), *PR-2* (Atg57260.1) and *PR-5* (Atg75040.1) encoding genes were designed using the LightCycler Probe Design software 2.0 (Roche). PCR amplification, melting curve analysis was performed using these primers to verify the amplification of a single PCR product. Real-time PCR was performed on a LightCycler® 480 II instrument (Roche) with the following program: 95°C for 5 min; 45 cycles split in 95°C for 10 sec, 60°C for 15 sec and 72°C for 15 sec; and a temperature gradient from 55°C to 95°C in 1 min. The relative amount of cDNA corresponding to the transcript level in the sample was calculated using the $2^{-\Delta\Delta Ct}$ (Pfaffl, 2001). The target gene expression level was normalized against internal reference genes, averaged over triplicate determinations, and shown as a relative value. The induction (or repression) factor of the target gene at the T_X time

was calculated with respect to a starting biological condition T_0 set at 1. Technical triplicates were performed for each sample.

Extraction and quantification of chlorophylls and carotenoids, total sterols, sterol esters and fatty acids from plant tissues

The plant material hitherto stored at -80°C was crushed in freshly prepared 80% acetone (Sigma Aldrich) in water (v/v). Samples were incubated in the dark at 4°C for 24 h. After incubation, 200 µL of the supernatant were transferred to a 96 well microplate (96 Well ELISA Microplates, PS, U-bottom, MICROLON®, Greiner Bio-one). For each sample, 3 wells were prepared for measurement. Optical density was measured for each well at 470 nm, 646 nm and 663 nm on FLUOstar Omega spectrometer (BMG Labtech). The concentration of chlorophylls and carotenoids in the samples was determined with the equations given by (Lichtenthaler and Buschmann, 2001), with c_a: concentration of chlorophyll a; c_b: concentration of chlorophyll b; $c_{(x+c)}$: concentration of xanthophylls and carotenes.

$$c_a\ (\mu g.mL^{-1}) = 12.25\ A663.2 - 2.79\ A646.8$$

$$c_b\ (\mu g.mL^{-1}) = 21.50\ A646.8 - 5.10\ A663.2$$

$$c_{(x+c)}\ (\mu g.mL^{-1}) = (1000\ A470 - 1.82\ c_a - 85.02\ c_b)/198$$

To extract total sterols, sterol esters and fatty acids, acetone was evaporated from the remaining 2.8 mL samples (1 h at 65°C), and samples were lyophilized. 3 mL of 6% KOH in methanol (Carlo Erba) was added to the lyophilized material to proceed saponification at 70°C for 2 h. After addition of 1.5 mL milliQ H_2O, 1.5 mL n-hexane (Roth) was added samples were mixed and centrifuged 5 min at 2500 g. The hexane upper phase was transferred in new tubes. This extraction was performed 3 times on the same samples and the 3 hexane phases were pooled and evaporated (at least 10 min at 70°C). Acetylation was then performed on the dried residue with 100 µL of toluene (Carlo Erba), 50 µL of acetic anhydride (Fluka) and 30 µL of pyridine (Fluka) in a glass vial at 70°C for 1 h. After evaporation at 70°C for 30 min, samples were resuspended in 300 µL n-hexane (Roth). To identify sterols, plant extracts were analyzed by gas chromatography (GC instrument, Agilent 6890) coupled to mass spectrometry (MS analyzer, Agilent 5973) using a HP-5MS column (5% PhenylMethyl Siloxane, 30 m x 250 µm x 0,25 µm, Agilent J&W). 2 µL of sample were injected. The helium flux was 1 $mL.min^{-1}$. The column temperature was hold at 60°C for 1 min, heated to 200°C with a gradient of 30°C per

min, and then reaching a maximum of 300°C with a gradient of 2°C per min, for a total run time of 56,33 min for each sample. The separated molecules were ionized by electronic impact at 70 eV. The identification of each species was made by the detection of specific daughter ions obtained after ionization and using the database.

Results

The WT microbiota changed during growth in the tested conditions

In a first set of experiments, we wanted to evaluate if the community changed during the growth of plants. We chose two stages, the "rosette" and the "siliques" stages in WT Col-0 and WS2 ecotypes (Supplementary figure 1). The Supplementary figure 3 shows that *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* were dominant phyla at both stages in the two ecotypes. Second, β-diversity analysis revealed that, at both stages, the composition of the microbiota of the phyllosphere was significantly different from that in the roots and rhizosphere, which were more closed together (Supplementary Figure 4). Finally, clustering and principal coordinate analyses (CoAP =MDS) (supplementary figure 4) revealed that the composition of the communities at both stages were significantly different. In particular, we observed that *Bacteroidetes* were more abundant in the two ecotypes at the silique stage (9.2 and 12.2% in Col-0 and WS2, respectively) as compared to rosette stage (5.7 and 4.2 %). Moreover, *Actinobacteria* were more abundant in WS2 at the rosette stage (18.9 and 14.1%) than at the siliques stage. Thus, these observations revealed that the community changed during plant growth in both ecotypes.

Mutations in isoprenoid pathways did not affect the global composition of the bacterial community associated to *A. thaliana*.

We had to choose one stage to compare the community in WT and mutants. The phenotype of the *hmg1-1* is observable at the "siliques" stage. At this stage, the stem size of *hmg1-1* plants is reduced and smaller siliques are observed as compared to the WT (Suzuki et al., 2004). The chlorotic phenotype of the *chs5* mutant is mainly observed at early growth stage when the rosette is less than 7 cm bright and when plants are grown in cold conditions (Araki et al., 2000). In these conditions where mutants' phenotypes are visible, bias due to difference of size and number of leaves may influence the comparison of the communities between WT and mutants.

Because *hmg1-1* has a smaller size at the "siliques" stage, we decided to compare WT and mutant microbiota at the early "rosette" stage. Because of the cold-sensitive phenotype of the *chs5* mutant, plants were grown at 21°C during the light phase and 18°C during the dark phase, and the sampling was performed with plants having a diameter higher than 7 cm. In these conditions, WT and mutants had similar shapes (Figure 1).

First, we observed that the relative abundances of the different phyla were similar in the three replicates in each condition, showing that our sampling conditions were relevant (Supplementary Figure 5). Second, the rarefaction curves revealed that the number of sequences was important enough to visualize most of the diversity (Supplementary figure 6). The community at the phyla level was similar in both Col-0 and WS2 ecotypes (Supplementary figure 5A). However, only 16% of the OTUs were found in both wild types (Supplementary figure 5B), showing that, in our tested conditions, both ecotypes shared a core microbiota but had also specific OTUs interacting with them. Third, in all plant genotypes, α- and β-diversity indexes revealed that microbiota richness and structures in phyllosphere were globally different from the rhizospheric or the root microbiota, which were more closed together (Figure 2 and Supplementary figure 7 and 8).

Finally, we compared each mutant with its corresponding WT. The α-diversity index shows that WT and mutants' microbiota had globally similar richness and were therefore comparable in each compartment (Supplementary figure 7). More interestingly, the β-diversity analysis revealed that the microbiota composition was less different between WT and mutant than when the compartment communities were compared (Figure 3 and supplementary figure 8). These global analyses revealed that the mutations did not globally affect the community composition or richness. Nevertheless, some specific species may be impacted by the mutations. Therefore, we further looked for OTUs that were significantly more or less abundant in mutant as compared to the WT.

Comparison of OTUs abundance revealed that mutation impacted a fraction of the microbiota

First, we compared the relative abundance of each OTU in Col-0 and *chs5*. The relative abundance of 94, 224, and 108 OTUs was significantly different (ANOVA, $p<0.01$) in *chs5* as compared to Col-0, in the phyllosphere, rhizosphere and roots, respectively (Figure 3 and Supplementary Table 2). We observed a majority of *Proteobacteria*, *Actinobacteria* and

Bacteroidetes as for the total microbiota (Figure 3). Interestingly, we observed that in the phyllosphere, the percentage of *Actinobacteria* and *Saccharibacteria* was higher and the percentage of *Proteobacteria* was lower, in the variable OTUs as compared to the total microbiota. In the roots, the percentage of *Proteobacteria* was higher in the variable OTUs as compared to the total microbiota, whereas no global differences were observed in the rhizosphere. Among these OTUs which are variable in *chs5* as compared to Col-0, we found a higher percentage of *Streptomyces* (13.8, 9.3 and 6.7 % in the phyllosphere, the roots and the rhizosphere, respectively) as compared to the total microbiota (2.3, 1.9 and 1.9 % in the phyllosphere, the roots and the rhizosphere, respectively). In addition, in the rhizosphere we found 8.9 % of *Rhizobium* in the variable community, higher than what observed in the total community (1.6%). These observations suggest that colonization of the plants by *Rhizobium* in the rhizosphere, and *Streptomyces* in all compartments were affected in *chs5* mutant.

Second, we compared the relative abundance of each OTU in WS2 and in *hmg1-1* in each compartment. The relative abundance of 76, 291, and 121 OTUs was significantly different (ANOVA, $p<0.01$) in *hmg1-1* as compared to WS2, in the phyllosphere, rhizosphere and roots, respectively (Figure 3 and Supplementary Table 3). Interestingly, we observed that in the phyllosphere, the percentage of *Actinobacteria* was higher and the percentage of *Proteobacteria* was lower, in the variable OTUs as compared to the total microbiota (Figure 3). In the roots, the percentage of *Proteobacteria* was higher in the variable OTUs as compared to the total microbiota, whereas, in the rhizosphere, the percentage of *Bacteroidetes* was higher in the variable OTUs as compared to the total microbiota. Among the OTUs which are more abundant in the WS2 than in *hmg1-1*, we found a large majority of *Streptomyces* (13.1, 7.4 and 7.2 % in the phyllosphere, the roots and the rhizosphere, respectively) as compared to the total microbiota (3, 2.8 and 2.9 % in the phyllosphere, the roots and the rhizosphere, respectively). In the rhizosphere we found 7.2 % of *Rhizobium* in the variable community, higher than what observed in the total community (1.3%). These suggest that colonization of the plants by *Rhizobium* in the rhizosphere, and *Streptomyces* in all compartments were affected in *hmg1-1* mutant.

Third, we compared data obtained in both analyses. We found OTUs whose abundance was different in Col-0 versus *chs5* comparison, but not in WS2 versus *hmg1-1* analysis, and vice versa (Figure 3, Supplementary Data 4 and 5). For example, among these OTUs whose abundance was higher in Col-0 than in *chs5*, in phyllosphere or roots, we found some

Actinobacteria (*Kribella*, *Streptomyces*), *Chloroflexi*, *Planctomycetes* (*Schlesneria*), *Saccharibacteria*, and one OTU affiliated to *Sphingomonas*. This OTU was not found to be differently abundant in the phyllosphere nor roots in the comparison WS2 versus *hmg1-1*. We compared the 16S rRNA sequence to genbank and found 100% identity with *Sphingomonas wittichii*, a bacterium which was shown to degrade plant hormone indole 3-acetic acid (IAA) (Leveau and Gerards, 2008). Finally, we observed that the relative abundance of 23, 56, and 17 OTUs was significantly different (ANOVA, $p<0.01$) in both analyses, in the phyllosphere, rhizosphere and roots, respectively (Figure 3). Among these OTUs, 24 were more abundant in the mutants as compared to their respective WT, with a majority of *Proteobacteria* (11 OTUs) and *Actinobacteria* (9 OTUs among which 4 *Streptomyces*) (Table 1). On the other hand, 14 OTUs were more abundant in the WT as compared to mutants, with a majority of *Actinobacteria* (7 OTUs among which 4 *Streptomyces*) and *Proteobacteria* (5 OTUs) (Table 2). The abundance of these OTUs is therefore influenced by both mutations in the MVA and in the MEP pathways (Figure 1). All these results revealed that the colonization of some specific OTUs was impacted by either the mutation in the MEP or the MVA pathways. Interestingly, the colonization by *Rhizobium* in the rhizosphere, and *Streptomyces* in all compartments, and the colonization of 38 OTUs is impacted whatever the isoprenoids pathway affected. Next we wondered if the difference of abundance of some of these specific OTUs may impact other plant-bacteria interaction, as for example the interactions with pathogens.

The *chs5* mutant is more sensitive than the WT to *P. syringae* DC3000 infection

To test the capacity of *chs5* and *hmg1-1* mutants to interact with pathogens, *Pst*DC3000, was used to infect leaves. It is important to notice that this experiment was performed with the same set of plants than the 16S barcoding experiments. Quantification was performed 6 days post infection (6dpi). We obtained no significant difference of pathogen development between the *hmg1-1* mutant altered in the MVA pathway and the WS2 wild-type (Figure 4A). In contrast, a significant increase of the pathogen colonization was observed in the *chs5* mutant affected in the MEP pathway, compared to the Col-0 wild-type (Figure 4B). Thus, an alteration of the MEP but not the MVA pathway leads to an increased colonization by the pathogen.

We repeated this experiment with other *chs5* and Col-0 plants grown in the same conditions but at an earlier growth stage (rosette diameter <7cm), when the chlorotic phenotype of *chs5* was still visible. In these conditions, the chlorophylls contents were slightly reduced in this mutant as compared to the WT (Supplementary figure 9). Indeed, Chlorophyll a abundance is

1.3-fold higher in Col-0 compared to *chs5*; chlorophyll b is 1.5-fold more abundant in Col-0 than *chs5*; and the abundance of carotenoids is unaffected by the mutation. In this case, we observed that 10-fold more pathogens colonized the *chs5* plants as compared to the wild-type. To elucidate whether this difference of sensitivity between Col-0 and *chs5* originated from a defect in plant immune response, we investigated the expression level of some key genes known as SAR markers (Supplementary figure 9). *Pathogenesis-related* (*PR*) genes *PR-1*, *PR-2* and *PR-5* are usually induced upon infection by (hemi)-biotrophic pathogens such as *Pst*DC3000 (Thomma et al., 2001). These SAR marker genes were particularly induced 3dpi and decreased at 6 dpi. No significant difference was observed for *PR-1* at 1, 3 or 6 dpi; for *PR-2* at 1 and 3 dpi, and for *PR-5* at 1 or 6dpi. A slight difference was observed for PR-5 expression at 3 dpi, (1.8-fold higher in Col-0 than in *chs5*), and for *PR-2* at 6 dpi (1.6-fold higher in Col-0 than in *chs5*). However, these differences were minor and could not explain the difference in pathogen colonization in these tested conditions. Finally, it has been previously shown that the content of stigmasterol, an isoprenoid synthetized via the MVA pathway, increased upon *Pst*DC3000 infection (Griebel and Zeier, 2010). An analysis of the stigmasterol content was therefore performed (Supplementary figure 9). As previously observed, there is a 7.5-fold increase of stigmasterol in infected compared to non-infected Col-0 (mock-infiltrated) and a 5-fold increase in infected compared to non-infected *chs5* (mock-infiltrated), suggesting that the mutant can synthetize stigmasterol after infection, in the tested conditions. 6 days after infection, the amount of stigmasterol quantified by GC-MS is not significantly different between Col-0 and *chs5*. All these results suggest that a decrease of the MEP pathway efficiency, but not of the MVA pathway efficiency, led to a better colonization by *Pst*DC3000. This better pathogen colonization could not be explained by a defect in the expression of *PR-1*, *PR-2* and *PR-5* genes, nor by a defect in stigmasterol synthesis.

Discussion

In this work, to decipher if isoprenoids were involved in plant-bacteria interactions, we used two complementary approaches. We compared the interaction of WT and mutants affected in isoprenoids biosynthesis with a model pathogen, *Pst*DC3000. In parallel, we compared the microbiota associated with WT or mutants using a 16S sRNA gene sequencing approach. These approaches allowed us to show that both *hmg1-1* and *chs5* mutants were impaired in association

with some specific bacteria, and the *chs5* but not the *hmg1-1* mutant was more colonized and thus more susceptible to infection by *Pst*DC3000 than WT.

We made several control to verify that our 16S rRNA gene sequencing approach was relevant. First, in a preliminary experiment, we tested the effect of the growth stage on the phenotype or on the microbiota composition. The community changed during plant growth in both ecotypes. Such changes were previously observed (Chaparro et al., 2014; Lundberg et al., 2012). In these previous studies, the authors demonstrated that even if a core microbiota was characterized at different stages of the plant development, a subset of microbes associated with plants varies at different stages of the development. Second, according to the preliminary study, we chose a similar growth stage for all the experiments, in order to prevent bias due to differences of plants size. Indeed, our study was performed on plants having similar shapes. We grew *hmg1-1* in conditions were the phenotype was not visible. The chlorotic phenotype that is characteristic of *chs5* when the plant grows at cold temperature or at early stages was poorly or not visible in our culture conditions (21°C/18°C) either (Araki et al., 2000; Hugly and Somerville, 1992; Schneider et al., 1995). When the chlorotic phenotype was slightly observed, only a slight decrease of chlorophylls and carotenoids contents in this mutant was observed (Supplementary figure 9). Nevertheless, in such conditions, we were able to observe differences in the interaction of specific OTUs with *chs5* and *hmg1-1* mutants as compared to WT, and with the pathogen *Pst*DC3000 in the case of *chs5*. Third, our results agreed with several previous analyses on bacterial microbiota of *A. thaliana*. Indeed, at both stages, in all plant genotypes, α- and β-diversity indexes revealed that microbiota richness and structures in phyllosphere were globally different from the rhizospheric or the root microbiota, which were more closed together. Such observations were previously made by others (Bai et al., 2015; Lundberg et al., 2012; Schlaeppi et al., 2014). Moreover, *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* were dominant phyla at both growth stages, in the two ecotypes, as previously described (Bai et al., 2015; Lundberg et al., 2012; Schlaeppi et al., 2014). However, only 16% of the OTUs were found in both wild-type, showing that, in our tested conditions, both ecotypes shared a core microbiota but had also specific OTUs interacting with them, as previously observed (Bai et al., 2015; Lundberg et al., 2012; Schlaeppi et al., 2014).

Using this 16S rRNA-based approach, we demonstrated that the mutations did not globally affect the community composition or richness. Nevertheless, colonization of some specific OTUs was impacted by either the mutation in the MEP or in the MVA pathways. Interestingly,

the colonization of 38 OTUs, among which *Rhizobium* in the rhizosphere, and *Streptomyces* in all compartments, was impacted whatever the isoprenoid biosynthesis pathway affected. Plants isoprenoids may therefore be required for these OTUs to colonize the plants. Interestingly, among the OTUs which are more abundant in the WT than in mutants, we found a large majority of *Streptomyces* as compared to the total microbiota, in the phyllosphere, the roots and the rhizosphere. These observations suggest that *Streptomyces* are selected directly or indirectly via isoprenoids. These bacteria are known to produce a large number of secondary metabolites and among them, isoprenoids (Kuzuyama, 2017). Plants isoprenoids may be attractant for these bacteria. On the other hand, bacterial isoprenoids may be interesting for the plant as precursors for their own biosynthesis.

Changes in the microbiota composition may impact the fitness of the plants since the *chs5* mutant is more colonized by the pathogen than the WT. This could not be explained only by a decrease of *PR* genes expression nor stigmasterol production. In contrast, the *hmg1-1* mutant was not affected. This suggests an implication of isoprenoids synthetized via the MEP pathway but not those synthetized through the MVA pathway, in the plant defense against pathogens. This implication could be direct, via an effect of isoprenoids themselves on the pathogen, or indirect, via the implication of specific members of the microbiota. Some bacteria may play a role to promote pathogen colonization and be more abundant in the mutant than in the WT. Others may play a role to prevent pathogen colonization and be more abundant in the WT than in the mutant. Both mutants were differently impaired in pathogen colonization, we therefore hypothesized that members of the microbiota may play a role to protect plants against *Pst*DC3000, and their selection may require isoprenoids synthetized via the MEP pathway. We tried to identify such bacteria, by looking for OTUs whose abundance was higher in Col-0 than in *chs5*, but whose abundance was not changed in *hmg1-1* compared to WS2. Several of these OTUs had a 16S rRNA gene sequences sharing more than 99% identity with *Actinobacteria* (*Kribbella*, *Pseudonocardia*, *Streptomyces*), *Sphingomonas*, known to produce antimicrobial molecules or to have biocontrol effect (Innerebner et al., 2011; Liu et al., 2019; Shan et al., 2018). For example, *Sphingomonas* spp. were shown to prevent *Pst* DC3000 colonization in *A. thaliana* (Innerebner et al., 2011). Several *Streptomyces* were shown to display antagonisms against plant pathogens (Dias et al., 2017; Newitt et al., 2019; Olanrewaju and Babalola, 2019; Suárez-Moreno et al., 2019) or have been found in disease-suppressive soil (Cordovez et al., 2015). For example, it was shown that *Streptomyces* sp. EN27 induces defense pathways in *A. thaliana* (Conn et al., 2008). Remarkably, these bacteria were found in the phyllosphere, where

*Pst*DC3000 was infecting the plant. These OTUs may therefore play a role to prevent pathogen colonization in Col-0, but may be present in a too low abundance in *chs5* to prevent pathogen colonization, leading to a better colonization of *Pst*DC3000 in this mutant. Such hypothesis could be tested in the next future by isolating such bacteria and testing their effect on plants *in vitro* as previously done by other laboratories (Vogel et al., 2012; Vorholt et al., 2017).

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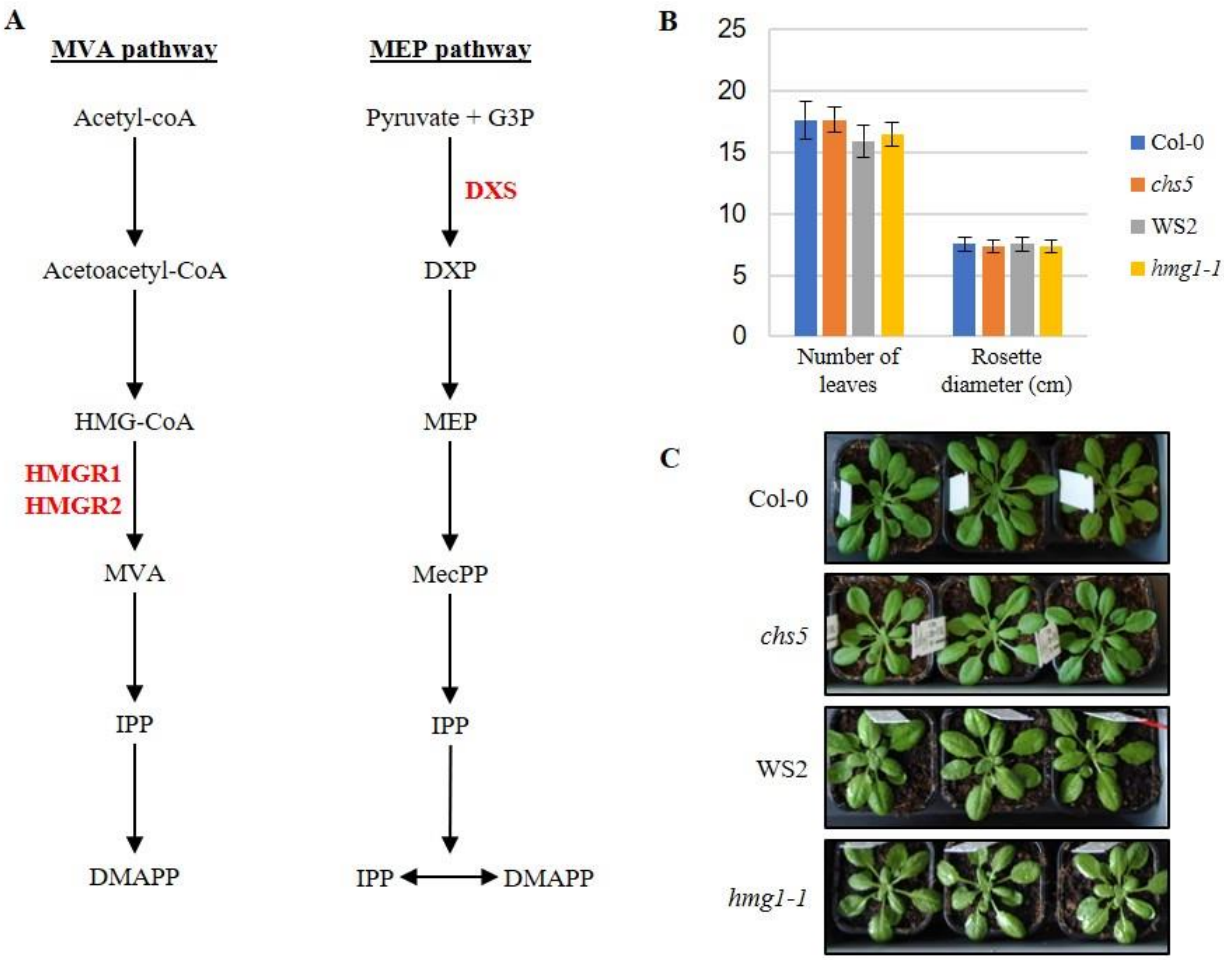


Figure 1. Simplified isoprenoid biosynthesis pathways in *Arabidopsis thaliana* and phenotype of *hmg1-1* and *chs5* affected in this biosynthesis. (A) The mevalonate (MVA) pathway occurs in the cytosol and requires the 3-hydroxyl-3-methylglutaryl CoA reductase (HMGR) as a key enzyme to obtain mevalonate. The 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway occurs specifically in plastids and requires the 1-deoxy-D-xylulose 5 phosphate synthase (DXS) as a key enzyme to form 1-deoxy-D-xylulose 5 phosphate (DXP). Both biosynthesis pathways lead to the formation of isopentenyl diphosphate (IPP) and its isoform dimethylallyl diphosphate (DMAPP), precursors of all isoprenoids. G3P: glyceraldehyde 3-phosphate; MEcPP: 2-*C*-methyl- D-erythritol 2,4-cyclodiphosphate; GGPP: geranylgeranyl diphosphate. Mutants in these biosynthesis pathways lead to a deficit in the formation of the isoprenoid precursors IPP and DMAPP. (B) and (C) *hmg1-1* is affected in the mevalonate biosynthesis pathway. The plant is characterized by a dwarf phenotype. *chs5* is affected in the methylerythritol phosphate biosynthesis pathway. The plant is characterized by a chlorotic phenotype in cold culture conditions (Araki *et al.*, 2000). In the tested conditions, this chlorotic phenotype is less visible and the chlorophyll content is only slightly reduced in this mutant as compared to the WT.

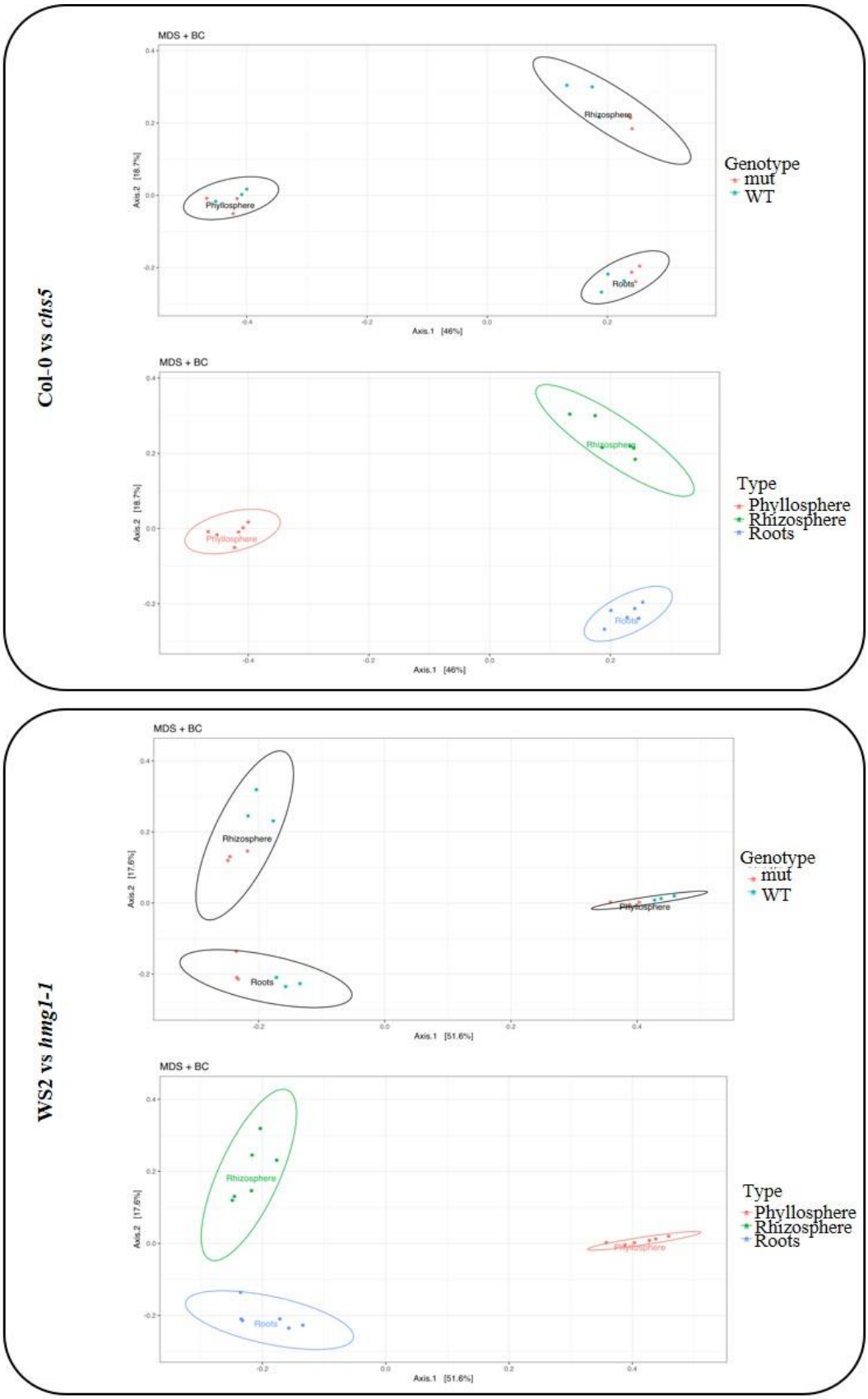


Figure 2. Principal coordinate analysis (CoAP =MDS). Principal coordinate analysis was produced using Bray-Curtis distances. The ellipses were drawn at the 95% confidence interval of standard error and the mean value of the groups. These figures revealed that the communities in the WT and in mutants ("mut") were less divergent than the communities in each compartment. Similar pattern were obtained based on Jaccard, Unifrac, or Weighted Unifrac indexes.

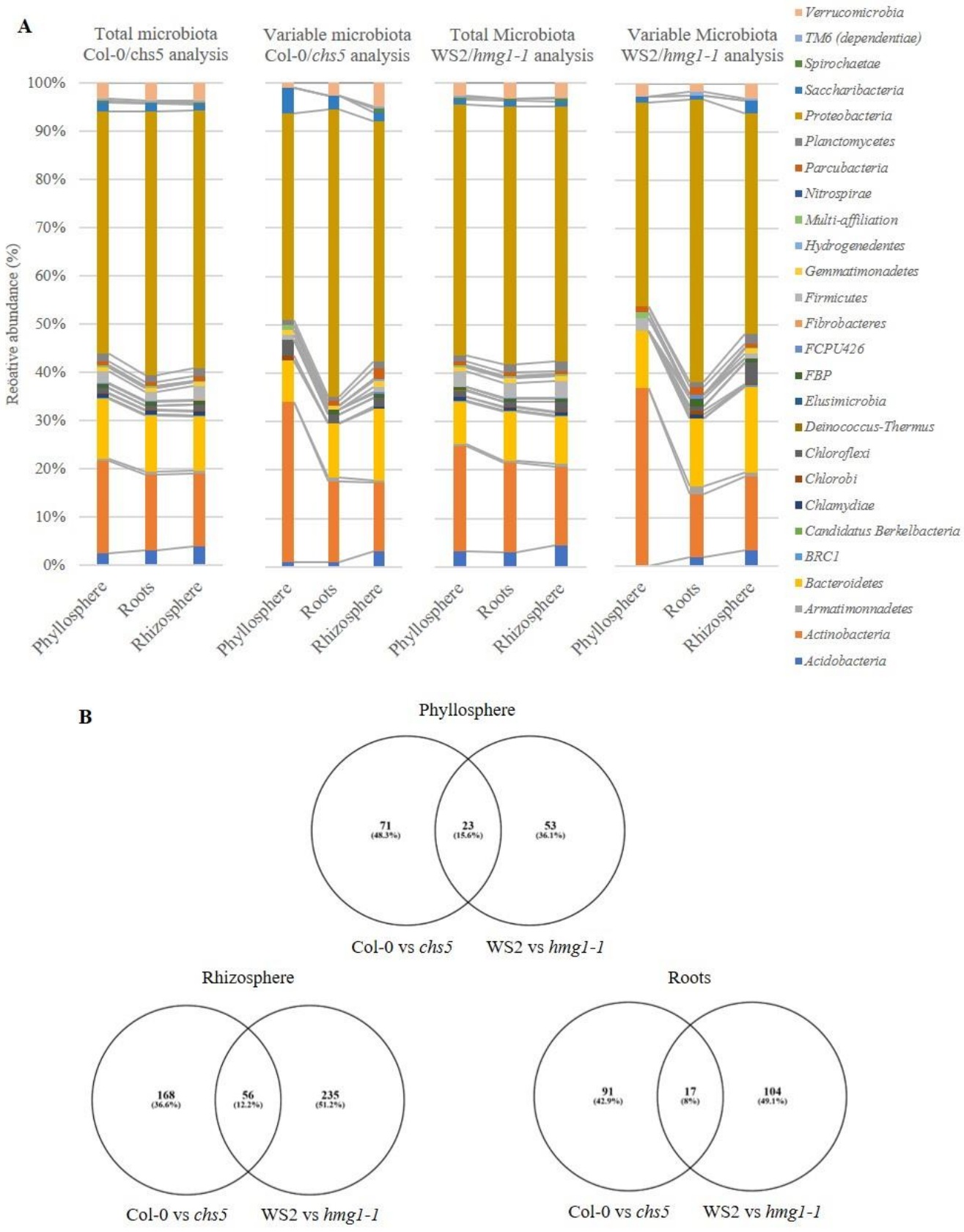


Figure 3. Relative abundance of different phyla in each compartments. (A) Relative abundance is expressed as a percentage. (B) Comparison of variable OTUs in both analysis, in the three compartments. 8 to 15% of the OTUs were differentially abundant in both mutants as compared to their respective WT. Among these OTUs, we found a majority of *Proteobacteria*, *Actinobacteria* and *Bacteroidetes*. Some OTUs were differentially abundant in both experiments, in each of the compartments.

