الجمهورية الجزائرية الديمقراطية الشعبية

République Algérienne Démocratique et Populaire

وزارة التعليم العالي و البحث العلمي

Ministère de l'Enseignement Supérieur et de la Recherche Scientifique

الجلفة-جامعة زيان عاشور

Université Ziane Achour – Djelfa

كلية علوم الطبيعة و الحياة

Faculté des Sciences de la Nature et de la Vie

قسم البيولوجيا

Département de Biologie

Projet de fin d'étude

En vue de l'obtention du Diplôme de Master

Filière : Biotechnologie, **Spécialité :** Biotechnologie Végétale

Thème

Manipulation of plants immunity by beneficial microbes

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2020/2021

Apreciation

Prophet mohamed ﷺ said’s in hadith “ who does not thank people does not thank Allah **“**

So in this context we want to take some time to thank and give the appreciations to those who helped us during our 5 years in the University , first of all we want to thank our parents and specially our fathers **khadir abderrahmane** and **sadki mokhtar** who gave us all we need to surpass the obstacles that we faced from the first day to this day, second we want to thank our amazing teachers that taught us everything we know and specially our supervisor **Berrabeh fathi** who opened our eyes to Horizons we never imagined and **Mr Adli benziane** who guide us and response to us whenever we needed him and **Mr Toumatia Omrane** for providing us a place where we could work comfortably and great amount of informations and **Ms Bencherif karima** for giving us some ressourses on rhizobia and PGPR which helped us a lot in our work, we want also to thank our dear friend **Senni Hamza** who helped us in several things during this 3years that we pass together and also a big thanks to **Bekai faiza** who gave us her pc when ours Breakdown , and finally we want to thank everyone that we met and knew during those amazing 5years it wouldn’t be that good without you people .

So to all of you .and to those we forgot to mention.

Thank you

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Introduction

In their environment, the plants interact with various range of microbes, certain are pathogens, while others are benefic. To distinguish between bad and good microorganisms, plants have acquired an arsenal of genes implicated in all steps of the interaction: from the recognition to the responses. By contrast microbes also evolved to encounter the plants responses and to establish effective interaction.

Plants can recognize pathogen directly by perception of conserved molecule called Pathogen Associated Molecular Patterns (PAMPs), or indirectly through the perception of Damage Associated Molecular Patterns (DAMPs) those represent host molecules released upon the action of lytic enzymes secreted by the pathogens. D/PAMPs are perceived by membrane associated receptors, the Pathogen Recognition Pattern (PRRs). Stimulation of PRRs by their ligands leads to the activation of PAMP Triggered Immunity (PTI). This defense response is characterized by the activation of specific signaling proteins, an oxidative burst and the expression of defense genes. PTI aim to suppress efficiently the pathogens, however, certain pathogenic strains inject effectors into the host cell through the secretion systems. In the cell, the effectors short-circuit the PTI by inhibition of signaling proteins. The inactivation of PTI results in the infection of the plants by the pathogens what's called Effector Triggered Sensibility (ETS). To avoid ETS, the plant produces intracellular receptors encoded by *R* genes. These receptors detect specifically the effectors and reactivate the immune signaling. In the case of the suppression of the pathogens, the response is called Effector Triggered Immunity (ETI).

Despite that symbiosis produce an opposite behavior than pathogenesis, beneficial microbes and pathogens use similar strategy to infect their host. The rhizobia represent a group of bacteria that are able to establish nitrogen-fixation symbiosis with the leguminous plants. These microbes are initially detected as pathogens by their host and it use different strategies to overcome the activation of the plant defenses. Certain rhizobium reduces the defense signaling by modification or masking their Microbial Associated Molecular Pattern (MAMPs), whereas others inject into the host cells effectors to block the MAMPs Triggered Immunity (MTI).

Another important group of beneficial bacteria is the Plant Growth Promoting Rhizobacteria (PGPR), which encompasses a large group of taxa establishing beneficial interaction with a various plants species. Actually, the manipulation of the host immunity by PGPRs is poorly understood; however, the available studies indicate that PGPRs seems to use similar strategies with the known processes used by the rhizobia for the manipulation of the host immunity.

In this work, we analyze the bibliography and the available data talking about the control of the immunity by beneficial bacteria. In addition, we analyze the evolutionary process of PGPRs genes implicated in the control of defenses, for that, the phylogeny of two key genes implicated in the immune control by bacteria: the secretion system II and III were analyzed. To determine if the secretions systems fellow the evolution of the bacterial strain or they was potentially subjected to a specific evolutionary pressure due to their role during plant-microbe interaction, we compared the phylogeny of studied genes, to the 16S ribosomal sequence, that used for bacteria classification.

Chapter I. Introduction to plants-microbes interactions

The plants interact with a wide range of microorganisms, some of them are pathogens and can lead to disease, whereas others are benefic and participate on plant adaptation to the environment. In this context, it's essential for the plants to distinguish between the beneficial and pathogenic microbes (Zamioudis and Pieterse, 2012). During their evolution the vegetables have acquired several numbers of genes involved in organizing and orchestrating the host responses to microbes (Jones and Dangl, 2006; Pieterse et al., 2012a; Tsuda and Somssich, 2015a). By contrast the microbes also develop different strategies to infect and establish the interaction with their hosts (Rey and Jacquet, 2018).