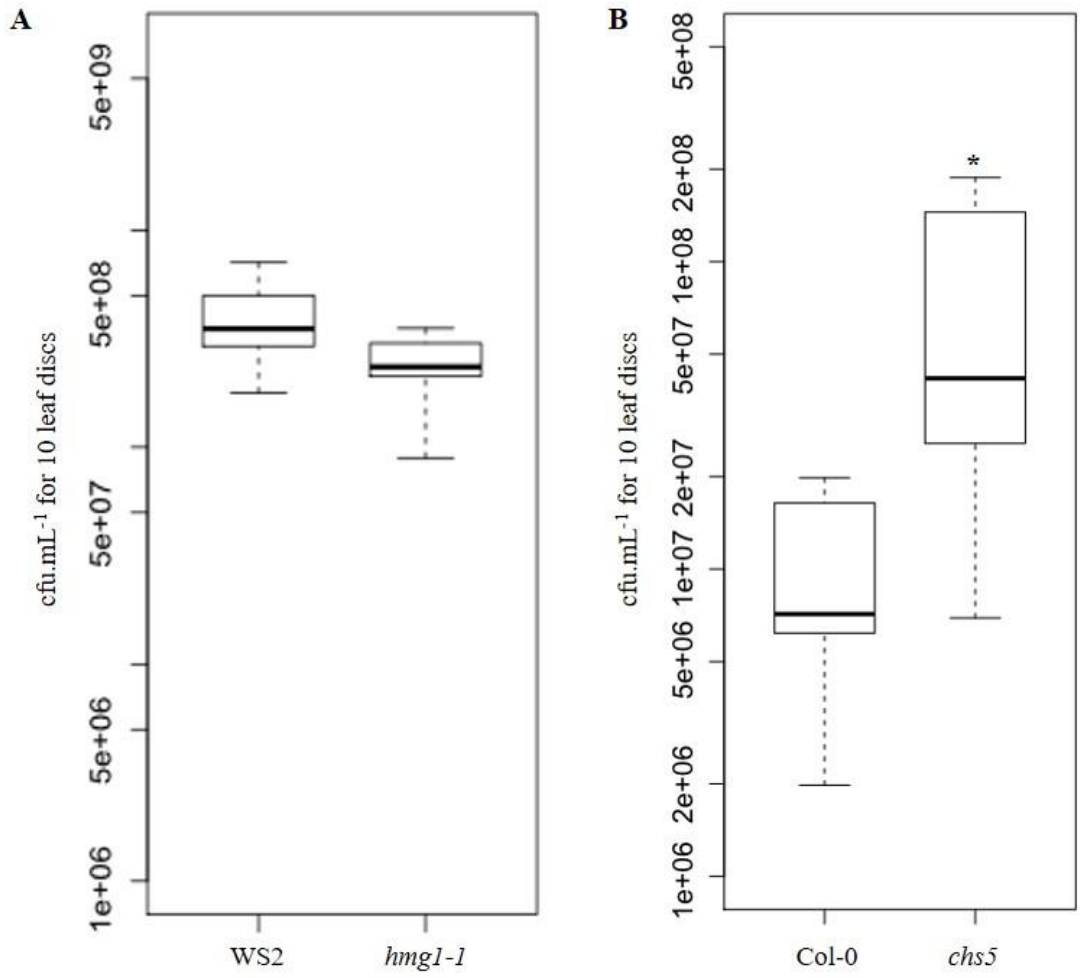
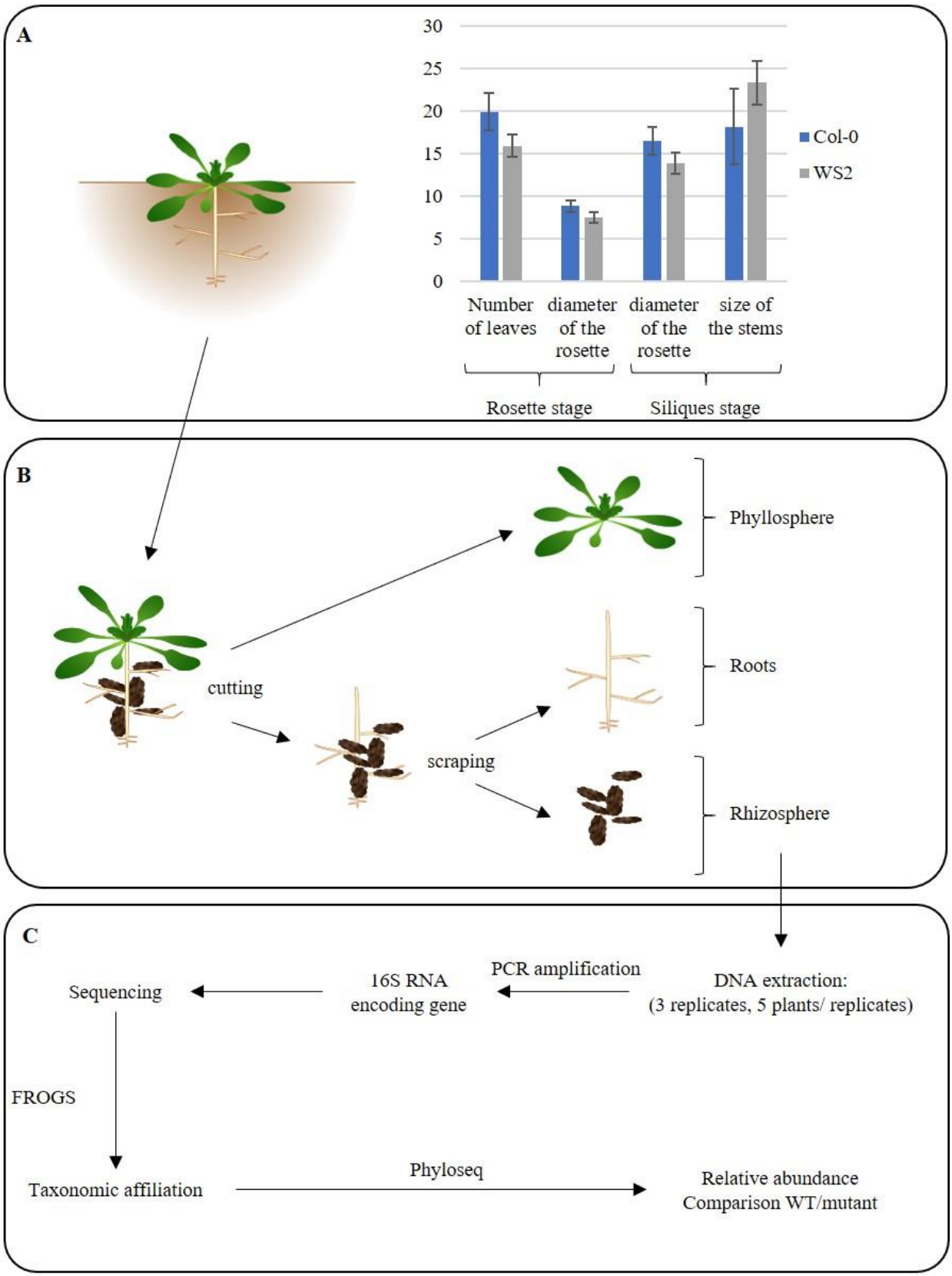
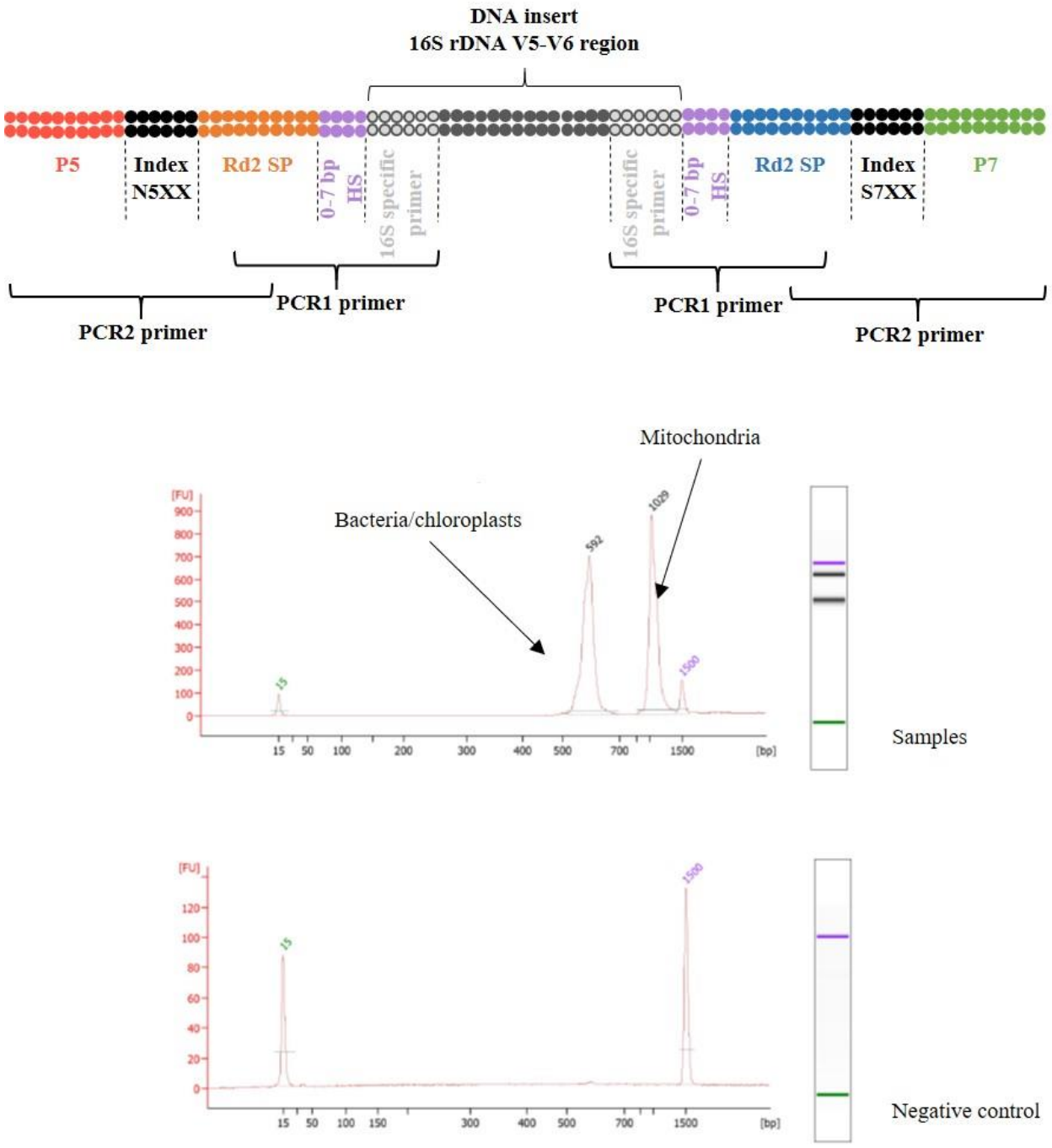


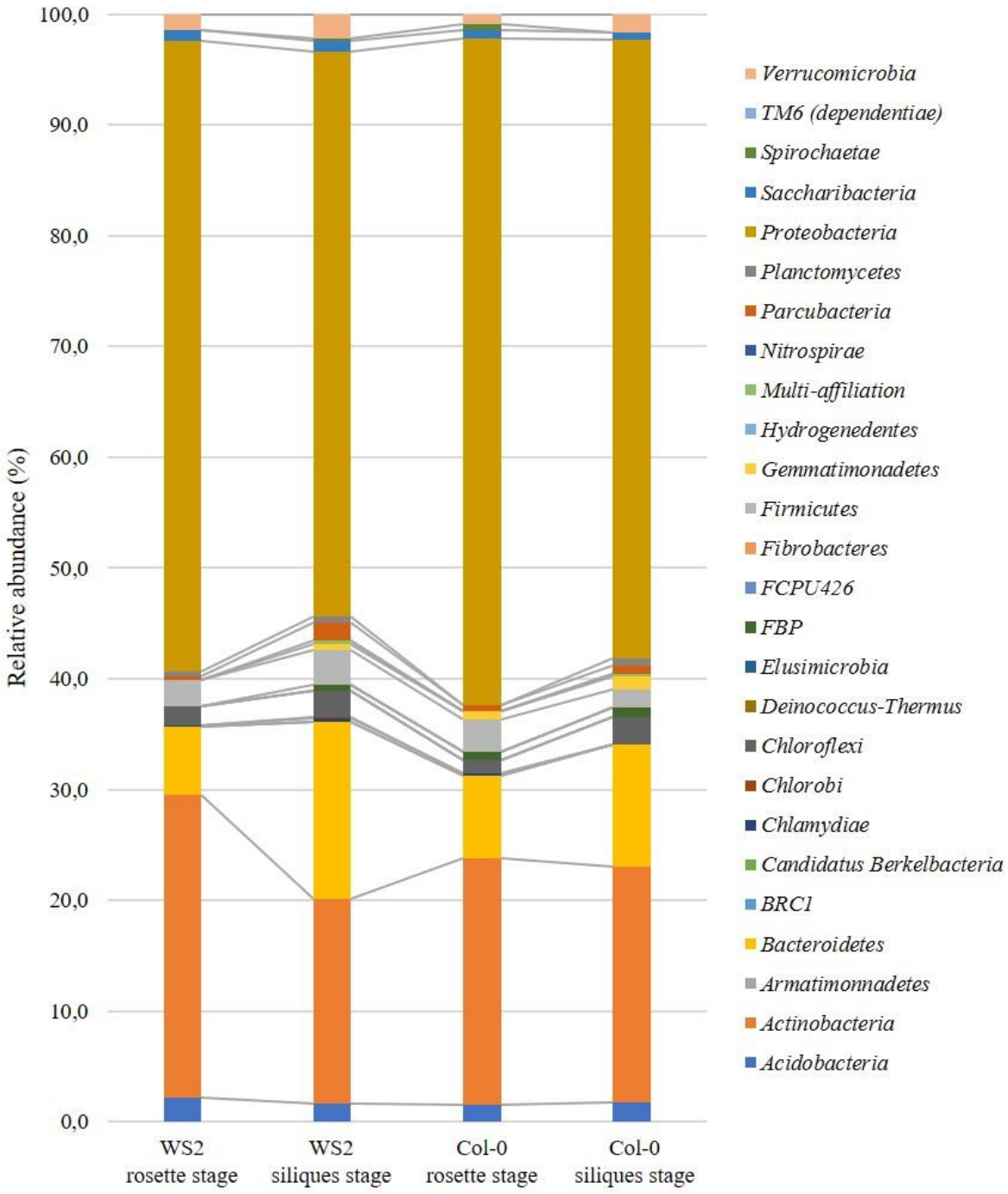
Figure 4. Pathogen numerations upon the infection of *Arabidopsis thaliana* by *Pseudomonas syringae* pv. *tomato* DC3000. Pathogen numerations were done for (A) WS2 and *hmg1-1,* and (B) Col-0 and *chs5*, at 6dpi. N=12. There is no major difference of pathogen colonization between WS2 and *hmg1-1*, while there is significantly more pathogen colonization in *chs5* compared to Col-0 (*P < 0.01: Student's t test).



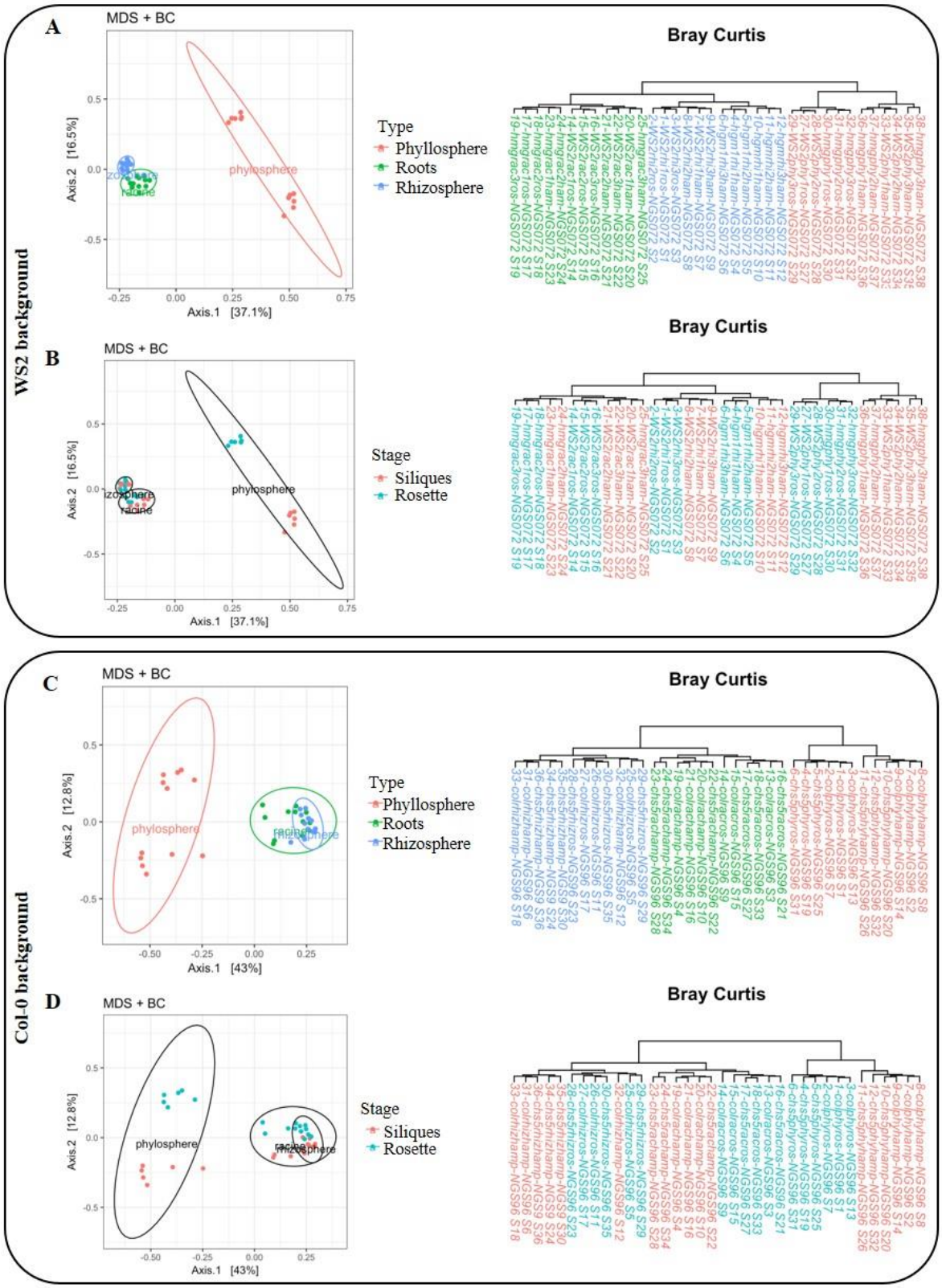
Supplementary figure 1. Strategy used to separate the 3 compartments (phyllosphere, roots and rhizosphere) and to analyse bacterial communities inhabiting these compartments. (A) Col-0 and WS2 were extracted from soil at the rosette and siliques stages according to the number of leafs, diameter of the rosettes and lengh of the stems. (B) Plants were shaken and roots shortly washed in sterile distilled water to remove soil. Then the plants were cutted in ordered to separate phyllosphere and roots/rhizosphere. Rhizosphere was separated from roots by scrapping them using a sterile scrapor. (C) DNA was extracted from these samples, 16S RNA encoding gene were amplified and sequenced using the Miseq Illumina Technology. Two pipeline were used to analyse sequences (FROG and Phyloseq, see material and methods).



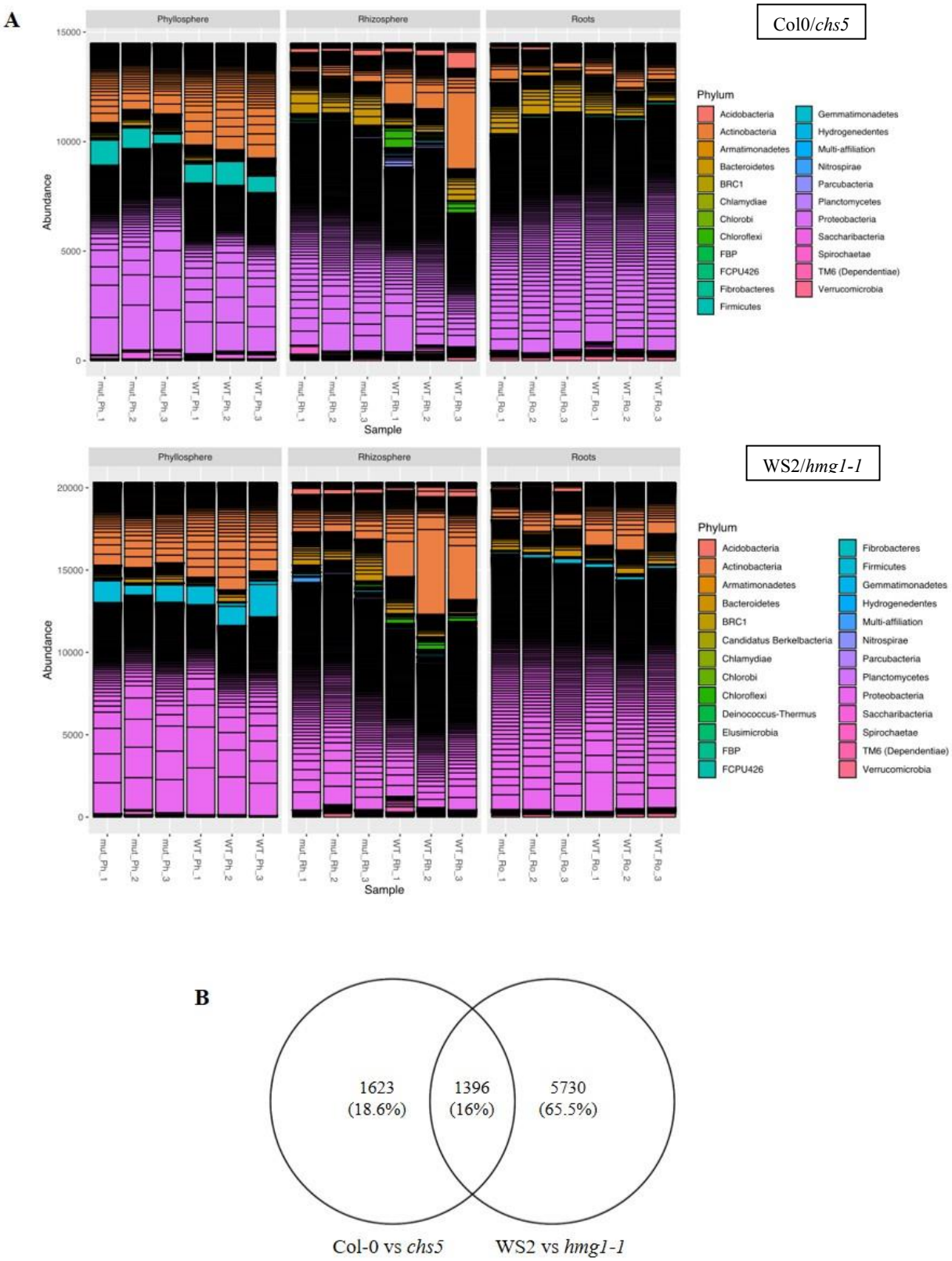
Supplementary figure 2. Primers used for the libraries generation. The PCR1 primers contain: the 16S V5-V6 specific sequence; the 0 to 7 bp heterogeneity spacer (0-7 bp HS); a part of the Read 1 or Read 2 specific sequences used for sequencing primer hybridization (Rd1 SP or Rd2 SP). The PCR2 primers contain: a part of the Read 1 or Read 2 specific sequences used for sequencing primer hybridization (Rd1 SP or Rd2 SP); an index (Nextera XT N5XX or S7XX); a P5 or P7 sequence used to hybridize the libraries on the flow cell and for cluster generation.



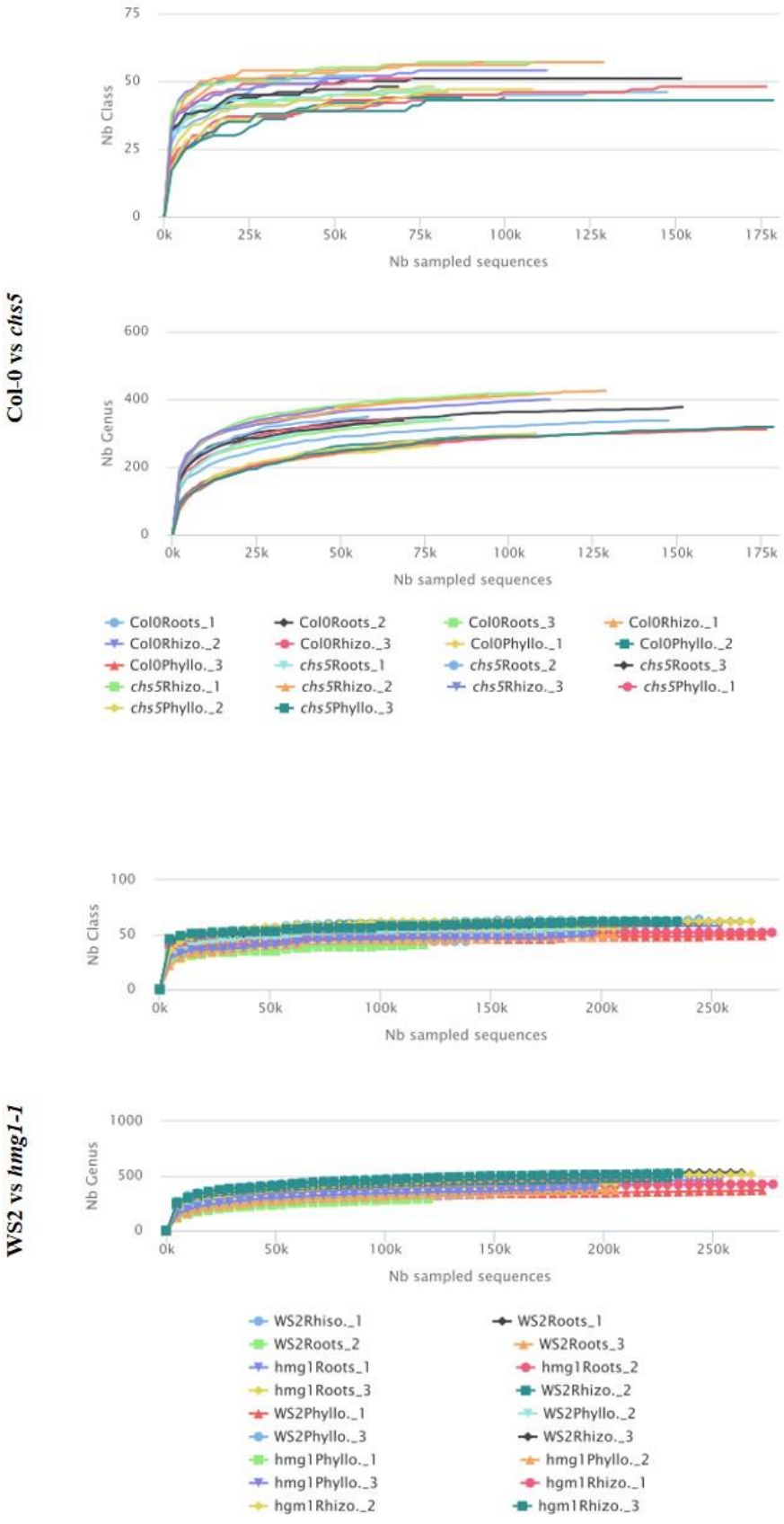
Supplementary figure 3. Relative abundance (%) of the different phyla at two different growth stages, in the two WT ecotypes.



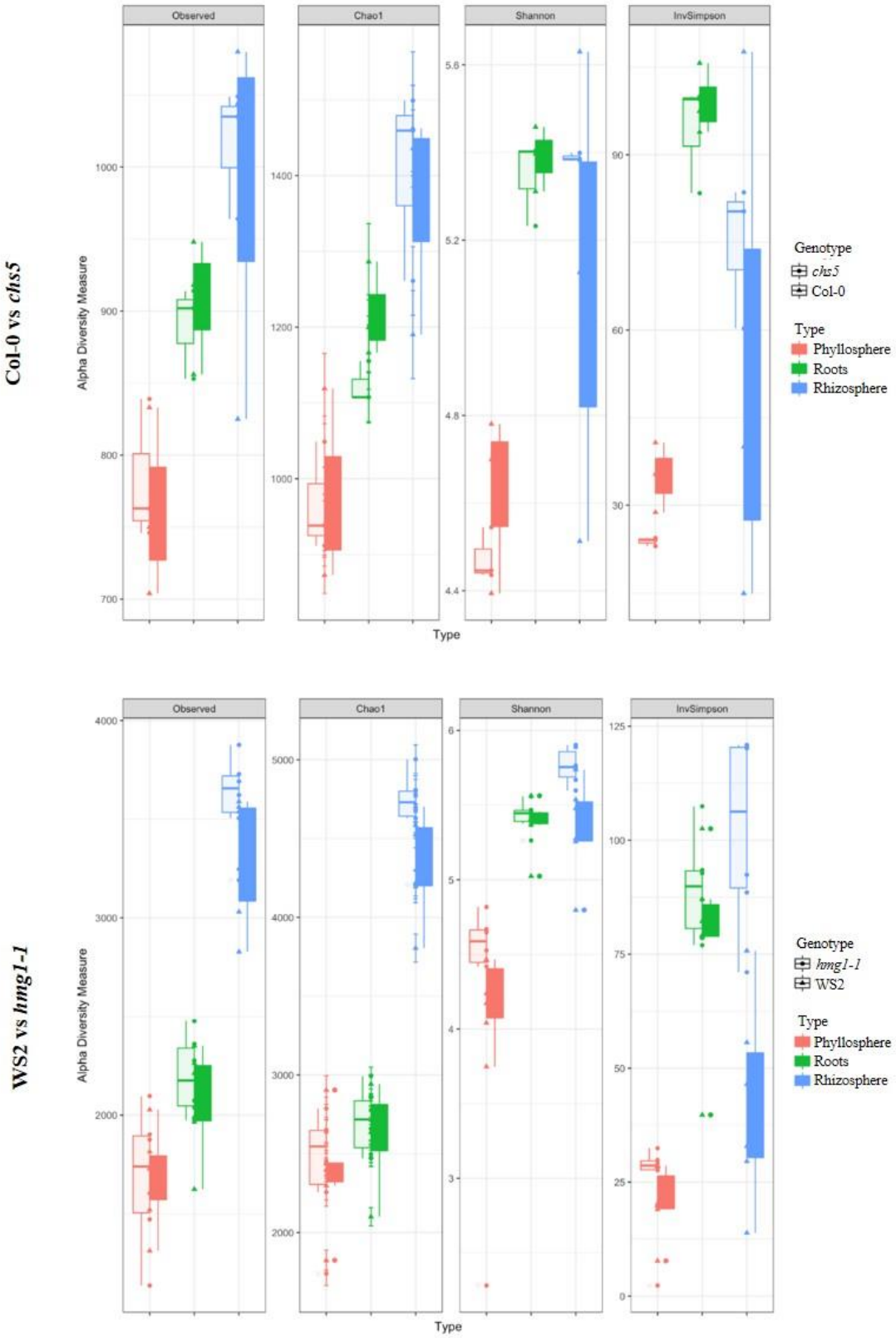
Supplementary figure 4. Clustering trees (right) and Principal coordinate analysis (CoAP =MDS) analysis (left). Comparison of the communities in three compartments ((A) and (C)) at two different stages ((B) and (D)) in *A. thaliana* WS2 and Col-0. Clustering trees were obtained with the Ward method, and the Bray-Curtis diversity index. Principal coordinate analysis was produced using Bray-Curtis distances. The ellipses were drawn at the 95% confidence interval of standard error and the mean value of the groups. These figures revealed that the community of the three compartments are specific. Moreover, we observed also that the communities were different at the two growth stages, particularly in the phyllosphere. Similar patterns were obtained based on Jaccard, Unifrac, or Weighted Unifrac indexes.



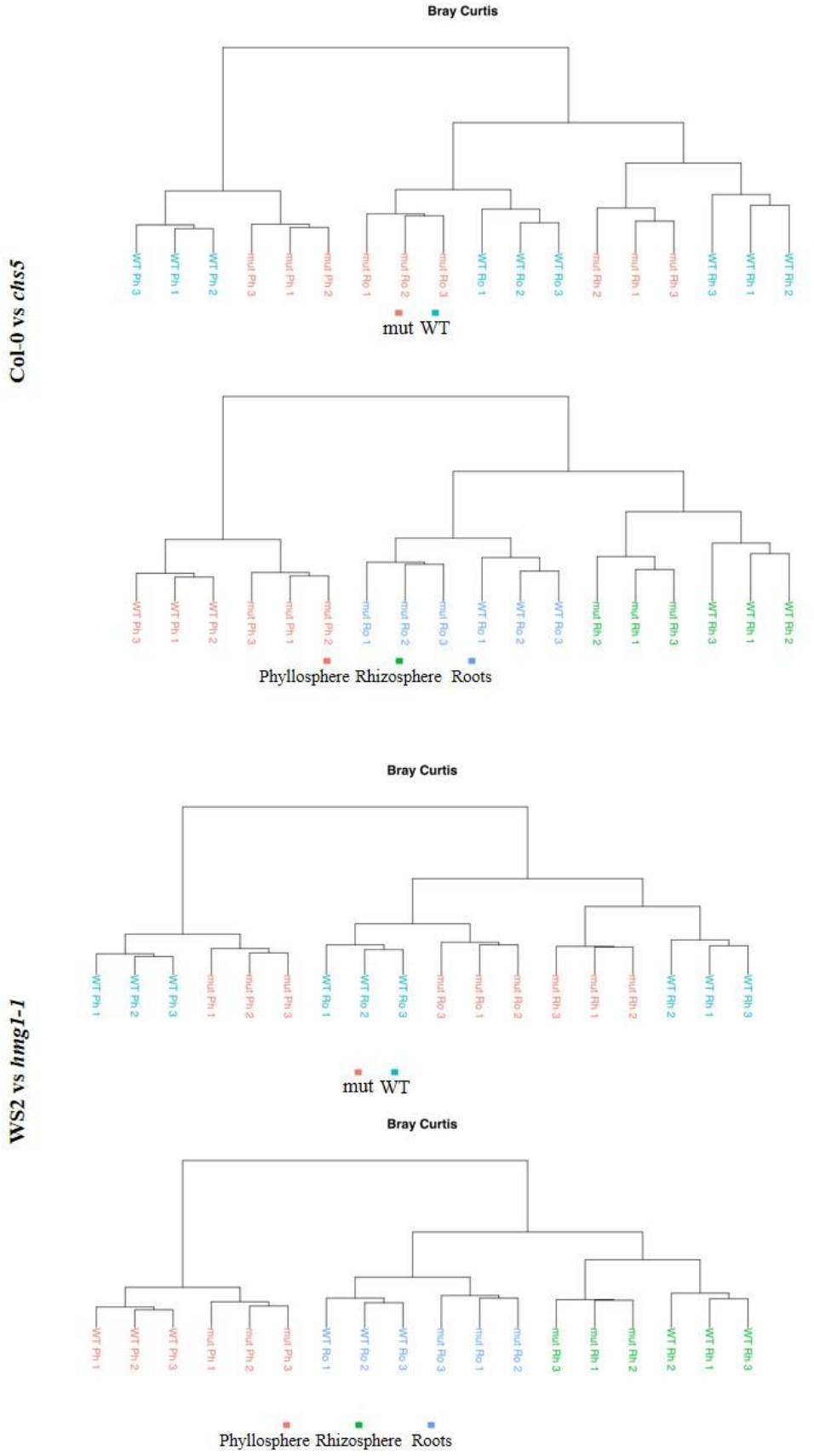
Supplementary figure 5. Comparison of the phyla and OTUs found in the two comparative analysis of Col-0 versus *chs5* and WS2 versus *hmg1-1*. (A) Relative abundance (%) of the different phyla at the rosette stage, in the two WT ecotypes WS2, Col-0 and the *chs5* and *hmg1-1* mutants. (B) The two analyses shared only 16% of the OTUs.



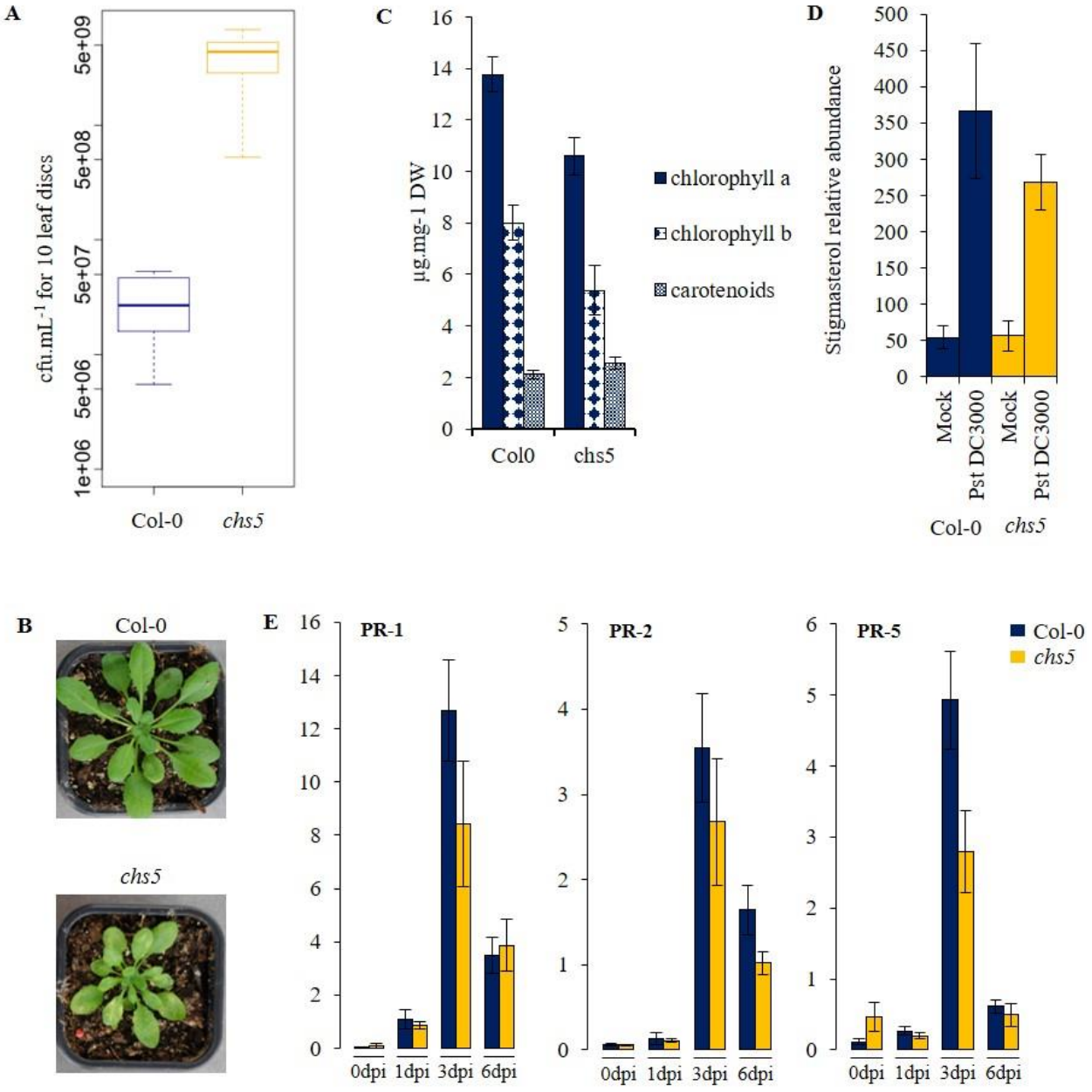
Supplementary figure 6. Rarefaction curves obtained from NGS sequencing data. This curve is a plot of the number of class (top) or genus (bottom) as a function of the number of samples. This rarefaction curve revealed that the richness were similar in the mutant as compared to the WT in both experiments.



Supplementary figure 7. Diversity analysis (Observed, Chao1, Shannon and inversed Simpson indexes) of the bacterial communities in 3 compartments and in Col-0 as compared to *chs5* or in WS2 as compared to *hmg1*. The bacterial community of the phyllosphere displayed significantly lower richness and α-diversity compared to the roots and rhizosphere (Anova Test, $P < 0.01$). In contrast, the bacterial community of mutants did not display significant differences in richness and α-diversity compared to WT.



Supplementary figure 8. Clustering trees showing the similarity degrees between communities. Clustering trees were obtained with the Ward method, and the Bray-Curtis diversity index**.** These figures revealed that the communities in WT and mutants ("mut") were less divergent than communities in each compartment. Similar patterns were obtained based on Jaccard, Unifrac, or Weighted Unifrac indexes.



Supplementary figure 9. Infection of WT and *chs5* by *Pseudomonas syringae* pv. *tomato* DC3000. Pathogen numerations were done for wild-type Col-0 and mutant *chs5*, at 6dpi. N=12. In this experiments, the chlorotic phenotype was slightly visible in (B) and the Chlorophylls contents (C) were slightly reduced as compared to the WT. There is a significant enhancement of *chs5* susceptibility compared to Col-0. (*P < 0.01: Student's t test). (D) Stigmasterol content was quantified 6 days post-infection, by GC-MS. Relative abundances correspond to the ratio between the pic area and the sample dry weight. Histograms represent mean area ± SEM. (E) Relative expression of each target gene before infection, 1-, 3- or 6-days post infection (dpi) is represented. Gene expression was determined by real-time PCR using gene-specific primers and normalized using ACT2 and GADPH reference genes. Transcript quantification was performed on three biological replicates analyzed in triplicate. Histograms represent mean expression ± SEM.

Table 1. OTUs which were shown to be significantly more abundant in both mutants as compared to WT.

WS2/*hmg1-1*		Col-0/*chs5*		
Cluster name	More abundant in:	Cluster name	More abundant in:	Taxonomic affiliation
Cluster_106	mutant phyllosphere, rhizosphere	Cluster_65	mutant rhizosphere, roots phyllosphere	*Proteobacteria Alphaproteobacteria rhizospherebiales rhizospherebiaceae rhizospherebium Multi-affiliation*
Cluster_16	mutant phyllosphere	Cluster_25	mutant phyllosphere	*Actinobacteria Actinobacteria Streptomycetales Streptomycetaceae Streptomyces Multi-affiliation*
Cluster_1386	mutant phyllosphere	Cluster_660	mutant phyllosphere	*Actinobacteria Actinobacteria Propionibacteriales Nocardioidaceae Marmoricola Marmoricola ginsengisoli*
Cluster_137	mutant phyllosphere rhizosphere	Cluster_151	mutant phyllosphere	*Multi-affiliation*
Cluster_847	mutant phyllosphere rhizosphere roots	Cluster_365	mutant phyllosphere and rhizosphere	*Proteobacteria Betaproteobacteria Burkholderiales Oxalobacteraceae Undibacterium Multi-affiliation*
Cluster_74	mutant phyllosphere, rhizosphere, roots	Cluster_49	mutant phyllosphere, rhizosphere and roots	*Actinobacteria Actinobacteria Streptomycetales Streptomycetaceae Streptomyces Multi-affiliation*
Cluster_545	mutant phyllosphere, rhizosphere, roots	Cluster_451	mutant rhizosphere, roots and phyllosphere	*Actinobacteria Actinobacteria Streptomycetales Streptomycetaceae Streptomyces Streptomyces paucisporeus*
Cluster_84	mutant phyllosphere and rhizosphere	Cluster_73	mutant phyllosphere	*Actinobacteria Actinobacteria Streptomycetales Streptomycetaceae Streptomyces Multi-affiliation*
Cluster_23	mutant phyllosphere, roots	Cluster_12	mutant phyllosphere, roots	*Proteobacteria Betaproteobacteria Burkholderiales Burkholderiaceae Burkholderia-Paraburkholderia Multi-affiliation*
Cluster_565	mutant phyllosphere	Cluster_355	mutant phyllosphere	*Actinobacteria Actinobacteria Micrococcales Intrasporangiaceae Knoellia Multi-affiliation*
Cluster_89	mutant phyllosphere	Cluster_142	mutant phyllosphere, roots	*Actinobacteria Actinobacteria Micrococcales Promicromonosporaceae Xylanimonas Xylanimonas cellulosilytica*
Cluster_295	mutant phyllosphere and rhizosphere	Cluster_266	mutant phyllosphere, roots and rhizosphere	*Actinobacteria Actinobacteria Micrococcales Microbacteriaceae Glaciibacter Glaciibacter superstes*
Cluster_125	mutant phylosphere, roots and rhizosphere	Cluster_155	mutant phyllosphere, roots	*Actinobacteria Actinobacteria Micrococcales Promicromonosporaceae Myceligenerans Multi-affiliation*
Cluster_547	mutant phyllosphere, rhizosphere	Cluster_346	mutant phyllosphere, roots and rhizosphere	*Bacteroidetes Flavobacteriia Flavobacteriales Flavobacteriaceae Chryseobacterium Chryseobacterium sp.*
Cluster_213	mutant phyllosphere, roots and rhizosphere	Cluster_657	mutant phyllosphere, rhizosphere	*Proteobacteria Alphaproteobacteria rhizospherebiales rhizospherebiaceae rhizospherebium Multi-affiliation*
Cluster_3162	mutant phyllosphere	Cluster_2314	mutant phyllosphere	*Firmicutes Clostridia Clostridiales Peptococcaceae Desulfosporosinus Multi-affiliation*
Cluster_1319	mutant phyllosphere	Cluster_1358	mutant phyllosphere, rhizosphere	*Bacteroidetes Flavobacteriia Flavobacteriales Flavobacteriaceae Chryseobacterium Multi-affiliation*
Cluster_356	mutant rhizosphere	Cluster_226	mutant rhizosphere et phyllosphere	*Proteobacteria Betaproteobacteria Burkholderiales Comamonadaceae Ramlibacter Multi-affiliation*
Cluster_855	mutant roots	Cluster_388	mutant rhizosphere et phyllosphere	*Proteobacteria Gammaproteobacteria Xanthomonadales Xanthomonadaceae Multi-affiliation Multi-affiliation*
Cluster_623	mutant rhizosphere	Cluster_618	mutant phyllosphere, rhizosphere, roots	*Proteobacteria Alphaproteobacteria rhizospherebiales rhizospherebiaceae rhizospherebium rhizospherebium sp.*
Cluster_235	mutant roots	Cluster_117	mutant roots	*Proteobacteria Betaproteobacteria Burkholderiales Burkholderiaceae Burkholderia-Paraburkholderia Multi-affiliation*
Cluster_139	mutant roots	Cluster_115	mutant roots	*Proteobacteria Betaproteobacteria Burkholderiales Burkholderiaceae Burkholderia-Paraburkholderia Multi-affiliation*
Cluster_521	mutant rhizosphere	Cluster_352	mutant roots rhizosphere	*Proteobacteria Betaproteobacteria Burkholderiales Burkholderiaceae Burkholderia-Paraburkholderia Multi-affiliation*
Cluster_388	mutant roots	Cluster_516	mutant roots	*Proteobacteria Alphaproteobacteria Sphingomonadales Sphingomonadaceae Sphingobium unknown species*

Table 2. OTUs which were shown to be significantly less abundant in both mutants as compared to WT.

WS2/*hmg1-1*		Col-0/*chs5*		
Cluster name	More abundant in:	Cluster name	More abundant in:	Taxonomic affiliation
Cluster_153	WT phyllosphere	Cluster_165	WT phyllosphere, roots, rhizosphere	*Actinobacteria Actinobacteria Streptomycetales Streptomycetaceae Streptomyces Multi-affiliation*
Cluster_418	WT phyllosphere	Cluster_518	WT phyllosphere	*Actinobacteria Actinobacteria Pseudonocardiales Pseudonocardiaceae Pseudonocardia Multi-affiliation*
Cluster_24	WT phyllosphere	Cluster_43	WT phyllosphere, rhizosphere	*Actinobacteria Actinobacteria Micrococcales Microbacteriaceae Rudaibacter Multi-affiliation*
Cluster_34	WT phyllosphere, rhizosphere	Cluster_94	WT phyllosphere, roots	*Actinobacteria Actinobacteria Streptomycetales Streptomycetaceae Streptomyces Streptomyces sp.*
Cluster_42	WT phyllosphere, roots	Cluster_66	WT phyllosphere, roots, rhizosphere	*Actinobacteria Actinobacteria Streptomycetales Streptomycetaceae Streptomyces Multi-affiliation*
Cluster_52	WT phyllosphere, roots, rhizosphere	Cluster_118	WT phyllosphere, roots, rhizosphere	*Actinobacteria Actinobacteria Streptomycetales Streptomycetaceae Streptomyces Multi-affiliation*
Cluster_196	WT phyllosphere	Cluster_126	Mutant phyllosphere	*Proteobacteria Betaproteobacteria Methylophilales Methylophilaceae Multi-affiliation Multi-affiliation*
Cluster_1152	WT roots	Cluster_1335	WT roots	*Proteobacteria Deltaproteobacteria Myxococcales BIrii41 unknown genus unknown species*
Cluster_47	WT roots	Cluster_57	WT phyllosphere, roots and rhizosphere	*Chloroflexi Ktedonobacteria Ktedonobacterales Ktedonobacteraceae unknown genus unknown species*
Cluster_858	WT roots	Cluster_150	WT roots	*Proteobacteria Alphaproteobacteria Sphingomonadales Sphingomonadaceae Sphingomonas Multi-affiliation*
Cluster_459	WT roots	Cluster_809	WT roots	*Bacteroidetes Sphingobacteriia Sphingobacteriales Chitinophagaceae Multi-affiliation Multi-affiliation*
Cluster_327	WT roots	Cluster_319	WT roots	*Proteobacteria Alphaproteobacteria Rhodospirillales Rhodospirillaceae Ferrovibrio Phaeospirillum sp.*
Cluster_652	WT roots	Cluster_859	WT roots	*Proteobacteria Alphaproteobacteria Rhodospirillales Rhodospirillaceae Ferrovibrio Ferrovibrio sp.*
Cluster_15	WT rhizosphere and roots	Cluster_26	WT rhizosphere roots and phyllosphere	*Actinobacteria Actinobacteria Streptosporangiales Thermomonosporaceae Multi-affiliation Multi-affiliation*

Supplementary Table 1. Primers used for 16S RNA encoding gene amplification and qPCR reactions .

Name of the primer	Sequence (5'→3')	reference
16S RNA amplicon		
799F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAACMGGATTAGATACCCKG	Bulgarelli *et al.* Bodenhausen *et al.* Schlaeppi *et al.*
799F1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTAACMGGATTAGATACCCKG	This work
799F2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTAACMGGATTAGATACCCKG	This work
799F3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCGAAACMGGATTAGATACCCKG	This work
799F4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATGAAACMGGATTAGATACCCKG	This work
799F5	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGCGAAACMGGATTAGATACCCKG	This work
799F6	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGAGTGGAACMGGATTAGATACCCKG	This work
799F7	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTGTGGAACMGGATTAGATACCCKG	This work
1193R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACGTCATCCCCACCTTCC	Bulgarelli *et al.* Bodenhausen *et al.* Schlaeppi *et al.*
1193R1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTACGTCATCCCCACCTTCC	This work
1193R2	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTACGTCATCCCCACCTTCC	This work
1193R3	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCGAACGTCATCCCCACCTTCC	This work
1193R4	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATGAACGTCATCCCCACCTTCC	This work
1193R5	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGCGAACGTCATCCCCACCTTCC	This work
1193R6	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAGTGGACGTCATCCCCACCTTCC	This work
1193R7	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTTGTGGACGTCATCCCCACCTTCC	This work
qPCR		
ACT2 forward	CTTGCACCAAGCAGCATGAA	Czechowski *et al.*, 2005
ACT2 reverse	CCGATCCAGACACTGTACTTCCTT	Czechowski *et al.*, 2005
GADPH forward	TTGGTGACAACAGGTCAAGCA	Czechowski *et al.*, 2005
GADPH reverse	AAACTTGTCGCTCAATGCAATC	Czechowski *et al.*, 2005
PR1 forward	GGTCACTACACTCAAGTTGTTT	This work
PR1 reverse	GTTCCACCATTGTTACACCTC	This work
PR2 forward	TGACACCACCACTGATACG	This work
PR2 reverse	CTCTTATACTCATCCCTGAACCT	This work
PR5 forward	CTGACCTCAACGCGGCTTGC	This work
PR5 reverse	GGCGTCAGGGCAAGCGTTCT	This work
Primers targeting PR-1 (Atg14160.1), PR-2 (Atg57260.1) and PR-5 encoding genes (Atg75040.1) were designed using LightCycler Probe Design Software 2.0 (Roche Life Science).		

IV. Discussion

In this chapter, I presented few technical points since I tried to optimize the infection experiments. I wish to notice that the model interaction between *A. thaliana* and *P. syringae* is sometimes considered as artefactual because there is no naturally occurring infection of *A. thaliana* by this pathogen, and we have to employ artificial inoculation methods in the laboratory, which are not exactly representative of a natural infection (Katagiri et al., 2002). However, this is a good model for comparative study of the sensitivity of different plants to the same pathogen, in the same conditions. There are lots of protocols commonly used for *A. thaliana* infection by *Pst*DC3000, but the needle-less syringe infection is reliable since we can control the pathogen repartition on selected leaves. I tried to quantify the pathogen thanks to fluorescence or luminescence measurements, but I did not obtain any correlation between the measurements and the pathogen numerations after plating. This lack of correlation could be due to the device that we use in the institute for the measurements, since we encountered difficulties with both methods, for which detection was made on the same device. Thus, I decided to proceed bacterial quantification with the usual plating method. Nevertheless, for the last infection experiment, I managed to reduce the manipulation steps by plating droplets corresponding to different dilutions of the grinded leaves on the same plate, which also facilitates the numeration since less colonies need to be counted. However, since this method only requires 20 µL spots, the numeration is also less precise than with the classic plating method. This could explain the increased difference of sensitivity observed between Col-0 and *chs5* in the last infection experiment. In addition, the size of the plants (rosette diameter and number of leaves) and their phenotype were slightly different between the repeated experiments, as the growing chambers used for their culture. Thus, lots of factors should be taken into account and could be responsible for the slight variability between the different experiments.

Nevertheless, I always observed that *chs5* mutants impaired in the plastidial MEP pathway were more sensitive to the *Pst*DC3000 than Col-0. In contrast, *hmg1-1* mutants impaired in the cytosolic MVA pathway were not. Thus, isoprenoids from the MEP pathway appear to be important for the plant interaction with *Pst*DC3000. It still

remains to determine if isoprenoids are directly or indirectly implicated in the plant resistance to pathogens. These molecules could have a direct effect on the plant resistance to pathogens since *chs5* mutants are altered in the formation of chloroplastic isoprenoids. This biosynthesis pathway is at the origin of phytohormones like abscisic acid (ABA) and cytokinins which may play a role in the defense against pathogens, additionally to the well-studied salicylic acid, jasmonic acid and ethylene (Albrecht and Argueso, 2017; Asselbergh et al., 2008; Ton et al., 2009). But isoprenoids could also have an indirect effect on the plant resistance to pathogens, since we highlighted a difference of communities interacting with Col-0 and *chs5*. We can speculate that some bacteria from the microbiota could play a role in the interactions with pathogens. Some micro-organisms are less abundant in *chs5* which is more sensitive to the pathogen and could potentially play a role in the plant protection. Some others are more abundant in *chs5*. It is possible that these ones affect the plant health allowing the pathogen to infect the plant more easily, for instance.

In the paper, we showed that despite the existence of a core microbiota, that was also stated in previous studies (Bai et al., 2015; Bodenhausen et al., 2013; Bulgarelli et al., 2012, 2015; Lundberg et al., 2012; Schlaeppi et al., 2014), there are bacteria that are significantly more abundant in wild-types or in mutants. The presence of these bacteria in the microbiota may be influenced by the isoprenoid content of the plant, which suggests that isoprenoids may play a role in the interactions between plants and micro-organisms in general. These bacteria differentially abundant between Col-0 and *chs5* could be implicated in the difference of sensitivity between the two plants, as mentioned above.

Furthermore, I tried to obtain an additional control for our experiments. The idea was to work with *chs5* mutants that integrated an exogenous *DXS1* gene and thus compensate their isoprenoid deficit. This would be a great tool to test whether the difference of the sensitivity between Col-0 and *chs5* really is the consequence of a deficit in isoprenoid biosynthesis. If it is the case, such a line should exhibit the same sensitivity as Col-0, or they should be even less sensitive to *Pst*DC3000. For that purpose, in the laboratory, *A. thaliana* Col-0 line overexpressing the *DXS1* under the control of a 35S promotor (35S:DXS1^{OE}) was crossed with *chs5* mutant line which is mutated in the *DXS1* enzyme. F1 hybrids resulting from this crossing were self-fertilized to obtain the F2 generation. I sowed 132 seeds from the F2 generation, and

I followed their phenotype before genotyping the plants. I was looking for plants exhibiting a wild-type phenotype characterized by green leaves, but with the *dxs1* mutation of the *chs5* mutant. I only managed to obtain heterozygous (*DXS1/dxs1*) that integrated the overexpressor, but no homozygous. Thus, I sowed 192 seeds obtained from one heterozygous plant that integrated the overexpressor. After genotyping the descendants, I still did not obtain any homozygous *dxs1/dxs1* that integrated the overexpressor. Nevertheless, I think that such a line could be really useful to deepen the observations made in this chapter.

Chapter 2
Do some specific bacteria influence the plant health and resistance to pathogens?

I. Introduction

The previous chapter of my thesis presented the inventory of the communities interacting with wild-type and mutants of *A. thaliana* altered in isoprenoid biosynthesis. We highlighted that, despite the existence of a core microbiota, specific operational taxonomic units (OTUs) were significantly more abundant in wild-types or in mutants. Additionally, *chs5* plants, altered in the biosynthesis of isoprenoids via the MEP pathway have been shown to exhibit a higher sensitivity to the phytopathogen *Pst*DC3000 than Col-0. Together, these results indicate that isoprenoids may influence the interactions between plants and micro-organisms.

This led us to wonder if some bacteria that are more abundant in wild-type or in mutants, whose presence is favored or not by plant isoprenoids, are able to impact the plant health and its resistance to pathogens. To answer this question, we had to test the effect of these strains on the plant, and thus to have them available. We managed to build a strain collection of 230 bacteria isolated from *A. thaliana* and the soil. Among them, I had to determine which ones could be interesting. Thus, I sequenced their 16S rRNA gene and compared it to the ones of the variable OTUs highlighted in the first chapter of my thesis. We were particularly interested in bacteria that are differentially abundant between Col-0 and *chs5* since these two plants exhibit a difference of sensitivity to *Pst*DC3000 in holoxenic conditions, as showed in the previous chapter. Few strains that we isolated exhibit 100% of sequence identity with the variable OTUs, which led me to suppose that they could be the same bacteria, or phylogenetically close to bacteria whose abundance varies. Thereafter, I inoculated some of these strains to plants under controlled conditions in order to determine whether they influence the plant health or resistance to pathogens. Inoculations were proceeded in gnotoxenic culture *in vitro* to determine the impact of the tested strains in absence of the naturally associated microbiota. I also inoculated one of them *in vivo*, in holoxenic conditions, to determine its effect in the presence of the plant microbiota.

The present chapter is a synthesis of the work that I made in the isolation of bacteria and the in-depth study of five of them that were potentially more abundant in wild-type or in mutants.

Table 2. Quantity of isolated strains obtained for each isolation method. Isolated strains were obtained upon selection of colonies based on their morphologic features. Most of the isolated strains were finally able to grow on LB medium. 100 strains were isolated from WS2; 108 strains were isolated from Col-0; 22 strains were isolated from the soil.

WS2	Leaves	MYX	42
		MM + MeOH	7
		Total	**49**
	Phyllosphere	MYX	7
		MM + MeOH	1
		GYM	5
		Total	**13**
	Roots	YEM	9
		M408	9
		TYG	7
		TWYE	3
		GYM	6
		Total	**34**
	Rhizosphere	GYM	4
		Total	**4**
	Total		**100**

Soil	LB		12
	TWYE		10
	Total		**22**

Col-0	Leaves	MYX	6
		MM + MeOH	6
		Total	**12**
	Phyllosphere	MYX	12
		MM + MeOH	3
		Streptomyces medium	13
		Actinomycete medium	31
		Total	**59**
	Roots	YEM	6
		M408	10
		TYG	7
		TWYE	4
		Streptomyces medium	9
		Total	**36**
	Seeds	LB	1
		Total	**1**
	Total		**108**

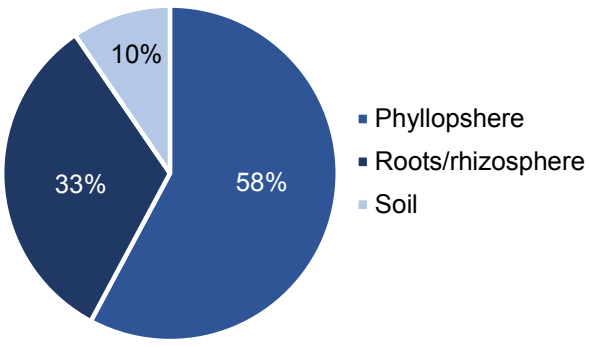


Figure 24. Proportions of isolated strains coming from the phyllosphere, the underground parts of the plant and the soil. We consider the number of strains from the phyllosphere as the number of strains isolated from either the leaves alone or the whole phyllosphere. The roots and the rhizosphere form together the underground parts of the plant. Bacteria isolated from the soil are considered separately.

1. Isolation and taxonomic affiliation of 230 strains

We isolated 230 strains from the different parts of wild-type Col-0 and WS2 (phyllosphere, roots, rhizosphere, seeds), and from the soil used in the institute for their culture. These strains were affiliated at the genus level and five of them were studied more in details. I selected them based on their partial 16S rRNA gene sequence identity with variable OTUs highlighted in the inventory of the communities exposed in chapter 1.

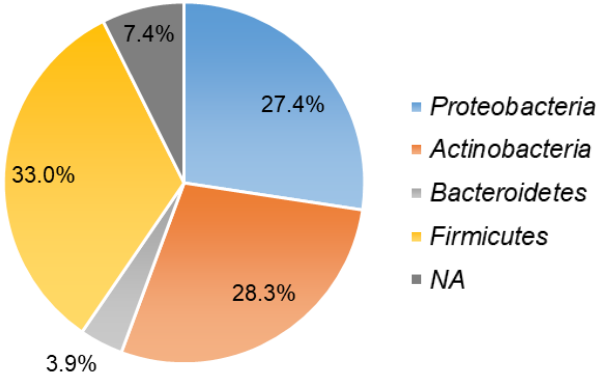
1.1. Strain collection

For the isolation of the strains from the plant, we decided to separate the aboveground parts of the plant (leaves or whole phyllosphere) from the roots and the rhizosphere that are the underground parts of the plant. We used different culture media depending of the plant material, based on a previous publication (Bai et al., 2015). We first plated the grinded plant material at different concentrations. We subsequently tried to mainly select colonies that were morphologically different from each other in terms of size, color, shape or aspect, to isolate them. Among the 230 strains that we isolated in the laboratory, 100 were isolated from *A. thaliana* WS2, 108 from Col-0, and 22 from the soil, as indicated in the **table 2**. The proportions of strains isolated from the aboveground parts of the plant, the underground parts of the plant, and the soil, are represented in **figure 24**. More than half of the strains that we isolated originated from the phyllosphere, and less from the rhizosphere and the soil.

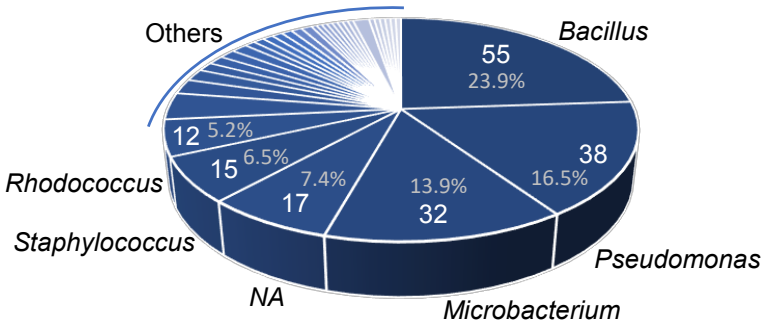
1.2. Taxonomic study of the isolated strains

The first step following the isolation of these strains was their identification. Taxonomy allows to find a taxon corresponding to a strain based on similarities criteria. One criteria commonly used in taxonomy and that could be rapidly observed is the 16S

A Library of 230 isolated strains



B



C

Bacterial genus	Number of strains
Glutamicibacter	9
Flavobacterium	5
Exiguobacterium	4
Stenotrophomonas	3
Shinella	3
Curtobacterium	3
Rhizobium	3
Chryseobacterium	3
Brevundimonas	2
Erwinia	2
Paracoccus	2
Gordonia	2
Paenibacillus	2
Acinetobacter	2
Burkholderia	2
Pseudoclavibacter	1
Lelliottia	1
Pseudonocardia	1
Homoserinibacter	1
Mesorhizobium	1
Providencia	1
Janibacter	1
Achromobacter	1
Enterobacter	1
Cellulomonas	1
Chitinophaga	1
Rhanella	1
Comamonas	1
Agromyces	1

D

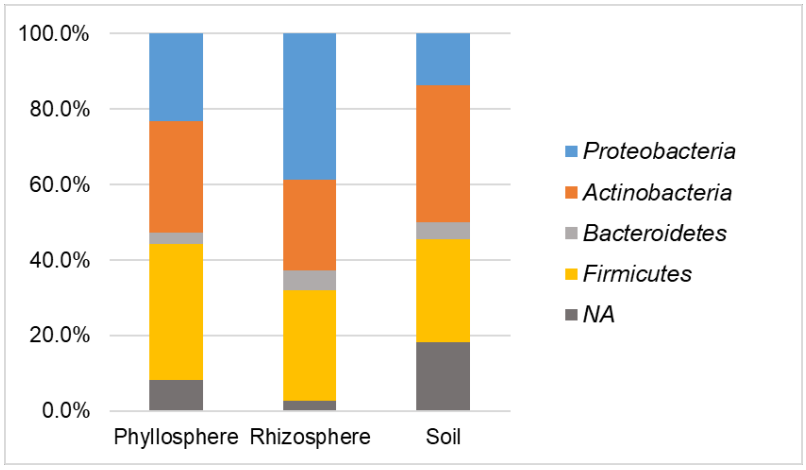


Figure 25. Composition of our strain collection. Strains were affiliated by 16S rRNA gene sequencing and comparison with the SILVA database. NA: non attributed. (A) Relative abundance of each phylum within our strain library. *Proteobacteria* are divided in 3.5% of *Alphaproteobacteria*, 1.7% of *Betaproteobacteria* and 22.2% of *Gamma-proteobacteria*. (B) Isolated strains were affiliated at the genus level. (C) Genus that are present in lower amounts in the "Others" category in (B) are given in this table. (D) Taxonomic distribution of our strains in the phyllosphere, the rhizosphere and the soil.

rRNA gene sequence. It could allow to find to which genus belongs each strain. Another criteria that can be assessed is the analysis of the fatty acid methyl esters (FAME).

1.2.1. 16S rRNA gene sequencing

The conserved regions of the 16S rRNA gene allow to hybridize universal primers for its amplification, independently of the organism, in order to amplify variable regions present in the gene. These variable regions enable to distinguish between different strains up the genus level. I amplified the entire 16S rRNA gene in order to sequence it and to affiliate our isolated strains to one genus by comparison of this sequence to the SILVA database. I chose this database since it is the same as the one allowing to identify the OTUs highlighted in the first chapter of my thesis.

As indicated in the **figure 25, A**, the most important phylum represented in our collection is the *Firmicutes*, followed by *Actinobacteria* and *Proteobacteria*. 17 strains are still unknown since we did not manage to properly extract their DNA or to sequence their 16S rRNA gene. We isolated a majority of *Bacillus*, *Pseudomonas* and *Microbacterium*, some *Staphylococcus* and *Rhodococcus*, and a wide variety of other bacteria in small amounts (0.2% to 2.6%) as shown in **figure 25, B**. A summary table of the number of bacteria that we isolated per genus in the “others” category from the **figure 25, B** is given in **figure 25, C**.

Finally, we can compare the proportions of each phylum in our library between each compartment of isolation that are the phyllosphere, the rhizosphere, and the soil as presented in **figure 25, D**. I wish to notice that I regrouped the bacteria isolated from the different parts of the phyllosphere (whole phyllosphere, leaves, stems, flowers) in one category, and I regrouped the bacteria from the roots and from the rhizosphere in a second category.

Table 3. Isolated strains exhibiting 100% of 16S rDNA sequence identity with OTUs differentially abundant between Col-0 and *chs5*. 16S rRNA gene sequence of our strains were compared to the partial 16S rRNA gene sequence of each variable OTU highlighted by the inventory of the communities. With 100% of sequence identity, the following strains are close to the varying OTUs. OTUs from the first, second, or third inventory of the communities are indicated by the number 1, 2 or 3, respectively, in the OTU name. Strains that we studied more in details are surrounded in red.

		Comparison with variable OTUs			
Isolated strain	SILVA affiliation	Sequence similarity	OTU	Compartment of the OTU	More abundant in
6H_MYX_WS2_F1C2	*Curtobacterium*	100%	1_2351	Phyllosphere	*chs5*
		99.76%	2_1240	Phyllosphere	*chs5*
5H_YEM_WS2_4-2	*Curtobacterium*	100%	1_2351	Phyllosphere	*chs5*
		100%	2_1240	Phyllosphere	*chs5*
2C_M408_WS2_Rc5	*Curtobacterium*	100%	1_2351	Phyllosphere	*chs5*
		100%	2_1240	Phyllosphere	*chs5*
10A_TYG_WS2_4-3	*Pseudomonas*	100%	2_51	Roots	*chs5*
		99.77%	1_65	Phyllosphere	*chs5*
		99.77%	1_65	Roots	*chs5*
2D_MYX_Col0_Phyllo_C5	*Pseudomonas*	100%	1_85	Phyllosphere	*chs5*
		100%	1_85	Roots	*chs5*
		100%	2_89	Phyllosphere	*chs5*
		100%	2_89	Roots	*chs5*
		99.1%	1_626	Roots	*chs5*
5B_MYX_WS2_F6C3	*Microbacterium*	100%	1_303	Phyllosphere	Col-0
Ced_4_2	*Microbacterium*	100%	1_303	Phyllosphere	Col-0
Ced_B1	*Rhizobium*	100%	3_657	Phyllosphere	*chs5*
		100%	3_657	Rhizosphere	*chs5*
Ced_5	*Pseudomonas*	100%	1_65	Phyllosphere	*chs5*
		100%	1_65	Roots	*chs5*
		100%	1_1049	Roots	*chs5*
		100%	2_51	Roots	*chs5*
9G_MYX_WS2_F5C3	*Pseudomonas*	100%	2_51	Roots	*chs5*
		99.77%	1_65	Phyllosphere	*chs5*
YEM_WS2_Rc3p	*Rhizobium*	100%	3_657	Phyllosphere	*chs5*

1.2.2. Comparison of the 16S rRNA gene sequences of our strains with those of the OTUs varying between wild-type and mutants

I compared the 16S rRNA gene sequences of our strains with those of the variable OTUs from the communities highlighted in the first chapter of my thesis (**table 3**). This inventory of the communities has been made three times for Col-0 and *chs5*, in conditions that were slightly different (same culture conditions but different growth chambers, leading to slight variations in the plant size). I performed a first comparison at the beginning of my thesis, with the sequences of the strains already isolated and those of the varying OTUs obtained in the first two test experiments comparing Col-0 and *chs5* communities available at this time. I only retained sequences that exhibit 100% of identity between the isolated strains and the variable OTUs to prevent to choose strains that are not belonging to the same species. The objective was to increase our probability to work with a strain that is phylogenetically close to the varying OTUs, in such a way that they could even correspond to the concerned OTU, or to a bacteria belonging to the same species. I selected 5 strains of interest that are surrounded in red in **table 3**. Later, during my thesis, I also compared the 16S rRNA gene sequence of all our strains to those obtained in the third inventory experiment of the communities interacting with Col-0 and *chs5* made in the lab. The results are also presented in the **table 3**. I also did it for the inventory of WS2 and *hmg1-1* communities (**Supplemental table S1**). These last data correspond the those exposed in the chapter 1. It allowed me to highlight some other bacteria that could be phylogenetically close to the varying OTUs.

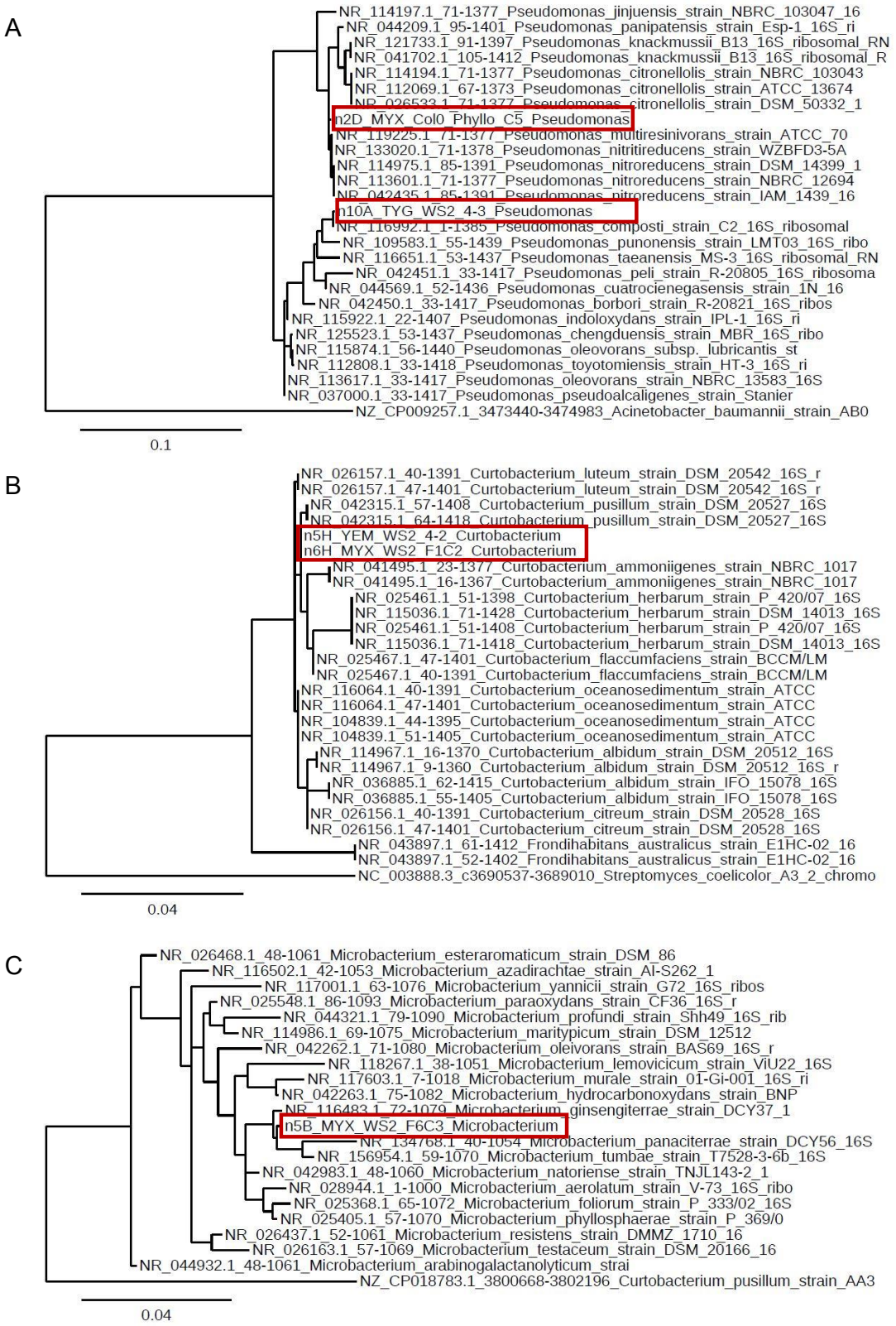


Figure 26. Phylogenetic trees of the studied strains. Phylogenetic trees were made after comparison of the 16S rRNA gene sequences of the isolated strains with the NCBI database. Trees were constructed on phylogeny.fr. Isolated strains are surrounded in red. (A) 10A_TYG_WS2_4-3 and 2D_MYX_Col0_PhylloC5 are phylogenetically close to *Pseudomonas composti* and *Pseudomonas multiresinivorans*, respectively. (B) 6H_MYX_WS2_F1C2 and 5H_YEM_WS2_4-2 are phylogenetically close to *Curtobacterium pusilum.* (C) MYX_WS2_F6C3 is phylogenetically close to *Microbacterium ginsengiterrae*.

1.2.3. Phylogenetic inference

To go further in the identification and eventually to affiliate our strains to known species, I also compared their 16S rRNA gene sequence with other phylogenetically close species. For this, I built phylogenetic trees to find the closest parent of the selected strains. I compared the 16S rRNA gene sequence of our strains with the NCBI database by BLAST (Basic Local Alignment Search Tool). This allowed me to find which bacteria of the database exhibit the highest sequence identity with our ones and to construct phylogenetic trees (**Figure 26**). The strains 10A_TYG_WS2_4-3 and 2D_MYX_Col0_PhylloC5 are phylogenetically close to *Pseudomonas composti* and *Pseudomonas multiresinivorans* or *nitroreducens*, respectively. These two isolated strains only exhibit 95.73% of 16S rRNA gene sequence identity, indicating that they are not the same species. This explains why they are found on different branches on the phylogram. The strains 6H_MYX_WS2_F1C2 and 5H_YEM_WS2_4-2 for their part are both phylogenetically close to *Curtobacterium pusillum.* Thus, I compared the 16S rRNA gene sequence of these two isolated strains and noticed that they were exhibiting 100% of identity. However, on LB medium, 6H_MYX_WS2_F1C2 formed small yellow and glossy colonies, while 5H_YEM_WS2_4-2 formed medium whitish glossy colonies. This suggests that they potentially have different capacities and impact on the plant. Finally, MYX_WS2_F6C3 is phylogenetically close to *Microbacterium ginsengiterrae*, *Microbacterium panaciterrae* and *Microbacterium tumbae*.

1.2.4. Fatty acid methyl esters (FAME) analyses

To go further in the identification of the 5 strains of interest indicated in the **table 3**, I analyzed their fatty acid methyl esters (FAME) profiles. More than 300 fatty acids can be found in bacteria, giving rise to unique profile from one species to another. The wealth of information contained in these compounds is both in the qualitative differences (usually at the genus level) and quantitative differences (commonly at the species level).

Table 4. Fatty acid methyl esters (FAME) analyses by GC/FID. Fatty acids (FA) content was studied by gas chromatography coupled with flame ionization detector (GC/FID). The major FA mentioned in the literature for each genus are indicated before the results of the analyses proceeded on our strains. 14:0 is miristic acid; 16:0 is palmitic acid; 16:1 *cis* is palmitoleic acid; 16:1 *trans* is palmitelaidic acid; 18:0 is stearic acid; 18:1 *cis* is *cis*-vaccenic acid; 18:1 *trans* is *trans*-vaccenic acid; 17cyclo and 19cyclo are cyclopropane FA.

***Pseudomonas* genus** (Heipieper and de Bont, 1994; Heipieper et al., 1992)									
16:0	16:1 *trans*	16:1 *cis*	17cyclo	18:0	18:1 *trans*	18:1 *cis*	19cyclo	Degree of Saturation	*trans*/*cis* *ratio*
Major FA			Traces – 2%			Major FA	Traces – 2%		
10A_TYG_WS2_4-3									
25.8%	8.4%	16.1%	0.0%	1.4%	4.8%	43.5%	0.0%	37%	0.22
2D_MYX_Col0_Phyllo_C5									
35.9%	9.0%	22.0%	0.1%	0.7%	3.2%	28.9%	0.1%	58%	0.24

***Curtobacterium* genus** (Kim et al., 2008; Suzuki and Komagata, 1983)								
14:0	15:0 iso	15:0 anteiso	16:0 iso	16:0	17:0 anteiso	18:0	18:1 *cis* Δ9	Anteiso/iso ratio
Traces – 4%		Major FA				Traces – 3%		
6H_MYX_WS2_F1C2								
0.0%	2.2%	41.1%	6.1%	8.5%	40.2%	1.9%	0.0%	9.76
5H_YEM_WS2_4-2								
0.0%	2.6%	35.8%	6.1%	2.6%	46.0%	2.4%	4.5%	9.44

***Microbacterium* genus** (Gorshkova et al., 2016; Yan et al., 2017; Zhu et al., 2019)								
14:0	15:0 iso	15:0 anteiso	16:0 iso	16:0	17:0 anteiso	18:0	18:1 *cis* Δ9	Anteiso/iso ratio
Traces	3-9%	Major FA		Traces – 4%	Major FA	Traces – 2.5%		
5B_MYX_WS2_F6C3								
0.0%	17.3%	33.0%	20.4%	4.1%	24.2%	0.9%	0.1%	1.52

This work has been made in Leipzig, in collaboration with Dr. Hermann Heipieper. Results from the analyses are shown in **table 4**. Based on the affiliations made by 16S rRNA gene sequencing, I searched data from the literature concerning *Pseudomonas*, *Curtobacterium* and *Microbacterium* strains. Our data confirm that our strains 10A_TYG_WS2_4-3 and 2D_MYX_Col0_PhylloC5 have a fatty acids composition similar to the *Pseudomonas* genus, with major fatty acids that are C16:0, C16:1 *cis*, C16:1 *trans* and C18:1 *cis* (Heipieper and de Bont, 1994; Heipieper et al., 1992). The strains 6H_MYX_WS2_F1C2 and 5H_YEM_WS2_4-2 have a fatty acids composition similar to the *Curtobacterium* genus with major fatty acids that are C15:0 anteiso, C17:0 anteiso, C16:0 iso and C16:0 (Kim et al., 2008; Suzuki and Komagata, 1983). Finally, the strain 5B_MYX_WS2_F6C3 has a fatty acids composition similar to the *Microbacterium* genus with majors fatty acids that are C15:0 anteiso, C17:0 anteiso and C16:0 iso (Gorshkova et al., 2016; Yan et al., 2017; Zhu et al., 2019).

Together, the 16S rRNA gene sequencing and the FAME analyses allowed to affiliate our strains to the previously mentioned genus. To simplify their further studies, I referred to 10A_TYG_WS2_4-3 as *Pseudomonas* sp. 10A, 2D_MYX_Col0_PhylloC5 as *Pseudomonas* sp. 2D, 6H_MYX_WS2_F1C2 as *Curtobacterium* sp. 6H, 5H_YEM_WS2_4-2 as *Curtobacterium* sp. 5H, and 5B_MYX_WS2_F6C3 as *Microbacterium* sp. 5B.

2. In-depth study of some isolated strains

First, I wondered if the 5 isolated strains mentioned above that could correspond to OTUs that are differentially abundant between Col-0 and *chs5* could be affected by plant isoprenoids. For this, I tested the effect of few isoprenoids found in *A. thaliana* on their bacterial growth. Then, I studied these strains to determine if they have an effect on the plant health and resistance to pathogens.

Figure 27. Culture of *Curtobacterium* sp. 5H in mineral medium + 4 g.L^{-1} succinate. Bacteria form aggregates which prevent the reliable measurement of the OD_{600nm}.

Table 5. Growing test in mineral medium supplemented with succinate or limonene. Bacteria were cultivated for ~48h at 28°C. An arbitrary scale is given by the colors with light blue as the minimal growth and dark blue as the highest growth, since OD_{600nm} measurements were not reliable due to the formation of bacterial aggregates.

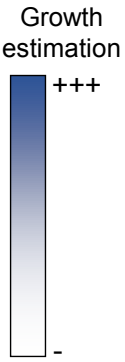
	Bacterial growth			
Mineral media supplemented with	Succinate 4 g.L^{-1}	Limonene 100 mg.L^{-1}	Limonene 250 mg.L^{-1}	Limonene 500 mg.L^{-1}
Curtobacterium sp. 6H				
Curtobacterium sp. 5H				
Pseudomonas sp. 10A				
Pseudomonas sp. 2D				
Microbacterium sp. 5B				

Growth estimation

2.1. Impact of isoprenoids on the bacterial growth

I first tested bacterial growth in liquid mineral medium containing succinate as a classic source of carbon, or limonene, an isoprenoid found in *A. thaliana*, at different concentrations. Despite their capacity to grow in the presence of limonene, strains formed aggregates. This was already the case for strains grown in mineral medium supplemented with succinate as we can see in **figure 27**. Since it prevents us to reliably measure the OD_{600nm}, and thus to properly quantify the bacterial growth, I tried to arbitrary quantify the growth of each strain based on the turbidity of the media and the quantity and size of aggregates. Results are shown in **table 5**. In short, each studied strain was able to grow in the mineral medium supplemented with limonene, but at high concentration, this isoprenoid impacted their growth.

Table 6 Growing test on mineral medium supplemented with isoprenoids. Isoprenoids were used as a carbon source. *Escherichia coli* Rosetta™ was used as a control. An arbitrary scale is given by the colors with white as the absence of growth and dark blue as the highest growth (strains grew well independently of the isoprenoid concentration).



	Compound	Concentration	*Curtobacterium* sp. 5H	*Curtobacterium* sp. 6H	*E. coli* (Rosetta™)
Mineral medium supplemented with	No carbon source				
	Succinate	4 $g.L^{-1}$			
	Limonene	100 $mg.L^{-1}$			
		250 $mg.L^{-1}$			
		500 $mg.L^{-1}$			
	β-caryophyllene	100 $mg.L^{-1}$			
		250 $mg.L^{-1}$			
		500 $mg.L^{-1}$			
	Farnesol	100 $mg.L^{-1}$			
		250 $mg.L^{-1}$			
		500 $mg.L^{-1}$			
	Myrcene	100 $mg.L^{-1}$			
		250 $mg.L^{-1}$			
		500 $mg.L^{-1}$			
	α-pinene	100 $mg.L^{-1}$			
		250 $mg.L^{-1}$			
		500 $mg.L^{-1}$			
	β-ocimene	100 $mg.L^{-1}$			
		250 $mg.L^{-1}$			
		500 $mg.L^{-1}$			
	α-humulene	100 $mg.L^{-1}$			
		250 $mg.L^{-1}$			
		500 $mg.L^{-1}$			
	(-)-linalool	100 $mg.L^{-1}$			
		250 $mg.L^{-1}$			
		500 $mg.L^{-1}$			
	Geraniol	100 $mg.L^{-1}$			
		250 $mg.L^{-1}$			
		500 $mg.L^{-1}$			
	Thujopsene	100 $mg.L^{-1}$			
		250 $mg.L^{-1}$			

I focused particularly on one isolated strain, *Curtobacterium* sp. 5H, that was particularly interesting for us, as explained in following paragraph II.2.2. For that strain, I also tested other isoprenoids as a carbon source in liquid mineral medium (geraniol, α-pinene, β-ocimene, farnesol, β-caryophyllene or α-humulene) and observations were the same in these conditions, the strain formed aggregates. Thus, I decided to test the growth of *Curtobacterium* sp. 5H and its phylogenetically close *Curtobacterium* sp. 6H on solid mineral medium containing isoprenoids as carbon source to avoid the quantification troubles. *Escherichia coli* Rosetta™ was used as a control since it is a bacteria that was not isolated from plants, but which is common in mammals. The aim was to see if this strain reacts like the ones isolated from plants to the different isoprenoids. The plated dilutions did not allow to count single colonies. However, this allowed us to confirm the capacity of the strains to grow on mineral medium supplemented with different isoprenoids, as shown in **table 6**. First of all, none of the tested strains was able to grow on mineral medium missing a carbon source, and they all grew well on mineral medium supplemented with succinate. Compared to *E. coli*, both *Curtobacterium* sp. 5H and *Curtobacterium* sp. 6H grew better on mineral media containing limonene, α-pinene, β-ocimene, geraniol, and particularly (-)-linalool at high concentrations. However, their growth was inhibited in the presence of high amounts of farnesol, compared to *E. coli* that grew well on this media, independently of the farnesol concentration. Finally, the tested bacteria isolated from plants were able to use plant isoprenoids to grow.

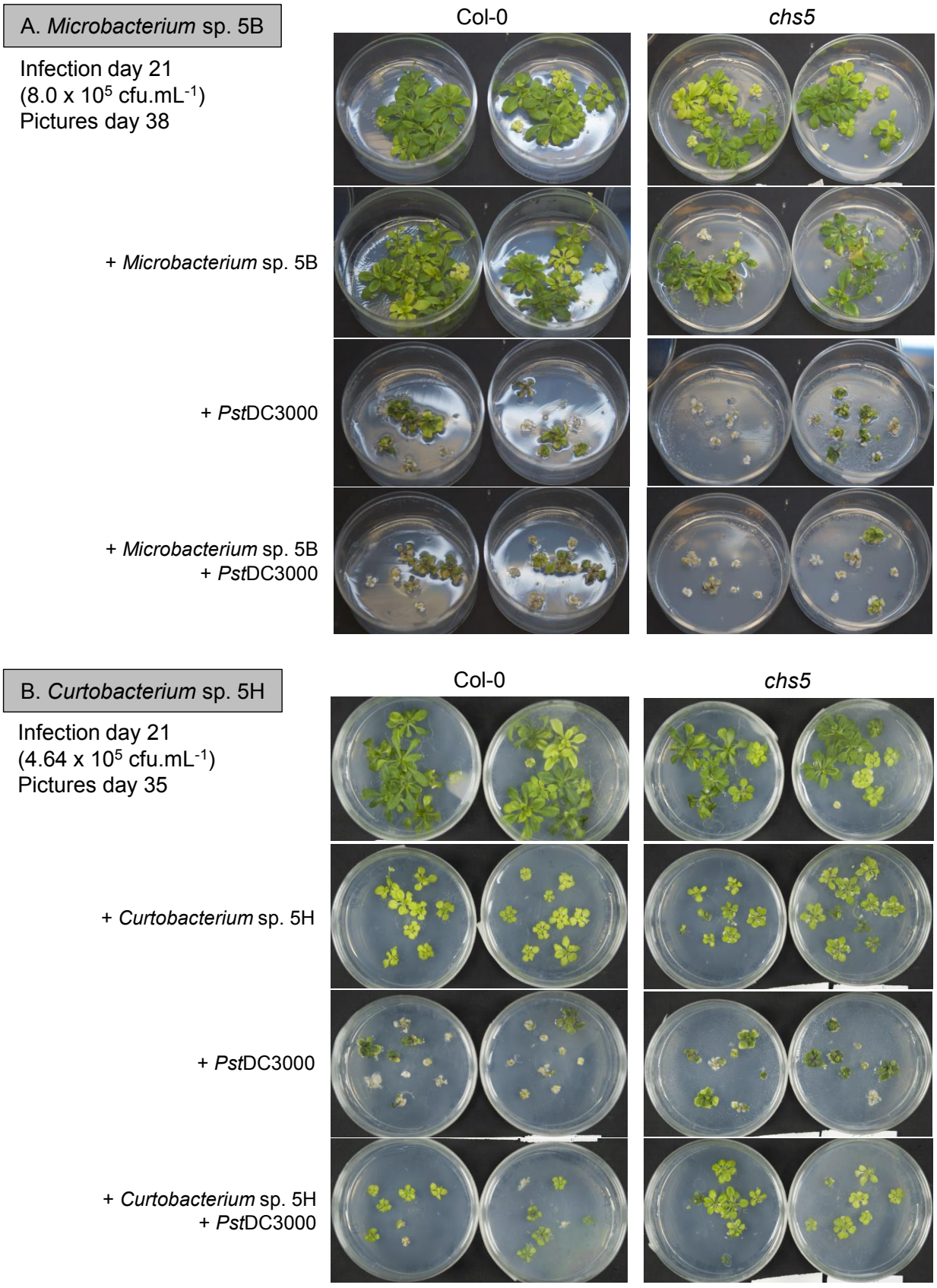


Figure 28. *In vitro* study of the impact of *Microbacterium* sp. 5B and *Curtobacterium* sp. 5H on Col-0 and *chs5* fitness and resistance to *Pst*DC3000. *Microbacterium* sp. 5B (A) and *Curtobacterium* sp. 5H (B) are shown as example of strains with no effect and potential protective traits, respectively. Strains were inoculated to Col-0 and *chs5* sterile seeds. Spray-infection by *Pst*DC3000 was proceeded after 3 weeks of growth. Pictures were taken by Cédric Jacob.

2.2. In vitro study of the effect of the strains on *A. thaliana*

The 5 strains previously mentioned were tested for their effect on Col-0 and *chs5* *in vitro*. The aim was to determine if these strains have an impact on the plant health and resistance to pathogens. For that purpose, sterile seeds of Col-0 and *chs5* were sown on solid MS medium allowing their culture in axenic conditions. Strains were inoculated on seeds, and after 21 days of growth, plants were infected or not with *Pst*DC3000. A preliminary experiment was conducted in the laboratory on several bacteria, among which *Curtobacterium* sp. 5H and *Microbacterium* sp. 5B shown in **figure 28**. The other strains of interest, *Curtobacterium* sp. 6H, *Pseudomonas* sp. 10A and *Pseudomonas* sp. 2D were also studied for their effect on Col-0 and *chs5*. Results are represented in the **table 7**.

In these experiments, we observed that plants inoculated with *Microbacterium* sp. 5B exhibit the same fitness as the non-inoculated ones, suggesting that the strain has neither negative effect, nor positive effect on Col-0 or *chs5* health. They also exhibit the same symptoms upon infection, whether or not they were seed-inoculated by *Microbacterium* sp. 5B, indicating that the strain does not induce any protective effect on the plants (**Figure 28, A**). As summarized in the **table 7**, *Pseudomonas* sp. 10A and *Pseudomonas* sp. 2D both exhibited a negative effect on the plant health characterized by a severe decrease in the size of the plants. *Pseudomonas* sp. 10A also induced more severe symptoms upon infection by *Pst*DC3000. *Pseudomonas* sp. 2D, for its part, did not impact the severity of the infection by *Pst*DC3000. In contrast, we observed that *Curtobacterium* sp. 5H induced a decrease in the size of the plants, but they did not exhibit lesions or other symptoms characteristic of pathogenic strains. In addition, we observed a potential protective effect of *Curtobacterium* sp. 5H upon infection by *Pst*DC3000 since plants that were inoculated before infection did not exhibit symptoms, compared to the plants that were not inoculated before infection. Indeed, the plants inoculated with *Curtobacterium* sp. 5H, and particularly *chs5*, exhibited the no particular phenotype compared to non-infected plants (**Figure 28, B**). Finally, *Curtobacterium* sp. 6H, which is phylogenetically close to *Curtobacterium* sp. 5H, also induced a slight decrease of the size of the plants, and potential protective traits upon infection by the pathogen. However, this effect is less obvious compared to *Curtobacterium* sp. 5H.

Table 7. Summary of the *in vitro* study of the impact of each strain of interest on Col-0 and *chs5* fitness and resistance to *Pst*DC3000. Strains were inoculated to Col-0 and *chs5* sterile seeds. Spray-infection by *Pst*DC3000 was proceeded after 3 weeks of growth. The effect of each strain is indicated by colors: blue characterizes a positive effect, red a negative effect, and white the absence of effect.

	Effect on the plant fitness		Effect on the protection against *Pst*DC3000	
	Col-0	*chs5*	Col-0	*chs5*
Pseudomonas sp. 10A				
Pseudomonas sp. 2D				
Curtobacterium sp. 5H				
Curtobacterium sp. 6H				
Microbacterium sp. 5B				

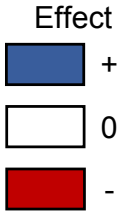


Table 8. Comparison of the sterilization protocols for *A. thaliana* seeds. A comparison of two sterilization protocols of the seeds has been made. The protocol number 1 is the one usually employed for my experiments: 2 minutes in 70% ethanol, a washing step of 1 minute in H_2O, 5 minutes in commercial bleach supplemented with 0.1% Tween 20, and 8 washing steps in sterile H_2O. The protocol number 2 is the one used for preliminary experiments: 1 minute in 70% ethanol, 5 minutes in a sterilization solution containing 4% of commercial bleach and 0.1% SDS, and 3 washing steps in sterile H_2O. Sterilized seeds were placed in different bacterial culture media or yeast culture media to test the sterilization efficiency.

		Sterilization 1	**Sterilization 2**
Germination efficiency		39%	38%
Contamination on solid MS medium		-	+
Bacterial growth in liquid culture medium	LB	-	+
	NYGB	-	+
	GYM	-	+
	YPD	-	+

I noticed however that the sterilization protocol previously used for this experiment was less stringent that the one that I usually employ in the laboratory. The usual protocol for seed sterilization (further referred as sterilization 1) requires a bath in ethanol followed by a bath in commercial bleach containing 4% sodium hypochlorite. In contrast, the previous sterilization method (further referred as sterilization 2) consists in a bath in ethanol followed by a bath in H_2O containing 4% of commercial bleach. Thus, it is possible that some micro-organisms remain on the seeds and could impact the effect of the tested strains. I decided to compare the efficiency of these two methods of sterilization. This comparison is summarized in **table 8**. There is no difference of seed germination efficiency following the sterilization. However, there was a fungal contamination on solid MS medium after the sterilization 2, and some micro-organisms were able to grow in different liquid culture media. Thus, the sterilization 2 protocol employed in the screening of the strains effect on *A. thaliana* was probably not completely efficient, suggesting that the observed effects could potentially result from interactions with other remaining micro-organisms.

I decided to test the effect of *Curtobacterium* sp. 5H, on seeds sterilized with the most stringent protocol of sterilization (sterilization 1). I obtained supplementary controls thanks to Pr. Julia Vorholt who provided me with two strains previously characterized in her laboratory: *Methylobacterium extorquens* PA1 which have no effect on disease development upon infection by *Pst*DC3000, and *Sphingomonas melonis* sp. FR1, that have been shown to exhibit protective traits against *Pst*DC3000 (Innerebner et al., 2011; Vogel et al., 2012). Moreover, I decided to proceed infection by pipette-inoculation, according to another protocol from her laboratory (Innerebner et al., 2011) because the spray we used for the preliminary experiments delivers 800 µL each time, which is much more than the pathogen supposed to be delivered (100 µL for 8 plants). I could have adapted the pathogen concentration, but I also noticed that the spray-infection did not allow a homogenous distribution. Indeed, since the plants are relatively close, each spray affected several plants. In addition, we though that the spray was too powerful, inducing mechanical stresses on the seedlings. In the new experiments, I observed slight protective traits of *Sphingomonas melonis* sp. Fr1 and the absence of effect of *Methylobacterium extorquens* PA1. However, I did not observe significant differences of sensitivity whether the plants were inoculated or not with *Curtobacterium* sp. 5H before infection by *Pst*DC3000.

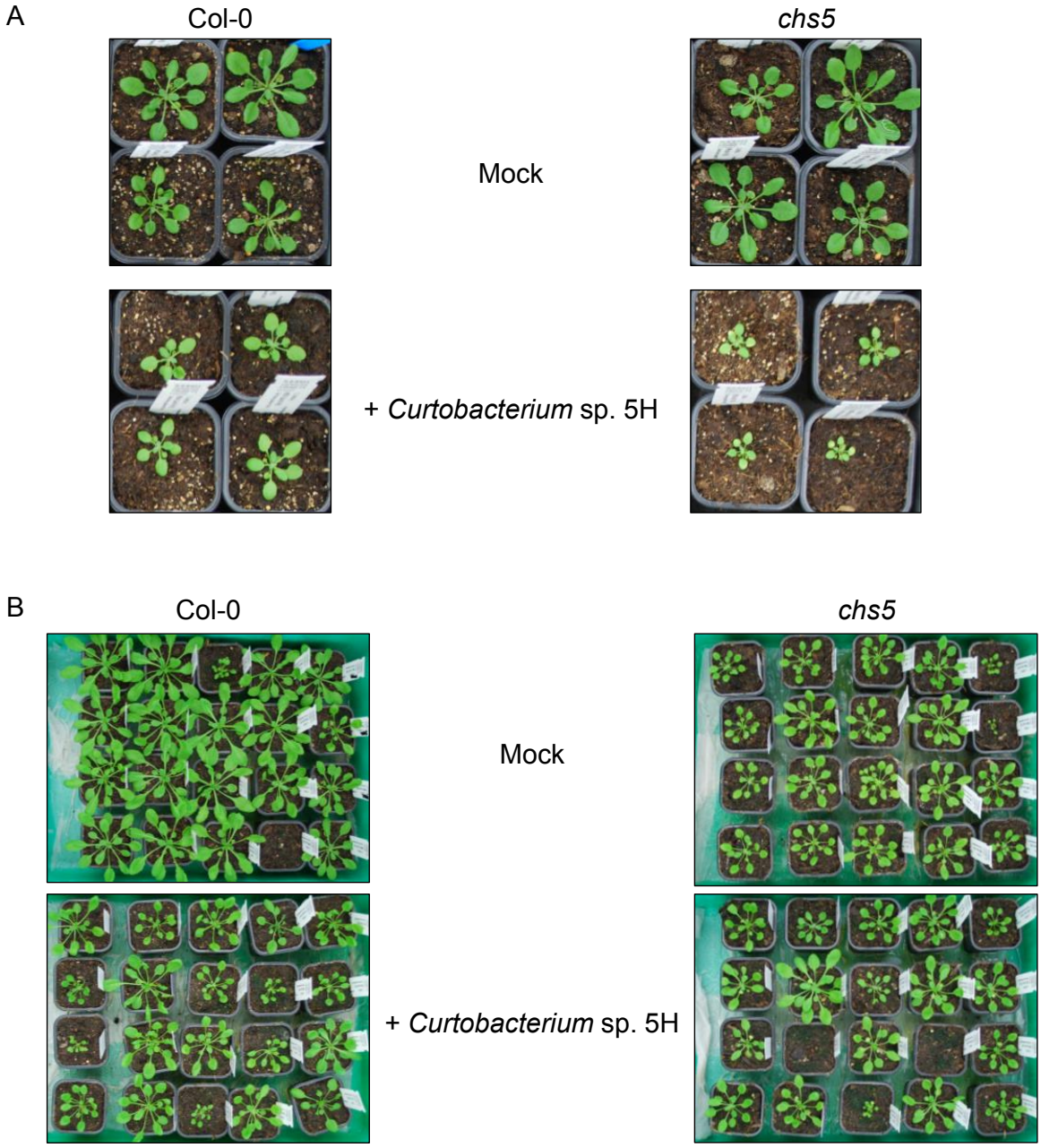


Figure 29. *In vivo* study of *Curtobacterium* sp. 5H effect on Col-0 and *chs5* fitness. (A) For seed inoculation, Col-0 and *chs5* seeds were immerged in a bath of *Curtobacterium* sp. 5H at a final concentration of 0.5 x 10^8 cfu.mL^{-1} for 15 minutes before sowing, or mock-treated with 10 mM $MgCl_2$. They are in a good shape, but their size is reduced compared to mock-treated plants. Pictures were taken after 6 weeks of culture. (B) 2 weeks-old Col-0 and *chs5* were inoculated with 5 mL of *Curtobacterium sp. 5H* at a final concentration of 1 x 10^8 cfu.mL^{-1}, or mock-treated with 10 mM $MgCl_2$. Pictures were taken after 6 weeks of culture before infection by *Pst*DC3000. Inoculated Col-0 are smaller than the non-inoculated ones but all plants appear healthy.

Nevertheless, I observed that plants inoculated were slightly smaller than the non-inoculated ones, suggesting that *Curtobacterium* sp. 5H partially inhibits the plant growth (**Supplemental figure S1**).

Finally, to get to the bottom of it, since we observed variable effects of *Curtobacterium* sp. 5H upon infection by *pst*DC3000, I decided to proceed inhibition tests between these two strains. The aim was to determine if *Curtobacterium* sp. 5H could directly impact the plant resistance by secreting antimicrobial molecules for instance. I did not observe direct inhibition of one strain by the other.

2.3. Effect of *Curtobacterium* sp. 5H in vivo

I supposed that the protective traits of *Curtobacterium* sp. 5H observed with the protocol of sterilization 2 but not repetitively observed with the protocol of sterilization 1, could result from interactions with other bacteria. Thus, I decided to test the inoculation of *Curtobacterium* sp. 5H on holoxenic plants, in pots. I first tested an inoculation method based on seed-coating, by putting the seeds in a bath of *Curtobacterium* sp. 5H. As we can see in **figure 29, A**, plants that were seed-inoculated exhibit a growth delay compared to the mock inoculated ones, indicating that *Curtobacterium* sp. 5H could alter their growth, as we already observed *in vitro*. With such a growth delay, it was complicated to perform syringe infection experiments to test the plants resistance to *Pst*DC3000.

In a second time, I decided to try another protocol based on a previous study of soybean (Park et al., 2017). I inoculated 5 mL of *Curtobacterium* sp. 5H suspension directly in the soil of 3-weeks old plants that were approximately the same size. In **figure 29, B**, we can see that after 6 weeks of culture, Col-0 plants inoculated with *Curtobacterium* sp. 5H exhibited a slight growth delay compared to the mock-treated, while *chs5* did not exhibit major differences whether they were inoculated or not. Thus, *Curtobacterium* sp. 5H may have an impact on the plant growth, at least on Col-0 in holoxenic conditions. I infected 5 plants previously inoculated with *Curtobacterium* sp. 5H or not to determine the effect of the strain against *Pst*DC3000 in holoxenic conditions. I did not observe major differences between inoculated or non-inoculated

Col-0 or *chs5* (**Supplemental figure S2**), suggesting that *Curtobacterium* sp. 5H has no protective capacities.

Altogether, our experiments revealed that *Curtobacterium* sp. 5H slightly inhibits the plant growth and had finally no obvious protective effect against *Pst*DC3000.

III. Discussion

The main outcome of this part of my work is undoubtedly the constitution of a strain collection composed of 230 bacteria isolated from *A. thaliana* and the soil. Since we selected the strains to keep based on morphological criteria, we probably missed lots of micro-organisms that look like the ones we chose, but that are not the same. For the same reason, it is probable that we isolated similar strains thanks to different media. By consequence, our strain collection is not necessary representative of the natural microbiota. This has been shown by comparison with the inventory of the communities proceeded in the laboratory. This is also confirmed by previous studies which highlighted a large predominance of *Proteobacteria*, followed by *Actinobacteria* and *Bacteroidetes*, and much less *Firmicutes* (Bai et al., 2015; Bodenhausen et al., 2013; Bulgarelli et al., 2012; Horton et al., 2014; Lundberg et al., 2012; Schlaeppi et al., 2014). This is not surprising since we only isolated a fraction of the bacteria compared to the cultivable part of the microbiota (Bai et al., 2015). However, the proportions of each phylum from our strain collection between the different plants compartments only exhibit slight variations. We showed that *Proteobacteria* are more abundant in the isolated strains coming from the rhizosphere than those isolated from the phyllosphere, which is consistent with the distribution observed in the natural microbiota, as indicated in the first chapter of my thesis. *Actinobacteria*, are more represented in the isolated strains coming from the soil, and from the phyllosphere as compared to those isolated from the rhizopshere in which they are less abundant. This is also in accordance with the phylum distribution observed in the natural communities.

Table 9. Comparison of the 16S rRNA gene sequence of the strains from our collection with the collection of Pr. Schulze-Lefert. Sequences of the strains from our collection were compared to those of the strains from collections of the laboratory of Pr. Schulze-Lefert (Bai *et al.*) (accession number PRJNA297956, PRJNA297942 and PRJNA298127 for leaf, root and soil collections, respectively). Only strains with >99% of sequence identity are considered in this table. The precise comparison of each isolated strain from our collection with those from Pr. Schulze-Lefert's collection is given in supplemental table S2.

>99% 16S rRNA gene sequence identity between our strains and strains from Bai *et al*.			
Affiliated genus from our collection	Lab Schulze-Lefert **Leaves collection**	Lab. Schulze-Lefert **Roots collection**	Lab Schulze-Lefert **Soil collection**
Microbacterium	6	21	
Exiguobacterium	1		
Rhanella	1		
Pseudomonas	3		
Stenotrophomonas	1		
Janibacter		1	1
Staphylococcus		1	
Bacillus		53 | 45 (100%)	
Rhizobium		2	
Pseudomonas		1	

Other strains collections already exist in laboratories studying the interactions between plants and micro-organisms. In comparison to our collection, the one of the laboratory of the Pr. Schulze-Lefert (Köln, Germany) is more substantial, with 433 strains. I compared the 16S rRNA gene sequence of our strains to this collection and found some phylogenetically close strains with almost 100% of sequence identity (**Table 9 and supplemental table 3**). 45 *Bacillus* within our collection exhibit 100% of 16S rRNA gene sequence similarity with bacteria from their roots collection. Other strains exhibit a high sequence identity, among which *Microbacterium*, *Pseudomonas*, and *Rhizobium*. In other words, it is possible that we have an overlap between our collections, but most of our isolated strains do not exhibit high similarity. This is probably partially due to the soil used for the plant culture since it is known that the soil type is important for the establishment of bacterial communities (Edwards et al., 2015; van der Heijden and Schlaeppi, 2015).

During my thesis, I studied more in details 5 strains from our collection. These candidates exhibit 100% of 16S rRNA gene sequence similarity with OTUs differentially abundant between Col-0 and *chs5*. Among them, I showed in the paragraph II.1.2.3 that 5H_YEM_WS2_4-2 (*Curtobacterium* sp. 5H) and 6H_MYX_WS2_F1C2 (*Curtobacterium* sp. 6H) had the same 16S rRNA gene sequence. However, they exhibit a different phenotype when cultivated on LB medium. Thus, the 16S rRNA gene sequence alone could allow us to differentiate between genus, but it is not precise enough to affiliate a strain at the species level. It seems that the two *Curtobacterium* that we isolated are not the same, thus, they potentially have different capacities. To determine the relativeness of these strains, it could be useful to proceed multilocus sequence typing (MLST) by sequencing other housekeeping genes such as *gyrB* or *rpoB* for instance (Stackebrandt et al., 2007). We should also be careful about the links we consider between our strains and the varying OTUs, since we cannot be sure that they are the same strains only on the base of the 16S rRNA gene sequence. I tried to use another identification method based on mass spectrometry analyses, since each bacteria exhibits a specific metabolic profile that can be compared to its identity card. However, to achieve this, the studied bacteria should already exist in the database, which is not the case for bacteria isolated from plants.

Once I affiliated our 5 strains of interest, I wondered if they needed isoprenoids to accommodate, or on the contrary, if isoprenoids could be toxic for them. Then, I tested their effect on *A. thaliana*.

Microbacterium sp. 5B was phylogenetically close to *Microbacterium* panaciterrae, *Microbacterium tumbae* and Microbacterium *ginsengiterrae* as shown in the paragraph II.1.2.3. We showed that *Microbacterium* sp. 5B was able to grow in mineral medium containing limonene, despite increased difficulties in the presence of high concentrations. It would have been interesting to test its growth on media containing other isoprenoids as carbon source to determine if they could favorize its growth, since this strain is potentially more abundant in Col-0 compared to *chs5*. Following inoculation on *A. thaliana*, we showed that *Microbacterium* sp. 5B did not impact the plant health or its resistance against *Pst*DC3000. Since no positive or negative effects of *Microbacterium* strains were reported in the literature, it is not surprising.

Pseudomonas sp. 2D, for its part, was phylogenetically close to *Pseudomonas multiresinivorans*, which could be considered as a synonym of *Pseudomonas nitroreducens* based on genetic and biochemichal similarities (Lang et al., 2007). Thus, it is not surprising that our strain was also close to *Pseudomonas nitroreducens* strains. It was interesting to test the effect of our *Pseudomonas* sp. 2D since *Pseudomonas nitroreducens* strain IHB B 13561 is considered as a PGPR by enhancing the growth of *A. thaliana*, and also *Lactuca sativa*, by stimulating the cell development and nitrate absorption (Trinh et al., 2018). However, *Pseudomonas* sp. 2D negatively affected the plants since it inhibited their growth and amplified the symptoms following the infection by *Pst*DC3000. *Pseudomonas* sp. 10A, was shown to be relatively close to *Pseudomonas composti*. No effects of that species were previously reported. As for the other *Pseudomonas* that we tested, *Pseudomonas* sp. 10A affected the plant development. However, no effect was observed upon infection by *Pst*DC3000. *Pseudomonas* sp. 2D and *Pseudomonas* sp. 10A grew easily in mineral medium containing limonene as a carbon source, but high concentrations appeared to be toxic for them.

Concerning *Curtobacterium* sp. 5H and *Curtobacterium* sp. 6H, both were phylogenetically close to *Curtobacterium pusillum*, *Curtobacterium ammoniigenes* and *Curtobacterium flaccumfaciens*. Only few effects of some of these strains are reported in the literature. For instance, *Curtobacterium flaccumfaciens* has been shown to

A

B

Figure 30. Hydroponic culture system. We optimized an axenic culture system where plants are grown in hydroponic conditions until the formation of siliques. Sterilized seeds are deposited on Eppendorf tubes containing Hoagland's medium + 0,6% agar in home-made nurseries containing liquid Hoagland's medium. After 1 month of growth, plants are transferred into closed big boxes containing the same medium, still under sterile conditions. Plant health is not affected by the culture conditions in liquid medium.

exhibit plant growth promoting effects on barley (Cardinale et al., 2015). *Curtobacterium flaccumfaciens* strain ME1 exhibited antagonistic effects against pathogens such as *P. syringae* (Horuz and Aysan, 2018). However, others are considered as pathogenic, such as *Curtobacterium flaccumfaciens* pv. flaccumfaciens that causes disease in soybean (Sammer and Reiher, 2012). I first observed that *Curtobacterium* sp. 5H and *Curtobacterium* sp. 6H were probably able to use few isoprenoids as a source of carbon to grow. This is not surprising since bacteria also need isoprenoids, and could be able to use the ones synthetized by their host (Kuzuyama and Seto, 2003; Pérez-Gil and Rodríguez-Concepción, 2013). However, we showed that *Curtobacterium* sp. 5H and *Curtobacterium* sp. 6H had difficulties to grow when the concentration in geraniol or farnesol were too high. Since the physiological concentrations of isoprenoids in *A. thaliana* are poorly documented, it is difficult to conclude about their implication in the bacterial growth. However, we can estimate that high concentrations of isoprenoids may be toxic for bacteria. The effect of *Curtobacterium* sp. 5H on *A. thaliana* was highly variable. After preliminary experiments, I made the choice to focus on this strain for its potential protective trait upon infection by *Pst*DC3000. I showed that this effect is not observable when the sterilization protocol was more stringent. This suggests that the potential protective traits of *Curtobacterium* sp. 5H could result from interactions with other micro-organisms. However, we always observed that the strain slightly inhibits the plant growth. Thus, it is difficult to conclude about the effect of the strain on the plant resistance to pathogens, but it is clear that it has a negative effect on the plant development. Since strains belonging to the *Curtobacterium* genus can be beneficial for plants while others are pathogenic, *Curtobacterium* sp. 5H requires further experiments to determine its impact on the plant.

From a technical point of view, different systems can be used for the study of plant-bacteria interactions, among which the *in vitro* culture used during my work is probably the most common. However, I observed a high variability of the effects following the inoculation of the isolated strains. Plants could be stressed by the different steps of manipulation, notably the transplantation after 2 weeks of growth, followed by the infection only one week later. To go further, other plant growth systems could be useful. During my thesis, I optimized a hydroponic system of culture (**Figure 30**) in order to process experiments in axenic conditions on plants that could grow until the formation

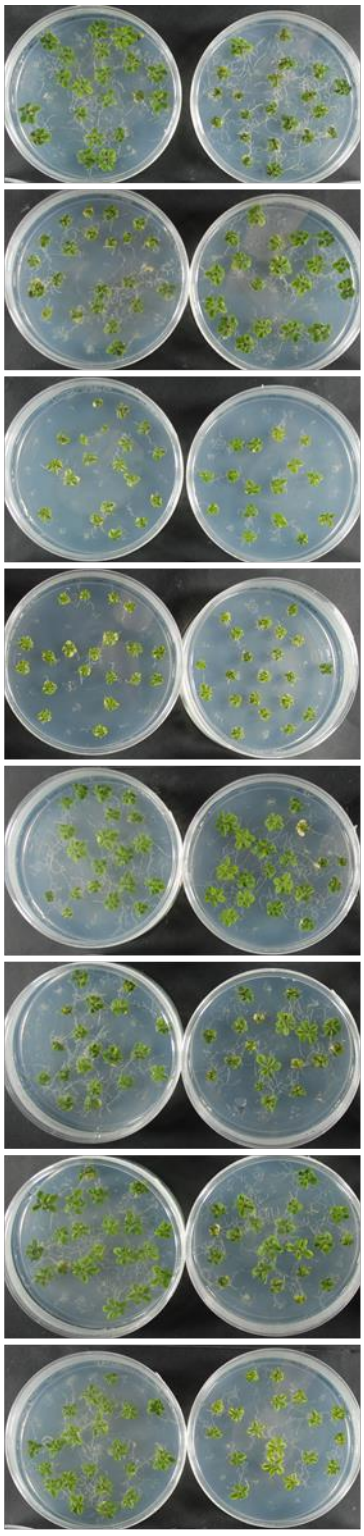
of the siliques, and not only on rosettes as it was possible on *in vitro* plates. This could also allow us to test the effect of the strains on bigger plants and to test the plant resistance against *Pst*DC3000. This would help us to understand if the difference of sensitivity observed between Col-0 and *chs5* in the chapter 1 requires the plant microbiota or not. To this day, the system is operational, but we are still encountering difficulties to obtain plants that are homogenous enough, since we can only grow 12 plants per system. Nevertheless, with several systems in parallel, it will be possible to compare different conditions on bigger batches of plants.

To finish, since I selected few strains of interest rapidly after the beginning of my thesis, I did not work on bacteria that could correspond to varying OTUs of the last inventory of Col-0 and *chs5* communities. This last inventory was made in the appropriate culture conditions in the institute and could better help us to understand which bacteria could be responsible for the difference of sensitivity between Col-0 and *chs5* observed in the first chapter. Thus, other strains mentioned in the **table 3** deserve further studies to determine their impact on the plant fitness and resistance to pathogens.

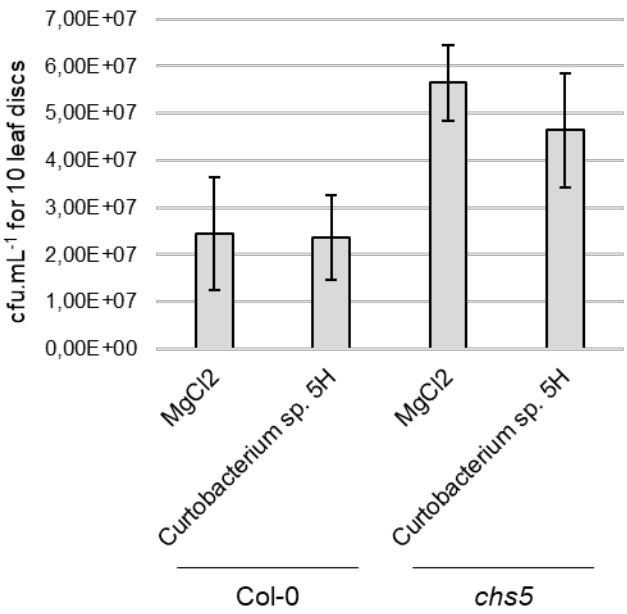
Supplemental figures and tables

Supplemental table S1. Isolated strains exhibiting 100% of 16S rDNA sequence identity with OTUs differentially abundant between WS2 and *hmg1-1*. 16S rDNA sequence of our isolated strains were compared to the partial 16S rDNA sequence of each variable OTU highlighted by the inventory of the communities. With 100% of sequence identity, the following isolated strains are close to the varying OTUs.

		Comparison with variable OTUs			
Isolated strain	SILVA affiliation	% 16s rDNA similarity	Cluster	Compartment	More abundant in
MM+MeOH_WS2_F 2C1	*Pseudonocardia*	100%	87	Phyllosphere	WS2
R2A_Ce	*Exiguobacterium*	100%	70	Phyllosphere	WS2
R1AA_Ce	*Exiguobacterium*	100%	70	Phyllosphere	WS2
T3AB_Ce	*Exiguobacterium*	100%	70	Phyllosphere	WS2
R2B_Ce	*Exiguobacterium*	100%	70	Phyllosphere	WS2
YEM_WS2_Rc3p	*Rhizobium*	100%	213	Phyllosphere	*hmg1-1*
		99.04%	213	Roots	*hmg1-1*
M408_Col0_Rc1-1	*NA*	100%	68	Phyllosphere	WS2
MM+MeOH_WS2_F 6C1	*Flavobacterium*	100%	123	Roots	*hmg1-1*
R3_Ce	*Pseudomonas*	100%	18	Roots	WS2
		99.03%	2629	Roots	WS2



Supplemental figure S1. Repeated *in vitro* study of *Curtobacterium* sp. 5H effect on *chs5* fitness and resistance to *Pst*DC3000. 5 µL of each strain were inoculated to Col-0 sterile seeds. 10 µL of *Pst*DC3000 at 4.27 x 10^4 cfu.mL^{-1} were pipette-inoculated to each plant after 3 weeks of growth. Plants were cultivated in Sanyo MLR-351H incubator with a regime of 16-hour light (160 µmol photon.s^{-1}.m^{-2}) at 18°C and 8-hour light (100 µmol photon.s^{-1}.m^{-2}) at 16°C. Pictures was taken after 31 days of culture.



Supplemental figure S2. *In vivo* study of *Curtobacterium* sp. 5H effect on Col-0 and *chs5* fitness and resistance to *Pst*DC3000 after inoculation on holoxenic 2-weeks old plants. 2 weeks-old Col-0 and *chs5* were inoculated with 5 mL of *Curtobacterium sp. 5H* at a final concentration of 1 x 10^8 cfu.mL^{-1}, or mock-treated with 10 mM $MgCl_2$. Numerations of PstDC3000 were proceeded on Col-0 and chs5 6dpi. No differences were observed between plants inoculated or not with *Curtobacterium* sp. 5H.

Supplemental table S2. Comparison of the 16S rRNA gene sequence of our strains with those of the Pr. Schulze-Lefert's collection. 16S rRNA sequences of the strains from the collections of Pr. Schulze-Lefert's laboratory, were available thanks to accession number are PRJNA297956, PRJNA297942 and PRJNA298127 for leaf, root and soil, respectively. Only strains with >99% of sequence identity are considered in this table.

		Leaf collection	
Strain	**Affiliation**	**% sequence identity**	**With**
YEM_WS2_Rc1-2	*Microbacterium*	99,91	Leaf320
			Leaf159
			Leaf161
		99,17	Leaf179
			Leaf436
			Leaf439
			Leaf441
		99,26	Leaf203
MYX_WS2_F6C2	*Microbacterium*	99,39	Leaf320
			Leaf159
		99,17	Leaf161
MYX_WS2_F5C1_1	*Microbacterium*	99,28	Leaf320
			Leaf159
			Leaf161
TYG_Col0_Rc6	*Microbacterium*	99,04	Leaf320
			Leaf159
MYX_Col0_PhylloC1	*Microbacterium*	99,38	Leaf320
			Leaf159
		99,14	Leaf161
MYX_WS2_PhylloC2	*Microbacterium*	99,4	Leaf320
			Leaf159
			Leaf161
R2A_Ce	*Exiguobacterium*	99,36	Leaf187
		99,58	Leaf196
TWYE_PBS1	*Rahnella*	99,08	Leaf50
Cedric_2_1_entoure	*Pseudomonas*	99,07	Leaf15
R3_Ce	*Pseudomonas*	99,17	Leaf434
T1_Ce	*Pseudomonas*	99,38	Leaf434
MYX_WS2_F5C9	*Stenotrophomonas*	99,06	leaf70
		Roots collection	
Strain	**Affiliation**	**% sequence identity**	**With**
MM+MeOH_WS2_F2C2	*Microbacterium*	99,12	Root280D1
MYX_WS2_F5C6-2	*Microbacterium*	9,02	Root553
		99,32	Root280D1
YEM_WS2_Rc1-2	*Microbacterium*	99,63	Root553
		100	Root280D1
		99,91	Root1433D1
			Root322
Cedric_2	*Microbacterium*	99	Root280D1
		99,92	Root1433D1
			Root322
Cedric_1_1	*Microbacterium*	99,78	Root1433D1
			Root322
Cedric_4_2	*Microbacterium*	9,9	Root1433D1
			Root322
Cedric_3_entoure	*Microbacterium*	99,77	Root1433D1
			Root322
MYX_WS2_F1C1	*Microbacterium*	99,55	Root280D1
		99,26	Root553
TYG_WS2_Rc1	*Microbacterium*	99,56	Root280D1
		99,26	Root553
MYX_Col0_F3C1	*Microbacterium*	99,31	Root553
		99,62	Root280D1
TYG_Col0_Rc6	*Microbacterium*	99,33	Root553
		99,63	Root280D1
TYG_WS2_Rc3	*Microbacterium*	99,18	Root553
		99,48	Root280D1

		Roots collection	
Strain	**Affiliation**	**% sequence identity**	**With**
MYX_WS2_F5C6-3	*Microbacterium*	99,18	Root553
		99,48	Root280D1
MYX_WS2_F5C1_2	*Microbacterium*	99,37	Root553
		99,69	Root280D1
MYX_Col0_PhylloC1	*Microbacterium*	99,37	Root553
		99,69	Root280D1
MYX_WS2_F2C1	*Microbacterium*	99,19	Root553
		99,48	Root280D1
MYX_WS2_PhylloC2	*Microbacterium*	9,2	Root553
		9,5	Root280D1
			Root1433D1
			Root322
MYX_WS2_PhylloC1	*Microbacterium*	99,48	Root553
		99,78	Root280D1
		99,03	Root1433D1
			Root322
MYX_WS2_F6C2	*Microbacterium*	99,47	Root553
		99,77	Root280D1
		99,01	Root1433D1
			Root322
MYX_WS2_F5C5	*Microbacterium*	99,14	Root280D1
MYX_WS2_F5C1_1	*Microbacterium*	99,36	Root553
		99,6	Root280D1
		99,51	Root1433D1
			Root322
MYX_Col0_F3C4	*Janibacter*	99,29	Root728
Clara_B	*Staphyllococcus*	99,85	Root560
MYX_WS2_F4C1-3	*Bacillus*	99,75	Root131
		99,74	Root11
E2B_Ce	*Bacillus*	99,92	Root131
		99,91	Root11
MYX_WS2_F4C1	*Bacillus*	99,74	Root131
		99,72	Root11
MM+MeOH_Col0_F1C2	*Bacillus*	100	Root131
			Root11
Endo_Col0	*Bacillus*	100	Root131
			Root11
Col0_Rc2-1_Strep	*Bacillus*	100	Root131
			Root11
Col0_P1-2	*Bacillus*	100	Root131
			Root11
S2_Cecile	*Bacillus*	100	Root131
			Root11
TYG_WS2_Rc2g	*Bacillus*	100	Root131
			Root11
MYX_Col0_F3C2	*Bacillus*	100	Root131
			Root11
M408_Col0_Rc5	*Bacillus*	100	Root131
			Root11
MM+MeOH_Col0_F1C1	*Bacillus*	100	Root131
			Root11
MYX_WS2_F1C5	*Bacillus*	100	Root131
			Root11
TWYE_WS2_Rc1	*Bacillus*	100	Root131
			Root11
T5bis_Ce	*Bacillus*	100	Root131
			Root11
T5B_Ce	*Bacillus*	100	Root131
			Root11
T5A_Ce	*Bacillus*	100	Root131
			Root11
S1_Ce	*Bacillus*	100	Root131
			Root11
R1B_Ce	*Bacillus*	100	Root131
			Root11
F1-3B_Ce	*Bacillus*	100	Root131
			Root11

		Roots collection	
Strain	Affiliation	% sequence identity	With
E2A_Ce	*Bacillus*	100	Root131
			Root11
MYX_WS2_F6C5	*Bacillus*	100	Root131
			Root11
MYX_WS2_F5C6-4	*Bacillus*	100	Root131
			Root11
MYX_WS2_F3C4	*Bacillus*	100	Root131
			Root11
MYX_WS2_F5C6	*Bacillus*	100	Root131
			Root11
MYX_Col0_F1C1	*Bacillus*	100	Root131
			Root11
MYX_Col0_F2C1	*Bacillus*	100	Root131
			Root11
R1AB_Ce	*Bacillus*	100	Root131
			Root11
M408_WS2_Rc2	*Bacillus*	100	Root131
			Root11
YEM_Col0_Rc5	*Bacillus*	100	Root131
			Root11
Col0_P2-2	*Bacillus*	100	Root131
			Root11
Col0_Rc1-2_Strep	*Bacillus*	100	Root131
			Root11
Col0_Rc2-2_Strep	*Bacillus*	100	Root131
			Root11
Clara_A	*Bacillus*	100	Root131
			Root11
Clara_J	*Bacillus*	100	Root131
			Root11
Cedric_2_2	*Bacillus*	100	Root131
			Root11
M408_WS2_Rc6-2	*Bacillus*	100	Root131
			Root11
YEM_WS2_Rc3g	*Bacillus*	100	Root131
			Root11
MYX_WS2_F3C5-2	*Bacillus*	99,75	Root131
		99,74	Root11
MYX_WS2_F5C8	*Bacillus*	99,84	Root131
		99,83	Root11
YEM_Col0_Rc3p	*Bacillus*	99,75	Root131
		99,74	Root11
TWYE_Col0_Rc4p	*Bacillus*	99,83	Root131
		99,82	Root11
MYX_WS2_F2C2	*Bacillus*	99,82	Root131
		99,81	Root11
Clara_F	*Bacillus*	99,92	Root131
		99,91	Root11
Clara_D	*Bacillus*	100	Root131
			Root11
MYX_Col0_F3C3	*Bacillus*	100	Root131
			Root11
TYG_Col0_Rc5	*Bacillus*	100	Root131
			Root11
Clara_I	*Bacillus*	100	Root131
			Root11
Clara_E	*Bacillus*	100	Root131
			Root11
Clara_C	*Bacillus*	100	Root131
			Root11
TWYE_Col0_Rc2	*Bacillus*	100	Root131
			Root11
MYX_Col0_PhylloC8	*Bacillus*	100	Root131
			Root11
MYX_WS2_F5C6-1	*Bacillus*	100	Root131
			Root11

		Roots collection	
Strain	**Affiliation**	**% sequence identity**	**With**
YEM_WS2_Rc3p	*Rhizobium*	99,62	Root651
Cedric_B1	*Rhizobium*	99,18	Root651
Cedric_2_1_entoure	*Pseudomonas*	99,03	Root201
		99,55	Root401
		99,25	Root329
		99,31	Root68
		99,33	Root71
		Soil collection	
Strain	**Affiliation**	**% sequence identity**	**With**
MYX_Col0_F3C4	*Janibacter*	99,69	Soil728

Discussion

In nature, plants interact with a huge variety of micro-organisms. The study of these interactions is a field that has been gaining interest since few decades. This enthusiasm is partially related to the fact that certain bacteria exhibit beneficial traits for the host plant, such as plant growth promotion, tolerance to stresses, or defense against pathogens (Lugtenberg and Kamilova, 2009; Schlaeppi and Bulgarelli, 2015; Yang et al., 2008). Indeed, it is suggested that the holobiont which is composed of the plant and its associated microbiota is potentially more capable to deal with stresses than the plant itself (Jones et al., 2019).

Researchers often focus on how bacteria from the microbiota impact the plant health, but less on how the plant may influence the selection of its microbiota (Jones et al., 2019). This is probably due to the focus on how to optimize crop cultures in modern agriculture, in terms of sustainability and productivity (Finkel et al., 2017; Keven Vessey, 2003; Schütz et al., 2018), but also in the reduction of postharvest food loss (Buchholz et al., 2018). The utilization of bacteria that have plant protective effects against pathogens, or plant growth promoting effects, is described as a great alternative to the use of pesticides and chemical fertilizers. Thus, the comprehension of the mechanisms that govern the interactions between plants and bacteria is a key milestone to take advantage of the microbiota and represent one of the most interesting questions about plant-bacteria interactions (Bulgarelli et al., 2013; Jones et al., 2019).

It is already known that interactions between plants and bacteria are influenced by the plant environment, the soil type, the plant genotype but also root exudates, which contain organic acids, amino acids, vitamins and sterols for instance (Bulgarelli et al., 2013; Edwards et al., 2015; Hartmann et al., 2008; van der Heijden and Schlaeppi, 2015; Mendes et al., 2013). In this environment, the presence of some molecules is implicated in the accommodation of the microbiota. A well-known example of molecules implicated in the recruitment of bacteria from the soil is flavonoids that can be sensed by *Rhizobium* (Hartwig et al., 1991; Phillips and Tsai, 1992). These molecules are also known for their antimicrobial, antifungal and antiviral activities, which participate in the microbiota selection (Cushnie and Lamb, 2005, 2011). Thus, we wondered if isoprenoids, another class of metabolites studied in our laboratory, may also influence plant-bacteria interactions. Previous studies highlighted the impact of specific bacteria of the microbiota on the production of plants isoprenoids (Gargallo-Garriga et al., 2016; Del Giudice et al., 2008; Pandey et al., 2018; Salomon et al.,

2016). However, less is known about the importance of isoprenoids on these interactions, but it was shown that plants deficient in phytosterols, a class of isoprenic lipids, are more easily colonized by pathogens (Wang et al., 2012b). In the same vein, it has been shown that holaphyllamine is a steroid capable to trigger defense response in *A. thaliana* (Zahid et al., 2017).

During the last three years, we have tackled the question of whether isoprenoids are involved in plant-bacteria interactions. For that purpose, we chose to work with *A. thaliana* lines that were altered in the biosynthesis of isoprenoids precursors, IPP and DMAPP. We showed that the plant isoprenoid status may have an impact on the establishment of the microbiota, on one hand, and on the resistance to pathogens, on another hand. To go further in the understanding of the process involved in the difference of sensitivity observed between Col-0 and *chs5* mutant altered in the production of plastidial isoprenoid precursors (MEP pathway), we built a strain collection by isolation of bacteria interacting with *A. thaliana*. This collection contains strains that could be phylogenetically close to OTUs that were differentially abundant between Col-0 and *chs5*. We tested the impact of isoprenoids on five candidate strains, and the impact of these strains on the plant health and resistance to *Pst*DC3000. Notably, I showed that *Pseudomonas* sp. 10A and 2D, and *Curtobacterium* sp. 5H and 6H had a negative impact on the plant growth, while *Microbacterium* sp. 5B had no effect. I was particularly interested in the study of one strain, *Curtobacterium* sp. 5H, that had variable effects on the plant resistance against *Pst*DC3000. Finally, I will discuss the output of my experimental work and I will present some perspectives that deserve attention for future studies.

I. Isoprenoids may be involved in the interactions between plants and bacteria from their microbiota

The first chapter of my thesis was partly devoted to the study of bacteria interacting with *A. thaliana*, considering the metabolic status of the plants. We showed that isoprenoids may be important for the establishment of the plant microbiota. We

confirmed that despite the presence of a core microbiota, which is consistent with previous studies (Bai et al., 2015; Bulgarelli et al., 2012, 2015; Lundberg et al., 2012; Schlaeppi et al., 2014), some specific OTUs were significantly more abundant in wild-types or in isoprenoid deficient mutants.

First of all, we showed that the composition of the microbiota was globally different between the phyllosphere, on one hand, and the roots and rhizosphere, on another hand, which were closer from each other. Such observations are in accordance with previous studies (Bai et al., 2015; Lundberg et al., 2012; Schlaeppi et al., 2014). This is not surprising since the establishment of the microbiota colonizing the underground parts of the plant is mainly controlled by the root exudates and the soil composition (Edwards et al., 2015; van der Heijden and Schlaeppi, 2015). In contrast, the microbiota colonizing the aboveground parts of the plants is governed by the atmosphere and interacting macro-organisms, for instance (Fahlgren et al., 2010), but also by molecules emitted by the plant such as volatile organic compounds (VOCs) (Bitas et al., 2013). Even if a deficit in isoprenoid precursors biosynthesis did not affect the global community composition or richness, some specific species were apparently impacted by the plant isoprenoid status. It was notably the case of *Rhizobium*, *Streptomyces* and a *Sphingomonas* strains. This should be considered since previous data from the literature showed the impact of strains closely related to these OTUs, as described below.

Rhizobium were shown to be more abundant in both mutants compared to wild-type *A. thaliana*. These bacteria are well-known plant growth promoting rhizobacteria (van Rhijn and Vanderleyden, 1995). Thus, the variable OTUs corresponding to *Rhizobium* genus could impact the plant health, and potentially its resistance against pathogens. However, as we demonstrated a higher sensitivity of *chs5* to *Pst*DC3000 compared to Col-0, the considered OTUs should not be involved in the plant resistance against this pathogen, at least in holoxenic conditions. In our strain collection, we managed to isolate *Rhizobium* strains, among which few are phylogenetically close to OTUs that are more abundant in *chs5* than in Col-0. However, they are also found to be phylogenetically close to OTUs that are more abundant in *hmg1-1* compared to WS2. Thus, they are probably not implicated in the difference of sensitivity observed between Col-0 and *chs5*. Nevertheless, it could be interesting to determine their impact

on *A. thaliana*, independently from the natural microbiota, in gnotoxenic conditions, as proceeded for the five strains of interest studied during my thesis.

Few *Streptomyces*, for their part, were shown to be more abundant in Col-0 compared to *chs5*, and they were not found to be differentially abundant between WS2 and *hmg1-1*. This is particularly interesting regarding the difference of sensitivity between Col-0 and *chs5* to *Pst*DC3000, and not between WS2 and *hmg1-1*. *Streptomyces* genus includes species considered as PGPR by production of siderophores or ACC deaminase, solubilization of phosphate, or production of volatile organic compounds (Dias et al., 2017); and they are known to be a great source for the production of bioactive secondary metabolites and antibiotics (Kuzuyama and Seto, 2003; Pérez-Gil and Rodríguez-Concepción, 2013). Moreover, plant growth promoting bacteria are often able to stimulate the plant defense machinery by inducing the systemic resistance (ISR) (Conn et al., 2008; Dessaux et al., 2016; Glick, 2012; Olanrewaju et al., 2017). It is notably the case of strains phylogenetically close to *Streptomyces rochei*, that induces ISR in tomato, against *Fusarium oxysporum* f. sp. *Lycopersici* race 3 (*FOL*) additionally to its PGP effects on the roots length and weight (Abbasi et al., 2019). Other *Streptomyces* are known to possess prenyltransferases allowing the prenylation of molecules such as naphterpin conferring them antioxidant, antimicrobial or anti-inflammatory activities (Kuzuyama et al., 2005). Thus, we can speculate that they can be useful for the plant and impact its health and capacities to defend against pathogens.

A *Sphingomonas* strain was also shown to be more abundant in Col-0 compared to *chs5*, and not differentially abundant between WS2 and *hgm1-1*. This strain is phylogenetically close to *Sphingomonas wittichii*, a bacteria shown to degrade indole 3-acetic acid (IAA) (Leveau and Gerards, 2008). In plants, this hormone is implicated in diverse processes including cell enlargement and division, tissue differentiation, and responses to light. Thus, bacteria producing IAA have the capacity to enhance root proliferation. An increased root system enhances nutrients uptakes and root exudation, which in turn increases the colonization by bacteria from the soil (Bashan et al., 2004; Dobbelaere et al., 1999). In addition, a previous study highlighted that the inoculation of another *Sphingomonas* strain, *Sphingomonas melonis* sp. Fr1, on *A. thaliana* provides protective traits against *Pst*DC3000 (Innerebner et al., 2011; Vogel et al.,

2012). Since there is a *Sphingomonas* which is more abundant in Col-0 than in *chs5*, this strain could be implicated in the observed difference of sensitivity to *Pst*DC3000.

The question of how isoprenoids may influence the establishment of bacterial communities remains open. Isoprenoids may be used by some bacteria as nutrients and may be toxic for others. This could be assessed by using mineral media supplemented with isoprenoids, as described in the second chapter of my thesis. Another method could be the use of *A. thaliana* Col-0 or *chs5* extracts in mineral media, to determine if the bacteria differentially abundant between Col-0 and *chs5* preferentially grow on one or another medium. Some bacteria are able to metabolize isoprenoids (de Carvalho et al., 2005; Seubert and W., 1960; Soares-Castro et al., 2017), such as *Pseudomonas* sp. strain M1 which is capable to oxidize β-myrcene into myrcene-8-ol (Soares-Castro et al., 2017). Thus, it could be interesting to perform stable isotope probing (SIP) using ^{13}C-labelled isoprenoids to determine the capacity of a strain to use it. It is also possible that isoprenoids act as signal molecules or chemo-attractants, like flavonoids. Finally, isoprenoids could also impact the establishment of the plant microbiota by defense mechanisms, since abscisic acid or cytokinins are implicated in the plant defense (Jones and Dangl, 2006; Pieterse et al., 2012), or by direct antimicrobial activities (Brigham et al., 1999; Yazaki et al., 2017).

Since we studied mutants altered in the formation of isoprenoids precursors, we don't know which isoprenoids may be involved in the establishment of the microbiota, or in the interactions with pathogens. It would be interesting to work with mutants altered downstream in the isoprenoid biosynthesis pathways to determine more precisely which isoprenoids could be implicated in these observations.

II. Isoprenoids may be involved in plant-pathogen interactions

I was particularly interested in the interactions of wild-type and isoprenoid mutant plants with the phytopathogen *Pst*DC3000. In the first chapter of my thesis, I have shown that there was no difference of sensitivity to the pathogen between WS2 and

hmg1-1 altered in the MVA pathway. However, *chs5* mutants altered in the plastidial MEP pathway were significantly more sensitive to the pathogen than Col-0. These results suggest that isoprenoids synthetized via the MEP pathway are important for the plant resistance to *Pst*DC3000. This difference of sensitivity could be directly related to the isoprenoid status, or indirectly, via the interaction with other bacteria from the microbiota interacting with wild-type and mutants.

To test whether the observations are related to the mutation of the *DXS1* responsible for the deficit in isoprenoids precursors, it would be interesting to obtain *chs5* plant lines that are mutated in the *DXS1* gene required in the MEP pathway, but rescued by the expression of the enzyme. I tried to obtain *dxs1*/*dxs1 A. thaliana* (*chs5* homozygous mutant) that integrated a 35S:DXS1^{OE} to rescue the plant isoprenoid status. Following the infection by *Pst*DC3000, these plants should exhibit a sensitivity comparable to that of Col-0, or they could be even less affected by the pathogen since there is an overexpression of the *DXS1* gene. This should also allow to determine if the establishment of the communities is impacted by the activity of this enzyme and its effect on the isoprenoid status. Three possibilities then arise: the microbiota could be similar to that of Col-0 since the plant is rescued for the isoprenoid biosynthesis; the microbiota could be similar to that of *chs5* since they are the same genotype; or it could be different from the one of the two others. Moreover, to determine if the observations are really the consequence of the isoprenoid deficit in *chs5* mutant, it could be interesting to chemically block the MEP biosynthesis pathway. For that, ketoclomazone could be used as an inhibitor of the DXS, or fosmidomycin as an inhibitor of the DXR enzyme (Phillips et al., 2008). In such conditions, plants should be more affected by *Pst*DC3000 than the non-treated ones, as it was the case for *chs5*. Such controls would allow to determine if there is potentially another mutation in *chs5* which could impact the interactions between plants and micro-organisms.

It is important to consider that the MEP pathway leads to the synthesis of phytohormones that are known to be implicated in the plant defense: abscisic acid, gibberellins, or cytokinins. Since it is known that there is a complex network activating the plant defense (Jones and Dangl, 2006; Pieterse et al., 2012), we can speculate that plants deficient in the synthesis of these hormones are probably affected in the plant resistance against pathogens, including hemibiotrophic pathogens. Indeed, it has already been demonstrated that *Pst*DC3000 induces ABA and abiotic response genes

in *A. thaliana*, in addition to the main hormone salicylic acid (Thilmony et al., 2006). Moreover, cytokinins are known to play a role in the plant defense against biotrophic pathogens (Albrecht and Argueso, 2017). This has been suggested few years ago since the application of high concentrations of cytokinins to tobacco cell cultures induces the expression of defense genes and stress genes (Schäfer et al., 2000). Plant cytokinins have been shown to promote the resistance of *A. thaliana* against *Pst*DC3000 (Choi et al., 2010). They modulate the salicylic acid signaling in order to increase the plant resistance against the pathogen. It has been confirmed by studies made on *A. thaliana*, with application of cytokinins before infection by a biotrophic oomycete, *Hyaloperonospora arabidopsidis* that led to a decreased susceptibility of the plants (Argueso et al., 2012). However, cytokinins are also synthetized via the MVA pathway since the inhibition of the HMGR induces a decrease in cytokinin content (Suzuki et al., 2004). If these molecules were responsible, alone, for the difference of sensitivity to *Pst*DC3000, *hmg1-1* mutants should also be more sensitive to the pathogen than WS2.

Moreover, it is possible that some bacteria from the microbiota interacting with Col-0 before infection induce plant enhanced defensive capacity, or “priming”, characteristic of the ISR. This defense priming state to resist further attacks by pathogens is independent of the SAR and the accumulation of PR proteins (Hammerschmidt, 1999; Hoffland et al., 1995; Van Peer and Schippers, 1992; Pieterse et al., 1996, 2000). ISR is controlled by jasmonic acid and ethylene hormones and also requires ABA which acts as a signal to enhance callose deposition (Pieterse et al., 2014). Since we did not observe major difference of expression of *PR* genes that could explain the difference of sensitivity between Col-0 and *chs5*, we suggested that SAR is not affected in *chs5* mutants. However, we did not study the other immune responses, thus it could be interesting to quantify phytohormones in order to determine if there is a priming state due to the ISR in Col-0 compared to *chs5*.

Different defense signaling pathways could be activated depending on the pathogen which attacks the plant. Thus, the sensitivity of *A. thaliana* wild-types and mutants should be further investigated by infecting plants with other pathogens, like the necrotrophic fungus *Botrytis cinerea*, for instance. This pathogen mainly activates the plant defense through the JA signaling pathway, contrary to *Pst*DC3000 who preferentially activates the SA signaling pathway (Glazebrook, 2005; Pieterse et al.,

2009). Such experiments could determine if isoprenoids specifically impact the interactions leading to the activation of SA signaling pathway, or also those activating the JA signaling pathway.

III. Some specific bacteria from the microbiota could influence the plant growth and resistance to pathogens

To go further in the study of how isoprenoids impact the interactions with micro-organisms, and how bacteria from the microbiota may influence the plant health and resistance to pathogens, it was necessary to isolate bacteria. Among the 230 strains from our strain collection, we focused on those that were phylogenetically close to OTUs that are differentially abundant between wild-type and mutants, and particularly Col-0 and *chs5*. Even if our strain collection only represents a small amount of the cultivable part of the plant microbiota(Bai et al., 2015), it contains bacteria that could be interesting to study independently, or in synthetic communities.

To select the candidate bacteria for further experimentations, we decided to compare their 16S rRNA gene sequence with the ones of the OTUs differentially abundant between Col-0 and *chs5*. I wish to notice that most of the time, the genus affiliations made for our strains are in accordance with those of the varying clusters for every strain presented in my thesis. When the genus of a variable OTU was not affiliated due to the short length of the sequence (500 bp compared to 1500 bp for the isolated strains), the phylum affiliation still correlated with the isolated strains. This gives credibility to the taxonomic affiliations and allowed me to select some strains to test their effect on the plant fitness and resistance to pathogens. Among the strains phylogenetically close to variable OTUs, most of them were more abundant in *chs5* compared to Col-0, suggesting that they would rather negatively impact the plant than positively if they are implicated in the difference of sensitivity to plants against *Pst*DC3000. It is possible that they affect the plant health, helping the pathogen to infect *chs5*. Among the tested strains, some belongs to *Curtobacterium* and *Pseudomonas* genus, which are known to be composed of both PGPB and pathogenic

strains (Cardinale et al., 2015; Horuz and Aysan, 2018; Passera et al., 2019; Sammer and Reiher, 2012). Only 2 strains from our collection are closely related to OTUs which are more abundant in Col-0: *Microbacterium* sp. 5B which had no effect on the plant, and another strain phylogenetically close to *Microbacterium*. It should be interesting to test this candidate for potential protective traits that could be implicated in the difference of sensitivity, despite the absence of description of such effects in the literature. Other strains from our collection should have been tested for their impact on *A. thaliana*, even if they were not closely related to variable OTUs. For example, the previously mentioned *Rhizobium*, but also *Bacillus* or *Pseudomonas* are known PGPB (Bloemberg and Lugtenberg, 2001; Soltani et al., 2010; Sturz and Nowak, 2000). Finally, it is important to consider that the effect of a strain could be different depending of the presence of this strain in a community or alone. It could be interesting to inoculate a synthetic community to *A. thaliana* instead of a unique strain in order to determine its effect on the plant. Moreover, strains such as our *Curtobacterium* sp. 5H that exhibited variable effects on plants potentially require additional analyses to determine if they have different beneficial traits. They could be tested for their capacity to solubilize phosphate, nitrate or iron, to produce siderophores, or ACC deaminase, for instance (Beneduzi et al., 2012).

Even if conditions are simplified in comparison to the natural environmental conditions, gnotobiotic systems in which sterile plants are grown such as the one we used for our experiments can help to determine the impact of some bacteria or communities on plants. However, the beneficial effect of a strain or a community on the host are often specific of the plant species and cultivar (Rodriguez et al., 2019). Thus, we must be careful about the generalization of the observations made in the laboratory. It is possible to test the effect of strains of interest on other plants than *A. thaliana* in order to determine if this effect is similar or not, notably on plants of agronomical interest. In addition, the strains could be tested on older plants, in other culture systems such as our hydroponic culture system mentioned in the chapter 2, to determine if their effect needs them to be established for a long time or not.

To become a tool in agriculture for instance, a specific strain exhibiting promoting traits in the laboratory needs to be operative in the field, meaning that a plant growth promoting bacteria needs to invade the plant and persist in the nature, with the natural microbiota interacting with the host and the variable environmental conditions (Finkel

et al., 2017). A first step to go further could be to voluntary stress the plants in culture conditions (humidity, drought, temperatures, UV, touching…). Then, field experiments by inoculation on seeds or directly on plants cultivated outside would be the better solution to determine the effect of bacteria in natural conditions.

To close the loop, a last point that would deserve our attention is the impact of the inoculated bacteria on the plant isoprenoid status. We showed that plant isoprenoids influence the interactions with micro-organisms, but we don't know what is the impact of the differentially abundant OTUs on the plant metabolic profile. The goal would be to determine if they could influence the production of isoprenoids by the plant, as observed in previous studies on the Vetiver (Del Giudice et al., 2008) and *Vitis vinifera* L. cv. Malbec (Salomon et al., 2016). This could be assessed by quantification of some key metabolites such as chlorophylls and carotenoids, as proceeded for non-inoculated plants in the paper presented in the first chapter of my thesis. In addition, non-targeted metabolomics approach could be used to study the impact of a strain on both isoprenoids and other metabolites. If bacterial strains or communities influence the production of isoprenoids by the plant, it is then possible that they indirectly impact the plant resistance against pathogens.

Materials and methods

I. Materials

1. Plant material

Arabidopsis thaliana (*A. thaliana*) lines used during my thesis were called Col-0 VIL, *chs5* VIL, WS2 VIL and *hmg1-1* VIL since their genome was entirely sequenced for the purpose of Next Generation Sequencing (NGS) project started by Dr. Claire Villette during her thesis. Lines were maintained by self-fertilization to ensure a constant use of the same genetic backgrounds.

1.1. *Arabidopsis thaliana* Wassilewskija (WS2) ecotype

Seeds of the wild-type *A. thaliana* ecotype Wassilewskiia (WS2) were obtained by the laboratory from Prof. Toshiya Muranaka (Osaka University, Japan).

1.2. *hmg1-1* mutant (WS2 genetic background)

Seeds of the *hmg1-1* mutant in the WS2 genetic background were also obtained by the laboratory from Prof. Toshiya Muranaka (Osaka University, Japan). In the *hmg1-1* line, the first exon of the gene coding for the 3-hydroxy-3-methylglutaryl coenzyme A reductase 1 (*HMG1*, At1g76490) is carrying a T-DNA insertion. This mutant line was originally screened by PCR-based genetics (*A. thaliana* T-DNA insertional mutant facility of Madison University, Wisconsin, USA). This mutant is characterized by a very low expression of the *HMG1* gene, leading to a defect in plant growth and fertility, associated with a decrease of metabolites downstream of the MVA biosynthesis pathway (Heintz et al., 2012; Suzuki et al., 2004).

Table 10. Bacterial culture media. The different media used for bacterial isolation and further cultivation are listed here.

LB (Luria Bertani) medium (Sigma-Aldrich)	
Tryptone	10 $g.L^{-1}$
Yeast extract	5 $g.L^{-1}$
NaCl	5 $g.L^{-1}$
(Agar)	12 $g.L^{-1}$
King's medium B (KB)	
Proteose peptone (Difco or Conda)	20 $g.L^{-1}$
K_2HPO_4	1.5 $g.L^{-1}$
Glycerol	15 $g.L^{-1}$
$MgSO_4.7H_2O$	1.5 $g.L^{-1}$
(Agar)	15 $g.L^{-1}$
NYGB medium	
Proteose peptone	5 $g.L^{-1}$
Yeast extract	3 $g.L^{-1}$
Glycerol	20 $g.L^{-1}$
(Agar)	15 $g.L^{-1}$
Nutrient broth (NB) medium	
D(+)-glucose	1 $g.L^{-1}$
Proteose peptone	15 $g.L^{-1}$
NaCl	6 $g.L^{-1}$
Yeast extract	3 $g.L^{-1}$
(Agar)	15 $g.L^{-1}$
pH 7.5	
Actinomycete Isolation Agar Medium (Sigma-Aldrich)	
Asparagine	0.1 $g.L^{-1}$
K_2HPO_4	0.5 $g.L^{-1}$
$FeSO_4$	1 $mg.L^{-1}$
$MgSO_4$	0.1 $g.L^{-1}$
Sodium caseinate	2 $g.L^{-1}$
$C_3H_5NaO_2$	4 $g.L^{-1}$
Agar	15 $g.L^{-1}$
pH 8.1	
Streptomyces medium (Sigma-Aldrich)	
NA	
MYX medium	
Na_2-glutamate	5 $g.L^{-1}$
Yeast extract	1 $g.L^{-1}$
$MgSO_4.7H_2O$	1 $g.L^{-1}$
D-glucose	2 $g.L^{-1}$
(Agar)	20 $g.L^{-1}$
pH 7.0	

Mineral medium (MM)	
$NaHPO_4.2H_2O$	7 $g.L^{-1}$
KH_2PO_4	2.8 $g.L^{-1}$
NaCl	0.5 $g.L^{-1}$
NH_4Cl	1 $g.L^{-1}$
$MgSO_4.7H_2O$	100 $mg.L^{-1}$
$FeSO_4.7H_2O$	10 $mg.L^{-1}$
$MnSO_4.H_2O$	5 $mg.L^{-1}$
$ZnCl_2$	6.4 $mg.L^{-1}$
$CaCl_2.6H_2O$	1 $mg.L^{-1}$
$BaCl_2$	0.6 $mg.L^{-1}$
$CoSO_4.7H_2O$	0.36 $mg.L^{-1}$
$CuSO_4.5H_2O$	0.36 $mg.L^{-1}$
H_3BO_3	6.5 $mg.L^{-1}$
EDTA	10 $mg.L^{-1}$
HCl	37%
Carbon source	Usually 4 $g.L^{-1}$
(Agar)	15 $g.L^{-1}$
Tryptone Yeast extract Glucose (TYG) medium	
Tryptone	1 $g.L^{-1}$
Yeast extract	1 $g.L^{-1}$
D-glucose	0.5 $g.L^{-1}$
KCl	6.34 $g.L^{-1}$
NaCl	1.2 $g.L^{-1}$
$MgSO_4.7H_2O$	0.25 $g.L^{-1}$
K_2HPO_4	0.13 $g.L^{-1}$
$CaCl_2.2H_2O$	0.22 $g.L^{-1}$
K_2SO_4	0.17 $g.L^{-1}$
Na_2SO_4	2.4 $g.L^{-1}$
$NaHCO_3$	0.5 $g.L^{-1}$
Na_2CO_3	0.09 $g.L^{-1}$
Fe EDTA	0.07 $g.L^{-1}$
(Agar)	20 $g.L^{-1}$
pH 7.0	
M408 medium	
Yeast extract	1 $g.L^{-1}$
Mannitol	10 $g.L^{-1}$
K_2HPO_4	0.5 $g.L^{-1}$
$MgSO_4.7H_2O$	0.2 $g.L^{-1}$
NaCl	0.1 $g.L^{-1}$
(Agar)	20 $g.L^{-1}$
pH : 7.0	

1.3. *Arabidopsis thaliana* Columbia-0 (Col-0) ecotype

Seeds of the wild-type *A. thaliana* ecotype Columbia-0 (Col-0) were obtained by initial order to the ABRC stock center (*Arabidopsis* Biological Resource Center, http://abrc.osu.edu/).

1.4. *chs5* mutant (Col-0 genetic background)

Seeds of *chilling-sensitive 5* (*chs5*) mutant in the Col-0 genetic background were obtained by the laboratory from Dr. Koh Iba (Kyushu University, Japan). The *chs5* line was originally isolated from a genetic screen for chilling sensitive mutants. These mutants display a normal wild-type phenotype at 22°C and a chlorotic phenotype at lower temperatures (15°C) (Hugly and Somerville, 1992; Schneider et al., 1995). In the *chs5* line, the exon 8 of the gene coding for the 1-deoxy-D-xylulose 5-phosphate synthase (*DXS1*, At4g15560) is carrying a missense mutation (GAC to AAC) responsible for the change of an aspartic acid (D) to an asparagine (N) residue at position 627 (D627N) in the encoded protein. *chs5* mutant is characterized by a chlorotic phenotype due to a defect in plastidial 1-deoxy-D-xylulose 5-phosphate (DXP) biosynthesis (Araki et al., 2000).

2. Bacterial strains

Bacterial media used for culture and isolation of the strains during my thesis are listed in **table 10**.

2.1. *Pseudomonas syringae* pv. *tomato* DC3000

2.1.1. *Pst*DC3000

Pseudomonas syringae pv. *tomato* DC3000 (*Pst*DC3000) strain was used for infection experiments *in vivo* and *in vitro*, but also in hydroponic culture systems. The strain was obtained from Dr. Isabelle Caldelari (IBMC, Strasbourg, France).

Minimal Medium + methanol (MM + MeOH) medium	
NH_4Cl	1.62 $g.L^{-1}$
$MgSO_4.7H_2O$	0.2 $g.L^{-1}$
K_2HPO_4	2.4 $g.L^{-1}$
$NaH_2PO_4.2H_2O$	1.1 $g.L^{-1}$
Methanol	5 $ml.L^{-1}$
$Na_2EDTA.2H_2O$	15 $mg.L^{-1}$
$FeSO_4.7H_2O$	3.0 $mg.L^{-1}$
$ZnSO_4.7H_2O$	4.5 $mg.L^{-1}$
$CoCl_2.6H_2O$	3.0 $mg.L^{-1}$
$MnCl_2$	0.64 $mg.L^{-1}$
H_3BO_3	1.0 $mg.L^{-1}$
$Na_2MoO_4.2H_2O$	0.4 $mg.L^{-1}$
$CuSO_4.5H_2O$	0.3 $mg.L^{-1}$
$CaCl_2.2H_2O$	3.0 $mg.L^{-1}$
(Agar)	15 $g.L^{-1}$
pH 7.1	
Yeast Extract Mannitol (YEM) medium	
Yeast extract	0.5 $g.L^{-1}$
Mannitol	5 $g.L^{-1}$
K_2HPO_4	0.5 $g.L^{-1}$
$MgSO_4.7H_2O$	0.2 $g.L^{-1}$
NaCl	0.1 $g.L^{-1}$
(Agar)	20 $g.L^{-1}$
pH 7.0	

R2A medium	
Casein acid hydrolysate	0.5 $g.L^{-1}$
Yeast extract	0.5 $g.L^{-1}$
Proteose peptone	0.5 $g.L^{-1}$
Dextrose	0.5 $g.L^{-1}$
Starch	0.5 $g.L^{-1}$
Dipotassium phosphate	0.3 $g.L^{-1}$
Magnesium sulfate	0.024 $g.L^{-1}$
Sodium pyruvate	0.3 $g.L^{-1}$
(Agar)	15 $g.L^{-1}$
pH 7.2	
GYM medium	
Malt extract	10 $g.L^{-1}$
Yeast extract	4 $g.L^{-1}$
Glucose	4 $g.L^{-1}$
$CaCO_3$	4 $g.L^{-1}$
(Agar)	12 $g.L^{-1}$
pH 7.2	
Tap Water Yeast Extract (TWYE) medium	
Yeast extract	0.25 $g.L^{-1}$
K_2HPO_4	0.5 $g.L^{-1}$
(Agar)	18 $g.L^{-1}$
pH 7.0	

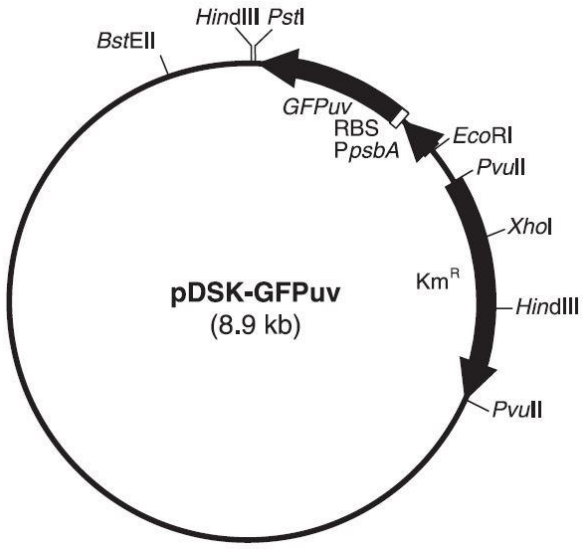


Figure 31. Schematic diagram of the plasmid pDSK-GFPuv. This plasmid can express the green fluorescent protein variant GFPuv at high levels under the constitutive chloroplast promoter psbA (*PpsbA*) and a ribosomal biding site (RBS) from *T7 gene10*. Extracted from Wang *et al.*, 2007

*Pst*DC3000 was cultivated on KB medium supplemented with 50 µg.mL^{-1} rifampicin (Sigma), or on NYGB medium supplemented with 50 µg.mL^{-1} rifampicin (Sigma) at 28°C.

2.1.2. *Pst*DC3000 GFPuv

*Pst*DC3000 GFPuv strain was tested for infection experiments. The plasmid pDSK-GFPuv (**Figure 31**) (Wang et al., 2007) was obtained from Dr. Jiangqi Wen (Noble Research Institute, Ardmore, USA) . The transformation protocol is described above, in paragraph II.3.10.1. *Pst*DC3000 GFPuv was cultivated on KB medium supplemented with 100 µg.mL^{-1} rifampicin (Sigma) and 50 µg.mL^{-1} kanamycin (Sigma) at 28°C.

2.1.3. *Pst*DC3000 lux

*Pst*DC3000 lux strain (Fan et al., 2007) was also tested for infection experiments. The strain was obtained from the laboratory of Chris Lamb (John Innes Centre, Norwich, UK) thanks to Pr. Julia Vorholt (ETH, Zurich, Switzerland). *Pst*DC3000 lux was cultivated on KB medium supplemented with 50 µg.mL^{-1} rifampicin (Sigma) and 25 µg.mL^{-1} kanamycin (Sigma), at 28°C.

2.1.4. *Sphingomonas melonis* sp. FR1 and *Methylobacterium extorquens* PA1

Sphingomonas melonis sp. FR1 and *Methylobacterium extorquens* PA1 were provided by Pr. Julia Vorholt (ETH, Zurich). *Sphingomonas melonis* sp. FR1 was used as a control for its protective effect against *Pst*DC3000 *in vitro* and *Methylobacterium extorquens* PA1 for its absence of effect against *Pst*DC3000 *in vitro* (Innerebner et al., 2011). *Methylobacterium extorquens* PA1 was cultivated in mineral medium with 0.5% succinate as the carbon source at 28°C, and *Sphingomonas melonis* sp. Fr1 was cultivated in NB medium at 28°C.

2.1.5. Other strains

All the other bacterial strains used during my thesis were isolated in the laboratory as described in paragraphs II.4.1 to II.4.3. thanks to the different media listed in **table 10**.

II. Methods

3. Plant culture in soil for seed production

All lines of *A. thaliana* were cultivated in 7 cm diameter pots in soil (LAT-Terra Standard Pikiererde, Hawita). Before sowing, seeds were kept at -20°C for 48 hours. WS2 and *hmg1-1* lines were cultivated in a 12-hour light regime under fluorescent light (6 Lumilux tubes T5, Osram) and 12-hour dark regime. Temperatures were set at 21°C during the light phase and 18°C during the dark phase. Col-0 and *chs5* lines were cultivated in a 16-hour light regime under fluorescent light (6 Lumilux tubes T5, Osram) and 8-hour dark regime. Temperatures were set at 16°C during the light phase and 13°C during the dark phase.

4. Protocols related to the inventory of the communities

4.1. Plant culture in soil for community inventory

Col-0, *chs5*, WS2 and *hmg1-1* were cultivated in 7 cm diameter pots in soil (LAT-Terra Standard Pikiererde, Hawita) and grown under 12-hour light regime under fluorescent light (6 Lumilux tubes T5, Osram) and 12-hour dark regime, until the formation of rosettes of an average size of 7 cm diameter. Temperatures were set at 21°C during the light phase and 18°C during the dark phase.

Table 11. Primers used for 16S rRNA gene amplification and qPCR reactions for the inventory of the communities.

Name of the primer	Sequence	reference
16S RNA amplicon		
799F	5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAACMG GATTAGATACCCKG-3′	Bulgarelli *et al.*, Bodenhausen *et al.,* Schlaeppi *et al.*
799F1	5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTAACMG GATTAGATACCCKG-3′	This work
799F2	5’-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTAACM GGATTAGATACCCKG-3′	This work
799F3	5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCGAAAC MGGATTAGATACCCKG-3′	This work
799F4	5’ –TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATGAA ACMGGATTAGATACCCKG- 3’	This work
799F5	5’-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGCGAA ACMGGATTAGATACCCKG- 3’	This work
799F6	5’ –TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGAGTG GAACMGGATTAGATACCCKG- 3’	This work
799F7	5’ –TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTGT GGAACMGGATTAGATACCCKG- 3’	This work
1193R	5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACGTC ATCCCCACCTTCC-3′	Bulgarelli *et al.*, Bodenhausen *et al.,* Schlaeppi *et al.*
1193R1	5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTACGT CATCCCCACCTTCC-3’	This work
1193R2	5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTACG TCATCCCCACCTTCC-3’	This work
1193R3	5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCGAAC GTCATCCCCACCTTCC-3’	This work
1193R4	5’ –GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATGA ACGTCATCCCCACCTTCC- 3’	This work
1193R5	5’ –GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGCG AACGTCATCCCCACCTTCC- 3’	This work
1193R6	5’ –GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAGT GGACGTCATCCCCACCTTCC- 3’	This work
1193R7	5’ –GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTTG TGGACGTCATCCCCACCTTCC- 3’	This work

Table 12. PCR program for 16S rRNA gene amplification for the inventory of the communities. DNA amplification was performed on a Mastercycler ep Gradient S (Eppendorf) using GoTaq polymerase (Promega).

	Initial denaturation	3 min	95°C
25 cycles	Denaturation	30 sec	95°C
	Priming	30 sec	55°C
	Elongation	30 sec	72°C
	Final elongation	5 min	72°C

4.2. Microbiota profiling

After 6 to 8 weeks, *A. thaliana* plants were extracted from soil, shaken, and roots were shortly washed in sterile distilled water to remove soil. Plant were cut in such a way to separate phyllosphere and roots/rhizosphere. Rhizosphere was separated from the roots by scrapping using a sterile scrapor. The inner root or leaf tissues and their surface were not discriminated and were referred as “root” and “phyllosphere” microbiota, respectively. Samples were crushed using a mortar and pestle and frozen at -20°C until DNA extraction. DNA was extracted from frozen rhizosphere (0.25 g), on one hand, or from powder obtained from phyllosphere and roots (50 mg) on the other hand using the PowerSoil DNA Isolation Kit and the PlantDNA Isolation kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA), respectively, according to the manufacturer’s instructions. The DNA concentration and quality were estimated by measuring the OD at 260 nm and 280 nm on a Nanodrop spectrophotometer (ThermoFisher Scientific).

Libraries were constructed according the 16S Metagenomic Sequencing Library Preparation protocol (Illumina Part # 15044223 Rev. B) except some modifications mentioned below. Briefly, 16S RNA encoding gene were amplified in duplicate from the extracted DNA using the primer listed in **table 11** that target the bacterial/archaeal 16 S rRNA gene variable region 5-6. The primers used for this first PCR were composed of (from 5’ to 3’ ends): 1) the Illumina overhang sequence (containing Read 1 and Read 2 specific sequences) described in the Illumina 16S protocol, 2) two 16S V5-V6 gene-specific sequences, 3) a 0 to 7pb heterogeneity spacer to increase the nucleotide diversity for sequencing, as described in Fadrosh *et al.* (Fadrosh et al., 2014). This first amplification (PCR1, 25 µl) was performed by mixing 25 ng genomic DNA, the KAPA HiFi HotStart ReadyMix PCR Kit (12.5 µl) (Kapabiosystems, Boston, United States) and primers (5 µl at 1 µM) and using the program indicated in **table 12**. PCR products were analyzed on 1% agarose gel to verify the success of amplification and duplicate amplified samples were pooled and purified using AMPure XP beads (Agencourt, Beckman-Coulter). The quantity and quality of these amplicons were controlled with the Bioanalyzer (Agilent). A second amplification (PCR2) was performed using the Nextera XT primers (Illumina) containing the full-length P5 and P7 sequences. Amplicons were purified using AMPure XP beads (Agencourt, Beckman-Coulter). Their size was controlled with the Bioanalyzer (Agilent). These libraries were

normalized, pooled together and 5% PhiX v3 (Illumina) was added. Sequencing was performed as paired end of 300 base pairs reads (2x300) on a Miseq platform.

The bioinformatics processing was performed using the FROGS pipeline under Galaxy environments (Escudié et al., 2018). Shortly, it included a pre-processing of the sequencing read data with “FLASH” (suppress PCR duplicates, too long or too short reads). Then the quality sequences were clustered to operational taxonomic units (OTUs, >97% sequence similarity, minimal coverage of 5 sequences) with “Swarm”. Chimeric OTU sequences were removed using “VSEARCH”. Filtering was performed to keep sequences present in at least X samples and suppress contaminants (phiX). Taxonomic assignments were done using multi-affiliation output with the Silva, Midas and Greengenes databases.

OTUs classified as mitochondrial or Cyanobacteria/chloroplasts sequences were removed. Data from WT and mutant were compared using the Phyloseq pipeline (McMurdie and Holmes, 2013).

5. Protocols related to the infection experiments by the phytopathogen *Pst*DC3000

5.1. Plant culture in soil for infection by *Pst*DC3000 experiments

For infection experiments, *A. thaliana* Col-0, *chs5*, WS2 and *hmg1-1* were cultivated in 7 cm diameter pots in soil (LAT-Terra Standard Pikiererde, Hawita) in growing chambers for about 6 weeks in the same conditions as for the inventory of the communities, under 12-hour photoperiod (6 Lumilux tubes T5, Osram). Temperatures were set at 21°C during the light phase and 18°C during the dark phase. The trays containing the pots were randomly rearranged every week to avoid plant growth heterogeneity. Particular attention was devoted to the use of plants that exhibit a similar size and number of leaves for each independent experiment.

5.2. Infection by *Pst*DC3000

For the infection experiments proceeded at the end of my thesis, NYGB medium replaced the KB medium usually used for *Pst*DC3000 culture since we encountered difficulties to grow the strain on KB medium after changing peptone supplier (Sigma-Aldrich instead of Difco).

The phytopathogen *Pst* DC3000 was streaked out from a -80°C glycerol stock onto a plate of KB medium or NYGB medium supplemented with 50 µg.mL^{-1} of rifampicin (Sigma) (KB+r or NYGB+r, respectively) and grown at 28°C for 2 days. Bacteria were transferred onto a liquid KB+r or NYGB+r culture and grown with shaking at 28°C until exponential growing phase. The culture was centrifuged at 2500 g for 10 minutes to pellet the bacteria. The supernatant was poured off and the bacteria were washed in 10 mM $MgCl_2$ twice. The pellet was resuspended in 10 mM $MgCl_2$ and the OD_{600nm} was adjusted to obtain 1 x 10^5 colony forming units per milliliter (cfu.mL^{-1}). Using a needle-less syringe, 10 leaves of each plant were pressure infiltrated with either 1 x 10^5 cfu.mL^{-1} of *Pst* DC3000, or mock infiltrated with sterile 10 mM $MgCl_2$ (adapted from Katagiri et al., 2002). Suspension of infection was serial diluted and plated on KB+r or NYGB+r for numerations after 2 days of incubation at 28°C.

5.3. Bacterial growth assays

Leaf discs from 10 infiltrated leaves were harvested at 6 days post infection (dpi) and grinded in 1 mL of KB+r or NYGB+r. After serial dilutions in physiological H_2O, samples were plated onto LB+r or NYGB+r agar plates and incubated at 28°C for 2 days until numerations. For each condition, numeration of the colonies was proceeded, and the pathogen titer was determined thanks to the following formula:

$$N = \frac{\sum colonies}{v \times (n_1 + 0.1 \times n_2 + 0.01 \times n_3)} \times \frac{1}{d_1}$$

N: Number of cfu.mL^{-1} ; $\sum$ colonies: total number of colonies counted for all the dilutions; v: volume plated; n_1: number of plates considered for the first dilution; n_2: number of plates considered for the second dilution; n_3: number of plates considered for the third dilution; d_1: first dilution considered.

Statistical analyses of the growth assays were proceeded with R software. Results were compared by Student t-test after verification of normality by Shapiro-Wilk test and the variance equality by Fisher test.

5.4. Total RNA extraction

For the last infection experiment, we decided to analyze the expression of some SAR marker genes. Infiltrated leaves from 9 infected plants and 9 mock-treated plants of *A. thaliana* Col-0 and *chs5* were harvested before infection by *Pst*DC3000 and at 1, 3 and 6 dpi, and grouped in bulks of 3 plants per condition. Plant material was grinded in liquid nitrogen and stored at -80°C until extractions.

1 mL of TRIzol reagent (Molecular Research Center) was added to approximately 60 mg of grinded material in 2 mL tubes containing glass beads before grinding 2 x 30 seconds with Precellys®. Samples were kept at room temperature for 5 minutes before adding 200 µL of chloroform (Sigma-Aldrich) and 15 seconds agitation. Samples were kept at room temperature for 2 minutes and 30 seconds before centrifugation at 4°C, 12 000 g for 15 minutes. 400 µL of supernatant were collected and 333 µL of isopropanol (Sigma-Aldrich) were added to the supernatant. Samples were kept at room temperature for 10 minutes before centrifugation at 4°C, 12 000 g for 20 minutes. The supernatant was poured off and 1 mL of 80% ethanol (Sigma-Aldrich) was added to the samples. Samples were centrifuged at 4°C, 12 000 g for 5 minutes and the supernatant was poured off. 1 mL of 100% ethanol was added to the samples before centrifugation at 4°C, 12 000 g for 5 minutes and elimination of the supernatant. Pellets were air-dried before addition of 50 µL of milliQ H_2O and incubation at 4°C for 30 minutes. Samples were then vortexed and incubated at 50°C for 5 minutes, twice. RNAs were finally stored at -20°C until reverse transcription.

5.5. Reverse transcription (RT)

A DNAse treatment was carried out on the RNAs before reverse transcription. For that, 1 µg of RNA was resuspended in H_2O supplemented with 10 µL of DNAse mix: 0.1 µL RNAse OUT (Promega); 6 µL H_2O; 2 µL DNAse 10X buffer (Promega); 2 µL

Table 13. Primers used for qPCR on *Arabidopsis thaliana* SAR marker genes. Primers for the amplification of target genes were designed on LightCycler Probe Design Software 2.0 (Roche Life Science). Primers for the amplification of reference genes were available on the sequencing platform from the IBMP.

Gene		Sequence (5'→3')	Type
ACT2 (At3g18780)	Forward	CTTGCACCAAGCAGCATGAA	Reference
	Reverse	CCGATCCAGACACTGTACTTCCTT	
GADPH (At1g13440)	Forward	TTGGTGACAACAG<GTCAAGCA	Reference
	Reverse	AAACTTGTCGCTCAATGCAATC	
PR1 (At2g14160.1)	Forward	GGTCACTACACTCAAGTTGTTT	Target
	Reverse	GTTCCACCATTGTTACACCTC	
PR2 (At3g57260.1)	Forward	TGACACCACCACTGATACG	Target
	Reverse	CTCTTATACTCATCCCTGAACCT	
PR5 (At1g75040.1)	Forward	CTGACCTCAACGCGGCTTGC	Target
	Reverse	GGCGTCAGGGCAAGCGTTCT	

Table 14. qPCR program for SAR marker genes expression analyses. DNA amplification was performed on a LightCycler® 480 II Instrument (Roche) using a SYBR® green mix (Roche).

	Initial denaturation	5 min	95°C
45 cycles	Denaturation	10 sec	95°C
	Priming	15 sec	60°C
	Elongation	15 sec	72°C
	Temperature gradient	1 min	55°C to 95°C

DNAse (Promega). The mix was incubated at 37°C for 45 minutes. Then the reaction was stopped by addition of 1 µL of Stop DNAse (EGTA, 20 mM, Promega) and incubation at 65°C for 10 minutes. After 5 minutes incubation on ice, samples were supplemented with 20 µL of RT mix: 6 µL H_2O; 8 µL 5X SuperScript IV buffer (Invitrogen), 2 µL 0.1 M DTT (Invitrogen); 2 µL 10 mM dNTPs (ThermoFisher Scientific); 2 µL 40 µM smart-Oligo-dT (ThermoFisher Scientific), and 0.5 µL of 200 $\mu g.\mu L^{-1}$ SuperScript IV (Invitrogen). Samples were incubated at 50°C for 10 minutes and at 80°C for 10 minutes. cDNAs were diluted by addition of 40 µL H_2O.

5.6. Quantitative PCR (qPCR) analyses

The reaction mix contained 1 µL of cDNA, 5 µL of SYBR® Green (Roche), 2 µL of H_2O, and the couple of primers (2.5 µM) for each gene. Primers for the amplification of target genes were designed with LightCycler Probe Design software 2.0 (Roche). Primers used for the qPCR are listed in **table 13**. Real-time PCR was performed on a LightCycler® 480 II instrument (Roche) following the program indicated in **table 14**.

The relative amount of cDNA corresponding to the transcript level in the sample was calculated using the $2^{-\Delta\Delta Ct}$ (Pfaffl, 2001). The expression level of a gene of interest is normalized with respect to the expression values of the two reference genes, listed in **table 13** and chosen for their stable expression in the studied material. The induction (or repression) factor of the target gene at the T_X time can be calculated with respect to a starting biological condition T_0 set at 1. Technical triplicates were performed for each sample.

5.7. Extraction of chlorophylls and carotenoids

The plant material hitherto stored at -80°C was grinded in freshly prepared 80% acetone (Sigma Aldrich) in water (v/v). Samples were incubated in the dark at 4°C for 24 h. After incubation, 200 µL of the supernatant were transferred to a 96-well microplate (96 Well ELISA Microplates, PS, U-bottom, MICROLON®, Greiner Bio-one). For each sample, 3 wells were prepared for measurement. Optical density was measured for each well at 470 nm, 646 nm and 663 nm on FLUOstar Omega

spectrometer (BMG Labtech). Concentrations of chlorophylls and carotenoids in the samples were determined with the equations given by Lichtenthaler and Buschmann (Lichtenthaler and Buschmann, 2001), with c_a: concentration of chlorophyll a; c_b: concentration of chlorophyll b; $c_{(x+c)}$: concentration of xanthophylls and carotenes.

c_a (µg/mL) = 12.25 A663.2 – 2.79 A646.8

c_b (µg/mL) = 21.50 A646.8 – 5.10 A663.2

$c_{(x+c)}$ (µg/mL) = (1000 A470 – 1.82 c_a – 85.02 c_b)/198

After measurements, acetone was evaporated at 65°C for 1 hour, and samples were lyophilized for further experiments.

5.8. Extraction of total sterols, sterol esters and fatty acids from plant tissues

3 mL of 6% KOH in methanol (Carlo Erba) were added to the lyophilized material to proceed saponification at 70°C for 2 h. After addition of 1.5 mL milliQ H_2O, 1.5 mL of n-hexane (Roth) were added and samples were mixed and centrifuged at 2500 g for 5 minutes. The hexane upper phase was transferred in new tubes. This extraction was performed 3 times for each sample and the 3 hexane phases were pooled and evaporated at 70°C for at least 10 minutes. Acetylation was then performed on the dried residue with 100 µL of toluene (Carlo Erba), 50 µL of acetic anhydride (Fluka) and 30 µL of pyridine (Fluka) in a glass vial at 70°C for 1 hour. After evaporation at 70°C for 30 minutes, samples were resuspended in 300 µL n-hexane (Roth).

5.9. Sterols analyses by gas chromatography coupled to mass spectrometry (GC-MS)

To identify sterols, plant extracts were analyzed by gas chromatography (GC instrument, Agilent 6890) coupled to mass spectrometry (MS analyzer, Agilent 5973) using a HP-5MS column (5% PhenylMethyl Siloxane, 30 m x 250 µm x 0,25 µm, Agilent J&W). 2 µL of sample were injected. The helium flux was 1 $mL.min^{-1}$. The column temperature was hold at 60°C for 1 minute, heated to 200°C with a gradient of 30°C per minute, and then reaching a maximum of 300°C with a gradient of 2°C per minute, for a total run time of 56.33 minutes for each sample. The separated molecules were

ionized by electronic impact at 70 eV. The identification of each species was made by the detection of specific daughter ions obtained after ionization using the NIST database.

5.10. Infection by variant of *Pst*DC3000

5.10.1. Preparation of the *Pst*DC3000 GFPuv strain

Preparation of calcium chloride competent *Escherichia coli* (*E. coli*) cells

5 mL of LB medium were inoculated with *E. coli* cells and grown at 37°C overnight. 100 mL of LB were then inoculated with 1 mL of the preculture and grown at 37°C for 3 hours. Cells were put on ice for 10 minutes and the following step were done at 4°C. Cells were centrifuged at 3500 g for 3 minutes and the supernatant was discarded before resuspension in 10 mL of cold 0.1 M $CaCl_2$. Cells were incubated on ice for 20 minutes and centrifuged at 3500 g for 3 minutes. The supernatant was discarded, and cells were resuspended in 5 mL of cold 0.1 M $CaCl_2$, 15% glycerol, before being divided in 100 µL aliquots. Aliquots are stored at -80°C.

E. coli heat shock transformation

100 µL of competent *E. coli* cells were transformed with 600 ng of plasmid pDSK-GFPuv. After 30 minutes incubation on ice, the heat shock was performed at 42°C for 2 minutes, and the cells were put back on ice. 900 µL of LB medium were added to the tubes before incubation at 37°C for 30 minutes. 100 µL were plated on LB supplemented with 100 $\mu g.mL^{-1}$ kanamycin and incubated overnight at 37°C.

Transfer of the pDSK-GFPuv from *E. coli* to *Pst*DC3000 by conjugation

15 mL of LB medium supplemented with 100 $\mu g.mL^{-1}$ kanamycin were inoculated with the donor, *E. coli* containing pDSK-GFPuv and grown at 37°C overnight. 15 mL of LB medium supplemented with 100 $\mu g.mL^{-1}$ kanamycin were inoculated with the helper, *E. coli* DH5α containing pRK2013 and grown at 37°C overnight. 15 mL of KB

medium supplemented with 50 µg.mL^{-1} rifampicin were inoculated with the receiver, *Pst*DC3000. Cultures were centrifuged and resuspended in the appropriate media. 0.5 mL of helper culture were added to the donor culture and resuspended gently. 2 drops spots of donor plus helper culture were plated on LB medium and dried. 2 drops spots of receiver culture were added to the previous spots of donor and dried. Plates were incubated at 37°C overnight. 1 mL of sterile physiological water was added on the plate to recover the maximum of bacteria with a pipette. 100 µL of the recovered liquid was plated on KB medium supplemented with 50 µg.mL^{-1} rifampicin and 100 µg.mL^{-1} kanamycin and incubated at 28°C overnight. Liquid culture in KB medium supplemented with 50 µg.mL^{-1} rifampicin and 100 µg.mL^{-1} kanamycin was inoculated with a colony and incubated at 28°C overnight. A glycerol stock was done and stored at -80°C.

Verification of the plasmid integration in *Pst*DC3000

Extraction of the plasmid integrated in *Pst*DC3000 was proceeded thanks to the kit NucleoSpin® Plasmid (Macherey-Nagel) following the supplier's recommendations. An enzymatic digestion of pDSK-GFPuv has been proceeded using FastDigest Eco RI (ThermoFisher scientific). The mix contained 2 µL of FastDigest buffer (ThermoFisher Scientific), 5 µL of DNA, 12 µL of H_2O, and 1 µL of Eco RI (ThermoFisher Scientific), and incubated at 37°C for 10 minutes. Digested fragment was analyzed on 1.5% agarose gel in 1X TAE.

5.10.2. Infection by *Pst*DC3000 GFPuv or *Pst*DC3000 lux

The protocol of infection was the same as described for the classic *Pst*DC3000 strain in paragraph II.3.2., except for the culture media. *Pst*DC3000 GFPuv was cultivated on KB medium supplemented with 100 µg.mL^{-1} rifampicin and 50 µg.mL^{-1} kanamycin, at 28°C. *Pst*DC3000 lux was cultivated on KB medium supplemented with 50 µg.mL^{-1} rifampicin and 25 µg.mL^{-1} kanamycin, at 28°C.

5.10.3. Detection of *Pst*DC3000 GFPuv fluorescence

Infected leaves were grinded in KB+r or in PBS 1X (140 mM NaCl; 2.7 mM KCl; 10 mM Na_2HPO_4; 1.8 mM KH_2PO_4; pH 7.4). The fluorescence of each sample was quantified using a FLUOstar Omega spectrometer (BMG Labtech) with a black 96-well microplate (96 well cell culture microplate, PS, F-bottom, CELLSTAR®, Greiner Bio-one) containing 200 µL of sample. The excitation filter was 485 nm and the emission filter 520 nm.

5.10.4. Detection of *Pst*DC3000 lux luminescence

Infected leaves were grinded in KB+r. The luminescence of each samples was quantified using a FLUOstar Omega spectrometer (BMG Labtech) with a white 96-well microplate (96 well microplate, PS, F-bottom, Nunc®, ThermoFisher Scientific) containing 200 µL of sample.

6. Protocols relative to bacteria isolation

6.1. Isolation of bacteria from the plant (Bai et al., 2015)

Wild-type Col-0 or WS-2 *A. thaliana* were cultivated in in 7 cm diameter pots in soil (LAT-Terra Standard Pikiererde, Hawita) until the end of their lifecycle in a 12-hour light regime under fluorescent light (6 Lumilux tubes T5, Osram) and 12-hour dark regime. Temperature were set at 21°C during the light phase and 18°C during the dark phase. Plants were removed from the soil, and the excess of soil was removed by manual agitation. Roots were washed in sterile physiological H_2O and the residual soil was eliminated using a sterile rake. Roots were then washed twice in sterile PBS for 20 minutes, and grinding was proceeded using 3 mm diameter beads and Disruptor Genie (Scientific Industries) at 1500 g for 2 minutes. Samples were plated on different media whose composition is given in **table 10**: TYG, YEM, TWYE and M408. For the phyllosphere, 6 leaves were grinded in PBS using a Potter, and the rest of the phyllosphere was grinded using a mortar and a pestle under sterile conditions.

Samples were plated on different media whose composition is given in **table 10**: MYX, MM + MeOH. Plates were incubated at 28°C until the apparition of colonies (2 days to 2 weeks). Colonies were isolated by successive subculturing. Isolated bacteria were then cultivated in the corresponding liquid media until their exponential growing phase to create a glycerol stock for their conservation at -80°C.

6.2. Isolation of bacteria from the soil

5 g of soil were mixed with PBS supplemented with 0.02% Silwet-77, or with 50X basal salts solution (23.2 mM Na_2SO_4; 170.3 mM $(NH_4)_2SO_4$; 33.5 mM KCl; 101.4 mM $MgSO_4.7H_2O$; 18.4 mM KH_2PO_4; 3 mM $Ca(NO_3)_2.4H_2O$) in a total volume of 50 mL. Samples were kept under agitation at 4°C overnight. After 10 minutes of decantation, 7.5 mL of supernatant were added in new tubes containing 17.5 mL Nycodenz®, at the surface of the liquid. Tubes were centrifuged at 10 000 g for 2 hours. The upper phase containing the bacterial cells was transferred in a new tube before addition of 2 volumes of PBS + Silwet-77 or basal salt solution. Samples were then centrifuged at 4°C, 10 000 g for 15 minutes. After centrifugation, the supernatant was poured off and the pellets were resuspended in 1 mL of physiological H_2O and transferred in Eppendorf tubes. Serial dilutions were made and plated on TWYE medium (**Table 10**) and LB medium for incubation at 28°C. Colonies were isolated by successive subculturing. Isolated bacteria were then cultivated in the corresponding liquid media until their exponential growing phase to make a glycerol stock for their conservation at -80°C.

6.3. Isolation of bacteria from the seeds (Truyens et al., 2013)

Seeds were sterilized in 0.1% sodium hypochlorite supplemented with 0.1% Tween 80 (Sigma-Aldrich) for 1 minute. Seeds were then washed in sterile distilled H_2O. Surface sterility was checked by incubation of the last washing solution on 869 (Mergeay et al., 1985) solid medium (10 $g.L^{-1}$ tryptone; 5 $g.L^{-1}$ yeast extract; 5 $g.L^{-1}$ NaCl; 1 $g.L^{-1}$ D-Glucose; 0.345 $g.L^{-1}$ $CaCl_2.2H_2O$ (pH 7)). Sterile seeds were then

Table 15. PCR program for the amplification of the 16S rRNA gene of the isolated strains. DNA amplification was performed on a Mastercycler ep Gradient S (Eppendorf) using Phusion polymerase (ThermoFisher Scientific).

	Initial denaturation	30 sec	98°C
32 cycles	Denaturation	10 sec	98°C
	Priming	15 sec	55°C
	Elongation	1 min 30	72°C
	Final elongation	5 min	72°C

grinded in a sterile mortar after adding 500 µL of 10 mM $MgSO_4$. Dilutions from 0 to 10^{-2} were plated on 1/10 869 medium in distilled H_2O and incubated for 1 week at 30°C.

6.4. Identification of the isolated bacteria

Extraction of total genomic DNA (gDNA)

3 mL of overnight culture was centrifuged at 13 000 g for 2 minutes before elimination of the supernatant. gDNA from pellets was extracted using "Wizard® Genomic DNA Purification Kit" (Promega), following the supplier's recommendations mentioned in the "Isolating genomic DNA from Gram positive and Gram negative bacteria" protocol. Once the gDNA extracted, it was resuspended in 50 µL of milliQ H_2O and the DNA concentration and quality were estimated by measuring the OD at 260 nm and 280 nm on a Nanodrop spectrophotometer (ThermoFisher Scientific).

16S rRNA gene amplification by PCR

Polymerase chain reaction (PCR) was performed for 16S rRNA gene amplification in order to identify isolated bacteria. 16S rRNA gene was amplified using the following primers: 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492r (5'-TACGGYTACCTTG TTACGACTT-3'). PCR was performed using Phusion High Fidelity DNA polymerase (ThermoFisher Scientific). For each reaction, the mix was composed of 150 ng of matrix DNA, 0.5 µM of each primer, 200 µM of each dNTP, 1X of Phusion HF Buffer, 1 U of Phusion polymerase, H_2O up to 50 µL. PCR amplification was performed on a Mastercycler ep Gradient S (Eppendorf), in the conditions indicated in **table 15**.

PCR fragments purification

Purification of the amplified fragments was proceeded using homemade purification beads following the AMpure XP (Agencourt) recommendations.

To prepare the home-made purification beads, Sera-mag SpeedBeads (Sigma-Aldrich) were vortexed and before the transfer of 1 mL to a 1.5 mL tube. The tube was placed on a magnetic rack for 2 minutes and the supernatant was poured off. To clean the beads, 1 mL of TE (10 mM Tris-HCl pH 7.5; 1 mM EDTA) was added to resuspend

the beads by pipetting and the tube was placed on a magnetic rack for 2 minutes before elimination of the supernatant. This step was repeated a second time. Beads were resuspended in 1 mL of TE and kept at room temperature until the next step. In a 50 mL Falcon tube, 9 g of PEG-8000, 2.92 g of NaCl, 500 µL of 1 M Tris-HCl and 100 µM of 0.5 M EDTA were added, and the volume was adjusted to 48 mL with nuclease free H_2O. The solution was mixed until it become clear, and 25 µL of Tween 20 (Sigma-Aldrich) were added to the tube. Then, beads were resuspended in TE by pipetting before they were transferred in the Falcon tube containing the 48 mL of solution. The volume was adjusted to 50 mL by addition of nuclease free H_2O. After homogenization, the volume was distributed in aliquots that were stored at 4°C in the dark.

PCR fragments analyses

Amplified PCR fragments were analyzed on 1.5% agarose gel in 1X TAE, using a 6X loading Dye Solution (Fermentas) and a MassRuler DNA ladder mix (ThermoFisher Scientific) as a size marker. DNA was stained with ethidium bromide (BET) and revealed on a UV transilluminator.

Amplified fragments were then sequenced at the IBMP sequencing platform by the Sanger method. For a total recovery of the 16S rDNA sequence, the following primers were used: 27f (5'-AGAGTTTGATCMTGGCTCAG-3'), 1193r (5'- ACGTCATCCCCAC CTTCC-3'), and 1492r (5'-TACGGYTACCTTGTTACGACTT-3'). Assembly of the sequences was proceeded using "CAP3 sequence assembly program" (http://doua.prabi.fr/software/cap3 (Huang and Madan, 1999)). Taxonomic affiliations were proceeded by comparison of the 16S rRNA gene sequence with the Silva database (https://www.arb-silva.de/ (Pruesse et al., 2012)). Sequences were also compared with the NCBI database by BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and phylogenetic trees have been made for some strains using phylogeny.fr (http://www.phylogeny.fr/simple_phylogeny.cgi (Dereeper et al., 2008)).

6.5. Comparison of the 16S rRNA gene sequences of the varying clusters with those of the strains from our collection

To determine which bacteria from our strain collection could be interesting for further studies, 16S rRNA gene sequences of the isolated strains were compared to 16S rRNA gene sequences of clusters whose abundance was variable between wild-type and mutants. An alignment of the sequences was made for each compartment in MUSCLE (https://www.ebi.ac.uk/Tools/msa/muscle/ (Edgar, 2004)). A matrix exhibiting the percentage of similarity between the sequences was obtained and analyzed in Excel. A selection of the sequences showing more than 99% of similarity was made to guide the choice of the strains to test, with a particular attention for strains with 100% of sequence identity.

7. Protocols related to the study of 5 selected strains

7.1. Fatty acids analyses by GC/FID

Fatty acids extraction

Bacterial fatty acids were analyzed by gas chromatography coupled to flame ionization detection (GC/FID). For that purpose, extraction of the membrane lipids was proceeded (Bligh and Dyer, 1959; Morrison and Smith, 1964). Bacteria were cultivated in 20 mL of LB medium until the end of their exponential growing phase. They were pelleted and resuspended in 0.5 mL H_2O; before addition of 1 mL methanol and 2 mL chloroform. Samples were shaken for 3 minutes before addition of 0.5 mL H_2O and an additional shaking for 30 seconds. Samples were centrifuged for 10 minutes at 1 000 g. the chloroform phase (lower liquid phase) was transferred in a glass vial and the liquid was removed under N_2-gasstream. 0.6 mL BF_3 in methanol were added to the dry sample to methylate the fatty acids to fatty acid methyl esters (FAME). Samples were incubated at 80°C for 15 minutes before addition of 0.3 mL H_2O and 0.5 mL hexane and shaking for 1 minute. The hexane phase (upper phase) was transferred to a new vial for GC analyses.

FAME analyses

The FAME were analyzed by gas chromatography coupled to a flame ionization detector (GC instrument, Agilent 6890N) using a CP-SIL 88 column (50 m x 250 µm x 0.25 µm, Agilent). 2 µL of sample were injected. The helium flux was 1 $mL.min^{-1}$. The column temperature was hold at 40°C for 2 minutes, followed by an increase of 8°C per minute up to 220°C, with a final hold at 220°C for 10 minutes. The peak areas were used to determine the relative amounts of each fatty acid. The degree of saturation of membrane fatty acids was defined as the ratio of saturated C16:0 and C18:0 to unsaturated C16:1 and C18:1 fatty acids. The *trans*/*cis* ratio of unsaturated fatty acids was defined as the ratio between the amounts of the two *trans* unsaturated fatty acids (16:1 *trans*, 18:1 *trans*) and the two *cis* unsaturated fatty acids (16:1 *cis*, 18:1 *cis*).

7.2. Growth on mineral medium supplemented with isoprenoids

Strains of interest were tested for their capacity to use isoprenoids as carbon source to grow in liquid or on solid mineral medium (**table 10**) supplemented with different isoprenoids as a carbon source, at different concentrations: 100 $mg.L^{-1}$, 250 $mg.L^{-1}$ and 500 $mg.L^{-1}$. As a vitamin supply, 200 $mg.L^{-1}$ of yeast extract were added to the medium. Isoprenoids tested were obtained from Dr. Nicolas Navrot (IBMP) and were the following: geraniol (Fluka), α-pinene (Sigma-Aldrich), β-ocimene (Fluka), farnesol (Fluka), (-)-linalool (Fluka), β-caryophyllene (not commercial), limonene (not commercial), α-humulene (not commercial), myrcene (not commercial) and thujopsen (not commercial).

Each testes strain was first grown in the appropriate culture medium until the exponential growing phase. The culture was centrifuged at 2500 g for 10 minutes and the supernatant was poured off. The pellet was washed with sterile 10 mM $MgCl_2$ and centrifuged at 2500 g for 10 minutes. The supernatant was poured off and the pellet was resuspended in sterile 10 mM $MgCl_2$ before adjusting the OD_{600nm} to 0.5. Serial dilutions were made in sterile 10 mM $MgCl_2$ until 10^{-4}. For each bacteria, a 5 µL drop was deposited on each medium.

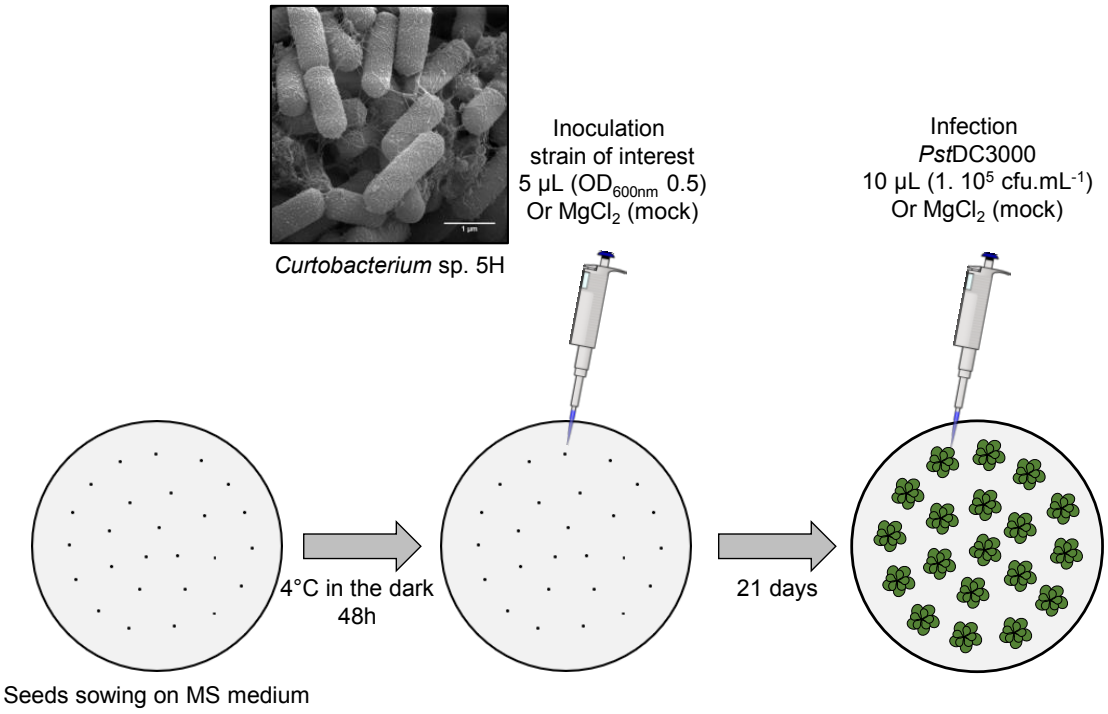


Figure 32. Pipeline of the *in vitro* experiments. *Curtobacterium* sp. 5H is given as an example of inoculated strain. Microscopy image was taken by Mathieu Erhardt (IBMP; microscopy platform). After 48h of stratification, seeds were pipette-inoculated with the strain of interest (OD_{600nm} 0.5). Infection was proceeded on 3-weeks old plants. Plants were observed day by day until the apparition of symptoms following the infection by *Pst*DC3000.

7.3. Inoculation of isolated strains and infection on plants cultivated in gnotoxenic conditions

The global protocol relative to the *in vitro* inoculation experiments is schematized in **figure 32**.

MS medium: 4.3 $g.L^{-1}$ Murashige & Skoog Medium M0221 (Duchefa Biochemie); 10 $g.L^{-1}$ sucrose (Euromedex); 100 $mg.L^{-1}$ myo-inositol (Duchefa Biochemie); 1 $mg.L^{-1}$ thiamine HCl (Duchefa); 0.5 $mg.L^{-1}$ pyridoxine HCl (Duchefa); 0.5 $mg.L^{-1}$ nicotinic acid (Duchefa); 7 $g.L^{-1}$ agar (Sigma); pH 5.8.

7.3.1. *In vitro* culture conditions

Autoclaved MS medium was poured in culture boxes. *A. thaliana* seeds were surface sterilized by treating them with 70% ethanol for 2 minutes before a washing step in sterile milliQ H_20 for 1 minute. The supernatant was removed, and the seeds were treated with a commercial sodium hypochlorite solution (4%) supplemented with 0.1% Tween 20 (Sigma-Aldrich) for 5 minutes. Seeds were washed 8 times with sterile milliQ H_2O. Then, they were placed on the surface of MS medium. Plates were placed at 4°C for 48h in the dark. Then, plants were cultivated in Sanyo MLR-351H incubator with a regime of 16-hour light (160 µmol photon.s^{-1}.m^{-2}) at 18°C and 8-hour light (100 µmol photon.s^{-1}.m^{-2}) at 16°C. Or they were cultivated in a culture chamber with a 16-hour light regime under fluorescent light (4 Lumilux tubes T8, Osram) and 8-hour dark regime, with temperatures set at 20.5°C during the light phase and 17°C during the dark phase. After 2 weeks of culture, plants of similar sizes were transplanted onto new plates.

7.3.2. Inoculation of the strains of interest

Each strain of interest was cultivated in the appropriate culture media at 28°C until the exponential growing phase. The culture was centrifuged at 2500 g for 10 minutes and the supernatant was poured off. The pellet was washed in sterile 10 mM $MgCl_2$ and centrifuged at 2500 g for 10 minutes. The supernatant was poured off and the

Table 16. Composition of the Hoagland medium for hydroponic culture.

$Ca(NO_3)_2.4H_2O$	$4{,}03.10^{-3}$ mol.L^{-1}
$NH_4H_2PO_4$	$5{,}22.10^{-4}$ mol.L^{-1}
KNO_3	$6{,}04.10^{-3}$ mol.L^{-1}
$MgSO_4.7H_2O$	$1{,}99.10^{-3}$ mol.L^{-1}
NaOH	$1{,}25.10^{-4}$ mol.L^{-1}
EDTA	$8{,}92.10^{-5}$ mol.L^{-1}
$FeSO_4.7H_2O$	$8{,}96.10^{-5}$ mol.L^{-1}
H_3BO_3	$9{,}68.10^{-6}$ mol.L^{-1}
$MnCl_2.4H_2O$	$2{,}03.10^{-6}$ mol.L^{-1}
$ZnSO_4.7H_2O$	$3{,}14.10^{-7}$ mol.L^{-1}
$CuSO_4.5H_2O$	$2{,}10.10^{-7}$ mol.L^{-1}
MoO_3	$1{,}39.10^{-7}$ mol.L^{-1}
$CoCl_2$	$8{,}59.10^{-8}$ mol.L^{-1}

pellet was resuspended in sterile 10 mM $MgCl_2$. For seed inoculation, the bacterial suspension OD_{600nm} was adjusted to 0.5 and 5 µL were pipette inoculated on each seed after 48 hours at 4°C in the dark. Mock were inoculated with 5 µL of sterile 10 mM $MgCl_2$. For seedling inoculation on 2-weeks old plants, OD_{600nm} was adjusted to 0.02 and 5 µL were pipette inoculated on each plant. Mock were inoculated with 5 µL of sterile 10 mM $MgCl_2$.

7.3.3. Infection by *Pst*DC3000

3-weeks old plants were infected by *Pst*DC3000. *Pst*DC3000 was cultivated in the appropriate culture media at 28°C until the exponential growing phase. The culture was centrifuged at 2500 g for 10 minutes and the supernatant was poured off. The pellet was washed in sterile 10 mM $MgCl_2$ and centrifuged at 2500 g for 10 minutes, twice. The pellet was resuspended in sterile 10 mM $MgCl_2$ and the OD_{600nm} was adjusted to obtain 1 x 10^5 cfu.mL^{-1}. For preliminary experiments, 800 µL of *Pst*DC3000 were sprayed to each plant. For further experiments, 10 µL of the infection suspension were pipette inoculated on the plants to allow a more uniform distribution. Mock were inoculated with 10 µL of sterile 10 mM $MgCl_2$.

7.3.4. Optimized hydroponic culture conditions

A hydroponic culture system was optimized in the laboratory in order to cultivate sterile plants until formation of the siliques.

For that purpose, *A. thaliana* seeds were surface sterilized by treating them with 70% ethanol for 2 minutes before a washing step in sterile milliQ H_20 for 1 minute. The supernatant was poured off, and the seeds were treated with a commercial bleach solution containing 4% sodium hypochlorite (Lacroix) supplemented with 0.1% Tween 20 (Sigma-Aldrich) for 5 minutes. Seeds were washed 8 times with sterile milliQ H_2O. A reusable autoclavable pipette tips box was used to create a nursery for the plants. 0.6 mL Eppendorf® tubes were cut at the basis to let the roots join the liquid medium and put inside this sterile box. They were filled with Hoagland medium (**Table 16**) supplemented with 0.6% agar and the box was filled with liquid Hoagland medium.

Sterile seeds were deposited on the surface of the solid medium using sterile toothpicks. The box was placed in a culture chamber with a 16-hour light regime at 20.5°C and a 8-hour dark regime at 17°C. Big boxes covered by glass containers were used to grow plants in hydroponic culture conditions after sterilization using commercial sodium hypochlorite solution (4%), followed by a wash with ethanol 70% under sterile conditions. Glass containers contain a hole that was recovered with sterile breathable self-adhesive films (Starlab) to allow gas exchange by avoiding contamination. After approximately 4 weeks in the nursery until the roots have reached the liquid culture medium, Eppendorf® tubes containing the plants were transferred in these bigger boxes containing Hoagland medium, using sterile tweezers. The system was closed with micropore and put back in the culture chamber. Sterility was checked by the presence of a LB plate in the system, and plating of 100 µL of Hoagland medium on LB plates for incubation at 28°C.

The inoculation of *Curtobacterium* sp. 5H was proceeded according to the protocol described in paragraph II.5.3.2. on 2-weeks old plants, except that 10 µL were pipette inoculated instead of 5 µL.

7.3.5. Comparison of two seeds sterilization methods for axenic experiments

The sterilization protocol usually employed for seed sterilization in my experiments requires a first washing step in 70% ethanol for 2 minutes, followed by a washing step in sterile milliQ H_2O for 1 minute. Then, seeds were washed in a commercial bleach solution containing 4% sodium hypochlorite (Lacroix) supplemented with 0.01% of Tween 20 (Sigma-Aldrich) for 5 minutes, before 8 washing steps in sterile milliQ H_2O. Seeds were air-dried in a sterile environment on sterile Whatman paper.

The second sterilization protocol requires a first washing step in 70% ethanol for 1 minute, followed by a washing step in a sterilization solution containing 4% of commercial bleach supplemented with 0.1% SDS for 5 minutes, before 3 washing steps in sterile milliQ H_2O. Seeds were air-dried in a sterile environment on sterile Whatman paper.

Sterilization efficiency was assessed by placing seeds on MS solid medium and in different liquid culture media: LB, NYGB and GYM whose composition is given in **table 10**, and YPD (10 $g.L^{-1}$ yeast extract; 20 $g.L^{-1}$ proteose peptone; 20 $g.L^{-1}$ D(+)-glucose) before incubation at 28°C and 37°C.

7.4. Inoculation of *Curtobacterium* sp. 5H and infection on plants in holoxenic conditions

7.4.1. *In vivo* culture conditions

Before sowing, Col-0 and *chs5* seeds were kept at -20°C for 48 hours. Plants were cultivated in 7 cm diameter pots in soil (LAT-Terra Standard Pikiererde, Hawita). The culture conditions for *in vivo* inoculation and further pathogen infection experiments were the same as mentioned in paragraph II.3.1 and II.3.2. Plants were cultivated in small greenhouses to avoid bacterial propagation to non-inoculated plants. For both methods of inoculation, plants were transplanted after 2 weeks of culture.

7.4.2. Inoculation of *Curtobacterium* sp. 5H

Curtobacterium sp. 5H was cultivated in LB at 28°C until the exponential growing phase. The culture was centrifuged at 2500 g for 10 minutes and the supernatant was poured off. The pellet was washed in sterile 10 mM $MgCl_2$ and centrifuged at 2500 g for 10 minutes. The supernatant was poured off and the pellet was resuspended in sterile 10 mM $MgCl_2$. For seed inoculation, OD_{600nm} was adjusted to 0.5 and seeds were immersed in the bacterial suspension for 15 minutes. Then, tubes were centrifuged, and the supernatant was poured off. Seeds were resuspended in 1 mL of sterile H_2O before sowing. For seedling inoculation on 2-weeks old plants, OD_{600nm} was adjusted to 1 and 5 mL of suspension were dispensed to each pot containing one plant. For both inoculation methods, mock treated plants were inoculated with the same quantity of sterile 10mM $MgCl_2$.

7.4.3. Infection by *Pst*DC3000

6-weeks old plants were infected by *Pst*DC3000. *Pst*DC3000 was cultivated in NYGB+r at 28°C until the exponential growing phase. The culture was centrifuged at 2500 g for 10 minutes and the supernatant was poured off. The pellet was washed in sterile 10 mM $MgCl_2$ and centrifuged at 2500 g for 10 minutes, twice. The pellet was resuspended in sterile 10 mM $MgCl_2$ and the OD_{600nm} was adjusted to obtain 1 x 10^5 cfu.mL^{-1}. 10 leaves of each plant were syringe infiltrated. Mock were syringe infiltrated with sterile 10 mM $MgCl_2$. Bacterial growth assays were proceeded as explained in paragraph II.3.3.

8. Protocols related to the screening of *DXS1* overexpressors

8.1. Plant culture conditions

Col-0 and *chs5* lines were cultivated in 7 cm diameter pots in soil (LAT-Terra Standard Pikiererde, Hawita). They were cultivated in a 16-hour light regime under fluorescent tubes (6 Lumilux tubes T5, Osram) and 8-hour dark regime. Temperatures were set at 16°C during the light phase and 13°C during the dark phase.

8.2. gDNA extraction from leaves

Edwards buffer: 200 mM Tris-HCl pH 7.5; 250 mM NaCl; 25 mM EDTA; 0.5% SDS.

Leaf discs of approximately 0.5 cm diameter were collected and placed in the wells of a 96-well plate containing metal beads. The plate was placed in liquid nitrogen and the samples were grinded with TissueLyser II (Qiagen), at 30 Hz for 1 minute, twice. The plate was kept at room temperature for 5 minutes. 300 µL of Edwards buffer were added to each well and the plate was inverted twice and kept on ice for the extraction. The plate was centrifuged at 16 000 g for 10 minutes at 4°C. 100 µL of supernatant was added to a new 96-well plate containing 80 µL of isopropanol. The plate was inverted twice and kept 5 minutes at room temperature before centrifugation at 16 000 g for 15 minutes at 4°C. The supernatant was removed, and the pellets were

Table 17. PCR and HRM program for *DXS1* genotyping. DNA amplification and HRM were performed on a LightCycler® 480 II instrument (Roche) a Mastercycler ep Gradient S (Eppendorf) using GoTaq polymerase (Promega).

	Initial denaturation	2 min	95°C
x 45 cycles	Denaturation	10 sec	95°C
	Annealing/extension	30 sec	60°C
HRM melting curve	Heteroduplex formation	30 sec	95°C
		1 min	60°C
	High resolution melting + plate read	10 sec	65°C
		0.02°C per second until 95°C	

Table 18. PCR program for 35S::DXS1^{OE} genotyping. DNA amplification was performed on a Mastercycler ep Gradient S (Eppendorf) using GoTaq polymerase (Promega).

	Initial denaturation	1 min	96°C
35 cycles	Denaturation	20 sec	96°C
	Priming	20 sec	50°C
	Elongation	45 sec	72°C
	Final elongation	1 min	72°C

washed with 70% ethanol. Samples were centrifuged for 15 minutes at 4°C and the supernatant was removed. The plate was air dried before resuspension of the pellets in 60 µL of H_2O.

8.3. Genotyping of *DXS1* by HRM

Genotyping of *DXS1* was proceeded by HRM on the extracted DNA using the following primers: CHS5-fw (5'-TAACTGTAGCGGATGCACG-3') and CHS5-rv (5'-GCTAAGCTGCGAATGAGAGg-3'). For each reaction, the mix was composed of 5 µL of Precision Melt Supermix (Bio-rad), 1 µL of 2 µM primers, and 4 µL of gDNA. The precision Melt Supermix (Bio-rad) contains hot-start iTaq™ DNA polymerase, dNTPs, $MgCl_2$, EvaGreen Dye, enhancers and stabilizers in a proprietary formulation optimized by Bio-rad for HRM applications. PCR and HRM were performed on LightCycler® 480 II instrument (Roche) with the program described in **table 17**.

Melting curves were analyzed on https://www.dna.utah.edu/ua/uanalyze.html.

8.4. PCR on 35S:DXS1^{OE}

Plants exhibiting a wild-type phenotype despite the presence of the *dxs1* mutation were controlled for the integration of the 35S:DXS1^{OE} using the following primers: P35Sf (5'-CAATCCCACTATCCTTCGC-3') and cDNA DXS1r (5'-GGCTTCATAAGCC TGTCCTG-3'). PCR was performed using GoTaq polymerase (Promega). For each reaction, the mix was composed of 2 µL of 5X GoTaq green buffer (Promega), 0.6 µL of 25 mM $MgCl_2$, 0.2 µL of 10 mM dNTPs, 0.1 µL of GoTaq polymerase (Promega), 0.5 µL of 10 µM forward primer, 0.5 µL of 10 µM reverse primer, 0.5 µL of DNA matrix, and H_2O up to 10 µL. PCR amplification was performed on a Mastercycler ep Gradient S (Eppendorf), in the conditions indicated in **table 18.**

9. Observation of *Curtobacterium* sp. 5H by electron microscopy

Curtobacterium sp. 5H was cultivated until the exponential growing phase. Mathieu Erhardt (Microscopy platform, IBMP) proceeded the fixation of the bacteria in glutaraldehyde 2% or 2.5% + phosphate buffer (Sorensen), pH 7.4 for 1 hour. The sample was washed in phosphate buffer (Sorensen) for 15 minutes, 3 times. Post-fixation of the sample was proceeded in 1% osmium tetroxide (OSO_4) in 0.1 M phosphate buffer (Sorensen) for 1 hour. The sample was washed in H_2O for 10 minutes, three times. Dehydration was proceeded in a series of alcohol baths (50%, 70%, 95% ethanol) 15 minutes each, and 3 baths of 30 minutes in 100% ethanol. Then, sample was placed in a bath of 100% ethanol + Hexamethyldisilazane (HMDS), 50:50, for 15 minutes. Bacteria were placed on a glass slide before a final bath in 100% HDMS was proceeded until evaporation overnight (desiccation). Sample became conductor under electron beam. Glass slide was attached with carbon tape on the sample part of the Scanning electron microscope (SEM) and objects were metallized under argon atmosphere, with deposition of a gold/palladium layer of few nanometers thick (20 mA, 240 seconds). Sample was observed on a Zeiss Sigma VP300 SEM under a tension of 15 kV.

Résumé du travail de thèse en français

I. Introduction

Les isoprénoïdes, aussi appelés terpénoïdes, constituent une importante classe de plus de 55 000 molécules que l'on retrouve chez tous les organismes vivants, y compris en grande diversité dans le monde végétal (Thulasiram et al., 2007). Chez les plantes, ces molécules sont impliquées dans des processus biologiques essentiels tels que la photosynthèse, la croissance, la respiration ou encore la réponse immunitaire.

Afin de synthétiser cette grande variété de molécules, deux précurseurs sont requis. Il s'agit de l'isopentenyl diphosphate (IPP) et du diméthylallyl pyrophosphate (DMAPP). Deux voies de biosynthèse permettant d'obtenir ces précurseurs sont connues à ce jour. Les animaux, les champignons et les archaea synthétisent leurs isoprénoïdes via la voie du mévalonate (MVA), tandis que les algues possèdent la voie du 2-C-méthyl-D-rythritol 4-phosphate (MEP). Les plantes supérieures, quant à elles, ont la particularité d'utiliser les deux voies de biosynthèse pour former l'IPP et le DMAPP. Elles ont en effet maintenu la voie du MVA considérée comme « classique » chez les eucaryotes, qui permet la synthèse des isoprénoïdes dans le cytoplasme ; et elles ont acquis plus tardivement la voie dite « alternative » du MEP qui permet leur biosynthèse dans les plastes (Rohmer, 1999, 2007). Les bactéries, pour leur part, synthétisent principalement leurs isoprénoïdes via la voie du MEP, mais certaines d'entre elles utilisent plutôt la voie du MVA, et parfois même les deux. Enfin, quelques exceptions ne possèdent aucune des voies de biosynthèse, probablement car il s'agit de parasites intracellulaires obligatoires qui peuvent donc obtenir leurs isoprénoïdes grâce à leur hôte (Kuzuyama and Seto, 2003; Pérez-Gil and Rodríguez-Concepción, 2013).

Au cours de ma thèse, j'ai travaillé sur la plante modèle *Arabidopsis thaliana*, qui possède les deux voies de biosynthèse des isoprénoïdes. Afin d'étudier de plus près l'impact de l'une ou l'autre de ces voies sur les interactions entre plantes et bactéries, j'ai travaillé sur des mutants affectés dans la voie du MEP ou du MVA. Le mutant *chs5* (fond génétique Col-0) qui présente un phénotype chlorotique est affecté au niveau d'une enzyme clé de la voie du MEP. En effet, il porte une mutation ponctuelle dans le gène codant la 1-désoxy-D-xylulose 5-phosphate synthase 1 (DXS1) nécessaire à la formation du MEP. Le mutant *hmg1-1* (fond génétique WS2) qui lui se caractérise par une diminution de croissance de la plante et du remplissage des siliques est affecté

au niveau d'une enzyme clé de la voie du MVA. L'allèle *hmg1-1* du gène codant la 3-hydroxy-3-méthylglutaryl-coenzyme A réductase 1 (HMGR1) nécessaire à la formation du MVA est porteur d'une insertion de T-DNA invalidante chez ce mutant. Dans les deux cas, il en résulte un déficit partiel de formation de l'IPP et du DMAPP, précurseurs nécessaires à la biosynthèse de tous les isoprénoïdes.

De récentes études ont suggéré que des isoprénoïdes bactériens (Silipo et al., 2014) ou végétaux (Wang et al., 2012) peuvent avoir un effet sur les interactions entre les plantes et les bactéries. D'autre part, certaines études ont mis en avant l'influence des interactions plantes-bactéries sur la régulation de la biosynthèse d'isoprénoïdes végétaux (Gargallo-Garriga et al., 2016; Del Giudice et al., 2008; Pandey et al., 2018; Salomon et al., 2016).

Ceci nous conduit à mon projet de thèse qui avait pour objectif d'étudier les interactions entre plantes et bactéries dans le contexte de la biosynthèse des isoprénoïdes chez *A. thaliana*. Mon projet pouvait se diviser en deux questions principales, déterminant mes objectifs de thèse :

- Les isoprénoïdes végétaux ont-ils une importance dans la sélection du microbiote associé à la plante ? Ont-ils aussi un effet sur les interactions entre plantes et pathogènes ?
- Est-ce que certaines bactéries du microbiote dont la présence dépend du statut isoprénique de la plante ont un impact sur la physiologie de la plante et sa résistance aux pathogènes ?

La première étape pour déterminer si les isoprénoïdes influencent les interactions entre plantes et bactéries impliquait d'établir un inventaire des communautés associées aux plantes sauvages et mutantes, altérées dans les voies de biosynthèse des isoprénoïdes. Le but était de déterminer si certaines bactéries sont différentiellement abondantes dépendamment du statut isoprénique de la plante. D'autre part, puisque les plantes sont soumises à de nombreux stress, incluant des interactions avec des pathogènes, j'ai également étudié l'impact des isoprénoïdes sur la colonisation de la plante par un pathogène bien connu, *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*DC3000).

Le second volet de ma thèse était consacré à l'étude de l'impact de certaines bactéries du microbiote dont la présence semble corrélée au statut isoprénique de la plante sur la physiologie de celle-ci et sa résistance aux pathogènes. Pour ce faire, nous avons d'abord isolé des bactéries interagissant avec *A. thaliana* pour créer une collection de souches. J'ai ensuite affilié ces souches à des genres connus, et comparé la séquence de leur gène codant l'ARNr 16S à celles des bactéries dont l'abondance varie entre les plantes sauvages et mutantes. J'ai pu mettre en évidence une proximité phylogénétique entre certaines souches isolées et certaines bactéries dont l'abondance varie. Ceci m'a conduite à tester l'effet de ces souches en particulier sur *A. thaliana*, ainsi que l'effet d'isoprénoïdes végétaux sur ces souches.

II. Etude de l'impact des isoprénoïdes dans la sélection du microbiote associé à la plante et dans sa colonisation par des pathogènes

Le premier volet de ma thèse consistait à déterminer l'impact des isoprénoïdes sur la colonisation de la plante par les bactéries en général, mais aussi sur les interactions de la plante avec des micro-organismes pathogènes.

1. Inventaire des communautés interagissant avec les plantes sauvages et mutantes affectées dans les voies de biosynthèse des isoprénoïdes

Un inventaire des communautés interagissant avec les plantes sauvages (Col-0 et WS2) et mutantes (*chs5* et *hmg1-1*) a été effectué au laboratoire afin de déterminer si les isoprénoïdes végétaux peuvent influencer la mise en place du microbiote de la plante. Cet inventaire a été réalisé par une approche « barcoding » basée sur l'amplification puis le séquençage d'une portion du gène codant l'ARNr 16S bactérien par Illumina.

L'inventaire ainsi réalisé nous a permis de confirmer l'existence d'un microbiote considéré comme un noyau commun indépendamment de l'écotype, et du génotype

de la plante, comme mentionné dans de précédentes études. (Bai et al., 2015; Bodenhausen et al., 2013; Bulgarelli et al., 2012, 2015; Lundberg et al., 2012; Schlaeppi et al., 2014). Ce microbiote est composé en grande majorité de *Proteobacteria*, ainsi que d'une part importante d'*Actinobacteria* et de *Bacteroidetes*, et une faible proportion de *Firmicutes*. Ensemble, ces trois phyla représentent plus de 80% du microbiote de la plante. Ces données correspondent aux proportions généralement retrouvées dans les précédentes études mentionnées ci-dessus.

Cependant, nous avons aussi pu mettre en évidence une différence significative d'abondance de certaines OTUs (Operational Taxonomic Units) spécifiques entre les plantes Col-0 et *chs5* ainsi qu'entre les plantes WS2 et *hmg1-1*, telles que des *Actinobacteria, Streptomyces*, ou *Proteobacteria*, par exemple. Cette différence de communautés suggère une potentielle implication des isoprénoïdes dans la mise en place du microbiote. Ainsi, la présence de certaines bactéries en particulier pourrait nécessiter les isoprénoïdes végétaux, de mêmes que ces derniers pourraient être toxiques pour d'autres micro-organismes.

Il est intéressant de noter que parmi les bactéries plus abondantes chez Col-0 que chez *chs5*, on retrouve des genres intéressants tels que des *Streptomyces* ou des *Rhizobium*. Les *Rhizobium* sont notamment connus pour leurs effets bénéfiques sur les plantes, on peut donc les considérer comme PGPB (Plant Growth Promoting Bacteria) (van Rhijn and Vanderleyden, 1995). Les *Streptomyces* incluent également des souches PGP, notamment par leur capacité à solubiliser le phosphate pour la plante, à produire des sidérophores, des antibiotiques, ou encore à induire la résistance systémique (ISR) de la plante (Abbasi et al., 2019; Dias et al., 2017; Kuzuyama and Seto, 2003; Pérez-Gil and Rodríguez-Concepción, 2013), la préparant à de futures attaques pathogènes. On peut donc supposer que la forte abondance de telles souches chez les plantes sauvages peut leur conférer des avantages, comme leur permettre une meilleure résistance aux pathogènes par exemple.

2. Etude de la sensibilité des plantes au phytopathogène *Pseudomonas syringae* pv. *tomato* DC3000

Comme mentionné ci-dessus, nous avons mis en évidence une possible implication des isoprénoïdes dans la sélection des bactéries associées aux hôtes de manière générale. Le microbiote étant important pour la santé de la plante et sa résistance aux pathogènes, nous nous sommes demandé si les isoprénoïdes peuvent également avoir un effet sur la résistance des plantes aux pathogènes. Ma stratégie a été de comparer la sensibilité des plantes Col-0 et *chs5*, mais aussi WS2 et *hmg1-1* au phytopathogène *Pseudomonas syringae* pv. *tomato* DC3000 en conditions holoxéniques, c'est-à-dire en présence du microbiote naturellement associé à chaque plante. Ce pathogène a notamment participé à la mise en place du modèle en « zigzag » du système immunitaire végétal (Jones and Dangl, 2006).

Les plantes WS2 et *hmg1-1* ne présentaient pas de différence de sensibilité majeure suite à l'infection par *Pst*DC3000, ce qui suggère que les isoprénoïdes synthétisés par la voie du MVA ne sont pas impliqués dans les mécanismes de défense de la plante contre ce pathogène. En revanche, j'ai pu mettre en évidence une sensibilité significativement plus importante des plantes *chs5* affectées dans la biosynthèse des isoprénoïdes plastidiaux (MEP) par rapport aux plantes sauvages, ce qui se caractérise par des lésions et une colonisation par le pathogène plus importantes. Afin d'étudier si cette différence de sensibilité se reflétait au niveau de la réponse immunitaire de la plante, j'ai analysé l'expression des gènes de défense *PR-1*, *PR-2* et *PR-5* (*Pathogenesis-Related* Genes) *d'A. thaliana* à différents temps post-infection. Leur expression est connue pour être induite en réponse à l'infection par *P. syringae*. J'ai ainsi pu observer par RT-qPCR une augmentation de l'expression de ces gènes, en particulier à 3 jours post-infection, où la réponse semble être la plus forte. On constate globalement une expression plus faible de ces gènes chez la plante mutante par rapport à la plante sauvage, mais cette faible diminution ne suffit probablement pas à expliquer à elle seule la différence de sensibilité entre Col-0 et *chs5*. J'ai donc voulu déterminer si cette différence de sensibilité se reflétait aussi au niveau métabolique en quantifiant notamment l'accumulation du stigmastérol, un isoprénoïde dont l'accumulation est décrite comme étant corrélée à l'infection par *Pst*DC3000 selon une précédente étude (Griebel and Zeier, 2010). J'ai en effet pu observer une accumulation plus importante du stigmastérol chez les plantes infectées

par rapport aux plantes témoins. Cependant, je n'ai pas noté de différence majeure d'accumulation de ce composé entre Col-0 et *chs5*. Ainsi, la différence de sensibilité observée ne résulte pas non plus d'une différence d'accumulation du stigmastérol.

Ensemble, ces résultats suggèrent un rôle des isoprénoïdes synthétisés par la voie du MEP dans les mécanismes de défense de la plante. Cet effet peut être direct, puisque certains isoprénoïdes sont connus pour être impliqués dans les mécanismes de défense de la plante, tels que l'acide abscissique ou les cytokinines qui sont synthétisées via la voie du MEP. Il est maintenant connu que les mécanismes de défense de la plante impliquent différentes hormones en plus des voies hormonales classiques de l'acide salicylique et de l'acide jasmonique et éthylène (Jones and Dangl, 2006; Kumar, 2014; Pieterse et al., 2012). Mais les isoprénoïdes peuvent aussi avoir un effet indirect sur la sensibilité de la plante, via la sélection du microbiote associé, puisque nous avons mis en évidence une différence de communautés entre les plantes sauvages et mutantes. En effet, au sein du microbiote des plantes Col-0, certaines bactéries en particulier pourraient avoir un effet protecteur en entrant en compétition avec le pathogène ou en sécrétant des composés antimicrobiens, par exemple, tout comme elles pourraient induire un état de « priming » des défenses de la plante par le mécanisme de la résistance systémique induite (Dessaux et al., 2016; Glick, 2012; Olanrewaju et al., 2017). D'autres bactéries interagissant avec *chs5* pourraient au contraire affecter la plante et ainsi favoriser l'infection par le pathogène. J'ai donc voulu tester l'effet de certaines bactéries isolées dans un second volet de ma thèse.

III. Etude de l'impact de bactéries isolées sur la physiologie et les défenses de la plante contre les pathogènes

Afin de déterminer l'effet du microbiote de la plante sur son état physiologique et sa sensibilité à l'infection par des pathogènes, j'ai décidé d'étudier dans un second volet de ma thèse l'impact de certaines bactéries isolées au laboratoire sur les plantes sauvages et mutantes.

1. Etablissement d'une collection de 230 souches isolées au laboratoire

Avec l'aide d'étudiants en stage au laboratoire, nous sommes parvenus à isoler 230 bactéries du microbiote d'*A. thaliana* à partir de différents compartiments (phyllosphère, racines, rhizosphère ou graines) de plantes sauvages Col-0 ou WS2, mais aussi à partir du terreau utilisé pour la culture de ces plantes. J'ai ainsi pu constituer une collection de souches dont l'effet peut être testé en conditions contrôlées. J'ai par la suite identifié le genre de ces bactéries sur base de la séquence du gène codant leur ARNr 16S obtenue par séquençage Sanger. Au sein de notre collection, on retrouve une majorité de *Firmicutes*, une grande proportion d'*Actinobacteria* et de *Proteobacteria*. Ainsi, notre collection n'est pas représentative de la composition du microbiote naturel en termes de proportions de chaque phylum. En effet, comme décrit précédemment, l'inventaire des communautés mettait en évidence une forte majorité de *Proteobacteria*, un quart d'*Actinobacteria* et un quart de *Bacteroidetes* et peu de *Firmicutes*. Ceci peut notamment s'expliquer par le fait que notre collection représente seulement une faible proportion des souches qui pourraient être cultivées en laboratoire (Bai et al., 2015) puisque nous nous sommes basés sur des critères morphologiques pour la sélection des souches à isoler. Cependant, de manière intéressante, notre collection contient des souches qui correspondent potentiellement à des bactéries dont l'abondance varie entre plantes sauvages et mutantes.

2. Comparaison des isolats aux OTUs différentiellement abondantes entre plantes sauvages et mutantes

J'ai procédé à une comparaison de la séquence du gène codant l'ARNr 16S de chacun de nos isolats aux séquences partielles des OTUs différentiellement abondantes entre les plantes sauvages et mutantes. Je me suis particulièrement intéressée aux OTUs dont l'abondance variait entre Col-0 et *chs5*, puisque ces plantes présentaient une différence de sensibilité significative suite à l'infection par *Pst*DC3000. Il est important de noter que plusieurs analyses des communautés ont été réalisées au cours de ces trois années de thèse. Au début de ma thèse, je disposais déjà des deux premières analyses, et d'une collection de souches plus

réduite que notre collection actuelle. J'ai donc effectué cette comparaison de séquences une première fois, ce qui m'a permis de mettre en évidence des souches qui pourraient correspondre (100% d'identité entre les séquences) à des bactéries dont l'abondance varie dépendamment du génotype de la plante. Leur présence serait donc potentiellement dépendante du statut isoprénique de la plante, et ce sont notamment ces souches qui pourraient en partie expliquer la différence de sensibilité au pathogène. Les isolats qui ressortent de cette étude comparative entre Col-0 et *chs5* sont ceux sur lesquels nous avons décidé de nous focaliser par la suite. Parmi eux, deux bactéries du genre *Pseudomonas*, deux *Curtobacterium*, et une *Microbacterium* ont retenu notre attention. Une comparaison plus récente entre les isolats de notre collection et les OTUs dont l'abondance varie entre plantes sauvages et mutantes a permis de mettre en évidence d'autres souches candidates, dont une *Rhizobium*.

J'ai décidé de caractériser ces cinq souches candidates plus finement. J'ai notamment procédé à des affiliations phylogénétiques, par comparaison des séquences du gène codant l'ARNr 16S aux séquences présentes dans la base de données du NCBI. A partir des séquences présentant la plus grande identité, j'ai pu établir des arbres phylogénétiques afin de mettre en évidence les plus proches parents de ces souches. Ceci m'a notamment permis de rechercher dans la littérature les effets positifs ou négatifs de bactéries du genre *Pseudomonas*, *Curtobacterium* et *Microbacterium* proches de nos souches. Aucun effet notable de bactéries du genre *Microbacterium* n'est reporté dans la littérature. En revanche, une précédente étude a montré des effets bénéfiques de *Pseudomonas nitroreducens* sur la croissance d'*A. thaliana* et *Lactuca sativa*, ce qui nous permet de la considérer comme PGPB (Trinh et al., 2018). Concernant *Curtobacterium*, certaines *Curtobacterium flaccumfaciens* sont connues pour être des PGPB (Cardinale et al., 2015; Horuz and Aysan, 2018), tandis que d'autres sont connues comme pathogènes (Sammer and Reiher, 2012). Il semblait donc intéressant de tester les effets de ces différentes souches sur *A. thaliana*.

De plus, chaque bactérie présentant un profil d'acides gras qui lui est propre, une analyse des acides gras est couramment utilisée pour affilier des souches à un genre précis. J'ai donc procédé à des analyses de spectrométrie de masse par GC/FID (Chromatographie gazeuse couplée à un détecteur à ionisation de flamme) afin

d'étudier les profils d'acides gras de chacune de ces souches, ce qui m'a permis de confirmer leurs affiliations préalablement réalisées par séquençage du gène codant l'ARNr 16S.

3. Etude approfondie de cinq isolats

3.1. Impact des isoprénoïdes sur la croissance des souches

J'ai voulu déterminer si ces souches candidates étaient capables d'utiliser des isoprénoïdes pour assurer leur développement, ou si au contraire, certains isoprénoïdes pouvaient être toxiques pour elles, expliquant ainsi leurs différences d'abondance entre plantes sauvages et mutantes. J'ai donc procédé à des tests de croissance en présence d'isoprénoïdes comme unique source de carbone. Dans un premier temps, une culture en milieu liquide a permis de mettre en évidence que les souches testées sont capables d'utiliser le limonène comme source de carbone mais que ce composé, en fortes concentrations, inhibe partiellement la croissance des bactéries. De plus, les conditions de croissances testées conduisaient les bactéries à former des agrégats qui rendaient difficile la mesure fiable de la DO_{600nm} permettant de quantifier la croissance bactérienne. Par la suite, j'ai donc décider de procéder à des tests de croissance sur milieu solide contenant des isoprénoïdes (limonène, β-caryophyllène, farnésol, myrcène, α-pinène, β-ocimène, α-humulène, (-)-linalool, géraniol et thujopsène). J'ai en particulier étudié la croissance des deux *Curtobacterium* précédemment citées. Ces souches sont capables d'utiliser tous les isoprénoïdes testés comme source de carbone pour assurer leur développement, même si cela ne correspond pas à leurs conditions optimales de croissance. Il semblerait même que certains isoprénoïdes tels que le farnésol ou le géraniol puissent être toxiques pour ces souches, puisqu'elles se développent moins facilement en leur présence à fortes concentrations. Ceci suggère une fois de plus un impact des isoprénoïdes sur les interactions entre plantes et bactéries.

4. Impact des souches sur le phénotype des plantes et leur résistance à *P. syringae*

Enfin, j'ai voulu étudier l'impact de ces cinq souches sur le phénotype et la croissance des plantes ainsi que sur leur résistance à l'infection par *P. syringae*. Pour ce faire, j'ai travaillé sur des plantes cultivées en conditions gnotoxéniques, c'est-à-dire stérilement avec inoculation d'une souche en particulier, d'une part, et en conditions holoxéniques, c'est-à-dire en présence de leur microbiote naturel, d'autre part.

En conditions gnotoxéniques, des plantes Col-0 et *chs5* ont été inoculées ou non par les souches d'intérêt, puis infectées par *P. syringae*. Nous n'avons pas observé d'effet de *Microbacterium* sp. 5B sur le développement de la plante ni sur sa résistance au pathogène. *Pseudomonas* sp. 10A et *Pseudomonas* sp. 2D, en revanche, affectent le développement de la plante. *Pseudomonas* sp. 10A a également un effet sur la résistance de la plante au pathogène, puisqu'elle amplifie les effets de l'infection. Enfin, *Curtobacterium* sp. 5H et *Curtobacterium* sp. 6H impactent légèrement le développement des plantes qui sont de taille réduite, mais elles semblent avoir un effet antagoniste plus ou moins important vis-à-vis du pathogène. Plus en détail, les résultats de ces tests ont attiré notre attention sur *Curtobacterium* sp. 5H, qui présentait un potentiel effet protecteur. Cependant, dépendamment de la stringence de la méthode de stérilisation utilisée, les effets observés n'étaient pas toujours les mêmes. En effet, avec l'utilisation d'une stérilisation plus poussée, nous n'avons plus observé ces traits protecteurs. Il en ressort que la souche induit systématiquement un retard de croissance des plantes inoculées, et variablement des traits protecteurs contre *P. syringae*. Nous avons supposé que l'effet protecteur de cette souche pouvait résulter de son interaction avec d'autres micro-organismes encore présents après une stérilisation incomplète.

Ceci m'a conduite à tester l'inoculation de *Curtobacterium* sp. 5H à des plantes sauvages et mutantes cultivées en conditions holoxéniques. Ainsi, je souhaitais déterminer si l'effet de cette souche pouvait être la conséquence de son interaction avec d'autres bactéries, et dans ce cas précis, avec le microbiote naturel. Les plantes inoculées étaient légèrement plus petites que les plantes non inoculées mais on n'observait pas de différence majeure de sensibilité au pathogène en conditions

holoxéniques. Le potentiel effet protecteur de cette souche est peut-être dépendant de la dose inoculée ainsi que des interactions avec un faible nombre de micro-organismes du microbiote, ou encore des conditions de cultures. Il est important de noter que lors des expériences *in vitro*, les plantes sont soumises à différentes étapes qui peuvent les stresser (inoculation, repiquage des plantes sur de nouvelles boîtes, infection), ce qui peut également avoir un impact sur celles-ci.

5. Systèmes d'étude

Au cours de ma thèse, j'ai optimisé un autre système de culture des plantes en conditions gnotoxéniques permettant leur culture jusqu'à la formation des siliques. Il s'agit d'un système de culture en hydroponie, dans lequel les plantes sont cultivées dans des tubes contenant un milieu gélosé permettant à leurs racines de les traverser et d'atteindre un milieu nutritif liquide nécessaire à leur croissance. Le système est opérationnel et chaque système permet la culture de 12 plantes simultanément. Il s'agit d'une bonne alternative au système *in vitro* sur milieu gélosé utilisé précédemment, qui semblait être difficile à maîtriser pour répéter les premières observations.

Ce système de culture en hydroponie permet de tester l'impact de souches en particulier sur des plantes plus grandes que ce qui était possible par l'usage des boîtes de culture *in vitro* précédemment utilisées. De même, il est possible d'étudier la sensibilité des plantes axéniques au pathogènes *P. syringae* afin de déterminer si la différence de sensibilité observée résulte du statut isoprénique de la plante lui-même, ou du microbiote normalement associé.

IV. Conclusions et perspectives

Les travaux effectués au cours de ma thèse suggèrent une implication des isoprénoïdes dans la mise en place des communautés bactériennes interagissant avec les plantes, ainsi que dans la résistance à un pathogène biotrophe. On peut donc

suggérer une implication des isoprénoïdes dans les interactions plantes-bactéries. Les communautés interagissant avec WS2 et *hmg1-1* sont elles aussi variables, mais les plantes mutantes ne présentent pas de différence de sensibilité par rapport aux sauvages. Ceci suggère l'implication plus spécifique d'isoprénoïdes synthétisés par la voie du MEP dans la défense au pathogène, directement ou indirectement. Il serait intéressant de tester la sensibilité des plantes sauvages et mutantes à un autre pathogène, tel que le champignon nécrotrophe *Botrytis cinerea*. En effet, les voies de défense activées par la plante suite à l'infection par un pathogène (hémi)-biotrophe tel que *Pst*DC3000 impliquent principalement l'acide salicylique ; tandis que l'infection par un pathogène nécrotrophe induit principalement les voies de l'acide jasmonique et de l'éthylène. Cependant, il est désormais accepté que les mécanismes de défense de la plante impliquent également d'autres hormones, telles que l'acide abscissique ou les cytokinines, synthétisées via la voie du MEP (Jones and Dangl, 2006; Pieterse et al., 2012). L'impact de ces molécules peut être différent dépendamment du type de pathogène qui interagit avec la plante. Les isoprénoïdes sont donc potentiellement capables d'influencer les interactions plantes-pathogènes à différentes échelles.

Dans le but d'étudier plus en détails le rôle des isoprénoïdes dans la résistance au pathogène entre les plantes Col-0 et *chs5,* j'ai essayé d'obtenir une plante mutante *chs5* chez laquelle le gène codant pour l'enzyme DXS1 serait exprimé sous contrôle d'un promoteur fort constitutif. Au laboratoire, des lignées d'*A. thaliana* portant ce gène sous promoteur fort ont été croisées avec des plantes *chs5*. Jusqu'à présent, j'ai pu obtenir des lignées hétérozygotes (*dxs1*/*DXS1*) ayant intégré le sur-expresseur, mais aucune lignée homozygote (*dxs1*/*dxs1*). L'obtention de telles lignées permettrait de vérifier que la surexpression de l'enzyme 1-désoxy-D-xylulose 5-phosphate synthase 1 restaure une meilleure résistance au pathogène chez le mutant, similaire ou peut-être même plus importante que chez Col-0, confirmant une implication des isoprénoïdes dans la différence de sensibilité aux pathogènes.

Enfin, d'autres bactéries de notre collection de souches pourraient être testées pour leur effet sur *A. thaliana*. Notamment, les *Rhizobium* présentant 100% d'identité de séquence du gène codant l'ARNr 16S avec des OTUs dont l'abondance varie entre les plantes sauvages et mutantes, pourraient être de bons candidats puisque ce genre bactérien est connu pour ses capacités promotrices, comme mentionné plus haut.

D’autres bactéries dont la séquence du gène codant l’ARNr 16S est différente de celle des OTUs variables pourraient également être de bons candidats.

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Deciphering the interactions between plants and bacteria in the context of isoprenoid biosynthesis in *Arabidopsis thaliana*

Les isoprénoïdes constituent une importante classe de molécules que l'on retrouve chez tous les organismes vivants. Chez les plantes, ils sont impliqués dans divers processus biologiques tels que la photosynthèse, la respiration ou la division cellulaire. Le but de ma thèse était de déterminer si ces molécules pouvaient être impliquées dans les interactions entre plantes et bactéries. J'ai comparé les interactions plantes-bactéries chez des plantes *Arabidopsis thaliana* sauvages et mutées dans chacune des deux voies de biosynthèse des isoprénoïdes existant chez les plantes supérieures. Un inventaire des communautés constituant le microbiote de ces plantes nous a permis de démontrer que malgré l'existence d'un microbiote commun, l'abondance de certaines bactéries varie entre plantes sauvages et mutantes. De plus, nous avons montré que les plantes affectées dans la voie de biosynthèse plastidiale, dite du méthylérythritol phosphate (MEP), sont plus sensibles que les plantes sauvages à l'infection par *Pseudomonas syringae*. D'autre part, nous avons isolé au laboratoire 230 souches interagissant avec *A. thaliana*, parmi lesquelles certaines ont été testées pour déterminer leur effet sur le phénotype et la résistance des plantes à *P. syringae*. Ensemble, les résultats obtenus suggèrent que les isoprénoïdes jouent un rôle dans les interactions entre les plantes et certaines bactéries du microbiote.

Mots-clés : *Arabidopsis thaliana*, interactions plantes-bactéries, isoprénoïdes, microbiote, *Pseudomonas syringae*.

Isoprenoids are a large class of molecules found in all living organisms. In plants, they are implicated in diverse biological processes such as photosynthesis, respiration or cell division. The aim of my thesis was to decipher if they could be involved in the interactions between plants and bacteria. Therefore, I compared plant-bacteria interactions in *Arabidopsis thaliana* wild-type and mutants altered in the two isoprenoid biosynthesis pathways occurring in higher plants. An inventory of the communities interacting with these plants highlighted that despite the existence of a core microbiota, some bacteria are differently abundant between wild-types and mutants. Moreover, plants affected in the plastidial biosynthesis pathway, referred as the methylerythritol phosphate (MEP) pathway, have been shown to be more affected by *Pseudomonas syringae* than wild-types. In addition, in the laboratory, we isolated 230 strains from *A. thaliana*, among which some of them were tested for their impact on the plant health and resistance to *P.* syringae. Together, these results suggest that isoprenoids may play a role in the interactions between plants and some bacteria from the microbiota.

Keywords: *Arabidopsis thaliana*, isoprenoids, microbiota, plant-bacteria interactions, *Pseudomonas syringae*.