I. The different types of plants-microbes interactions

Based on the results of the interaction between the plant and the microbe, at least three types of association can be distinguished:

i) Neutral interaction: this type of interaction doesn't affect the host.
ii) Negative interaction: this association involves pathogens and lead to negative effects on the plant, which are represented by alteration of the plant growth and the death in the extreme situations.
iii) Positive interaction: this association takes place between plants and beneficial microbes and lead to the improvement of the plant health and resistance (Berrabah, 2016).

II. Pathogenic interaction

The pathogenesis is process by which destruction or disorder occurs in the organism (Gellman and Turner, 2013). That can be produced by pathogenic microorganisms. Plant pathogens include bacteria, fungi, oomycetes and virus (Teixeira et al., 2019). A pathogen can infect one or multiple hosts and one plant can be infected by multiple pathogens. The virulence of the pathogenic strain and the resistance of the host to the pathogens are determined by genetic background including specific genes of virulence or resistance in respectively the pathogens and the plants (Jones and Dangl, 2006).

III. Beneficial microbes

Beneficial microbes (BC) include all microorganisms that show a benefit for the plants (Yan et al., 2019). These microbes are used in the agriculture fields to enhance the growth and the protection of cultivated plants. Based on the expected effects, we can distinguish two categories of BC applied to the agriculture:

i) The first class includes all microorganisms that show a direct effect on plant growth, nutrition and resistance to the abiotic stress. This category is called plant growth promoting microorganisms (or PGPM, (Ma et al., 2018)).

ii) The second category contains microbes that show antagonism with pathogens and it’s called bio-control (Farhana et al., 2017). This category is used for the control of plant disease.

Actual studied PGPM and bio-control are mainly belong to the bacteria and the fungi (Stępniewska and Kuåniar, 2013). These microbes are isolated and studied from various supports (plants, soil, and airborne particles….etc.) by classical microbiology technics or by more complex approach as the metagenomic (Vogel and Bai, 2016).

IV. The different level of plants-colonization by beneficial microbes

Beneficial microbes show various distributions into the plants. Due to the high density of microbes in the soil, the roots are the more infected plants organs (Lundberg et al., 2012). However, microbes can also colonize the upper parts of the plants through abiotic process consisting of the deposition of the microbes by exogenous factors (as the wind,(Yan et al., 2019)).

In addition, depending to the localization of the microbe on or in the plants we can distinguish the epiphytic microbes that live at the plants surface, whereas the endophytic microbes colonize the inner plants tissues (Bacon and White, 2016). Moreover, in certain symbiosis, a dedicated organ is formed by the plants as the nodules, a new roots organs produced by leguminous plants to host their symbionte, the rhizobium (Oldroyd et al., 2011). Finally into the plants, microbes can colonize intercellular or intracellular spaces (Haag et al., 2011; Groen et al., 2016).

Chapter II. Introduction to plant immunity

The immunity is the key system controlling the plants behaviors against microbes. This chapter aim to introduce general concepts and phenomenon's discovered in the plants immunity and controlling the plant-microbe associations.

I. The gene for gene relationship, a model for plants responses to pathogens

The model of gene for gene was proposed by M. Harold Henry Flor in 1942 (Flor, 1971). This model proposes that arrest or development of a disease caused by a pathogen is linked to the presence of two genes shared respectively by the host and the microbe and controlling the plant-pathogen compatibly. The host gene is called the *resistance* (*R*) and the pathogen gene is called the *Avriulence* (*Avr*). The presence of *R* genes in the host recognizing the *Avr* genes product lead to the activation of the immune response by the host and to the suppression of the microbe, which is lead to incompatible interaction. However, absence of the recognition between *R* and *Avr* product conduct to pathogenesis or compatible interaction (Table 1, (Flor, 1971)).

Table 1. Henry Flor gene for gene model. The presence in the plant of *R* genes recognizing the pathogen *Avr* genes lead to immune responses and to compatible interaction (C), whereas the absence of the *R* genes recognizing the *Avr* genes product lead to pathogenesis and to incompatible interaction (IC)

Pathogens Plants	*Avr*	*avr*
R	C	IC
r	IC	IC

II. Molecular basis of plants pathogens interaction

Plants can interact with various ranges of microbes, some of them are pathogens and they affect negatively the plant growth. The host develops a wide range of mechanism to recognize and to suppress the pathogens (van Loon et al., 2006).

II.1. Perception of the pathogens

Plants use diverse immune receptors to sense directly the pathogens through perception of pathogen-associated molecular patterns (PAMPs, (Cook et al., 2015)). Moreover plant can perceive indirectly the pathogen by the detection of compound released during the attacks called Damage-Associated molecular patterns (DAMPs, (Choi and Klessig, 2016)).

Recognition of D/PAMPs by pattern recognition receptors (PRR) localized on the plasma membrane leads to PAMP-triggered immunity (PTI, (Peng et al., 2018)). The PRR display extracellular domain involved in the perception of the PAMP, a trans membrane domain and an intracellular domain implicated in the activation of the signaling pathway. However, certain PRRs are devoid from one or two of the described domains (Dardick et al., 2012).

II.2. PTI signaling

During the PTI, a specific signaling pathway is activated after D/PAMPs perception and led to influx of the extracellular calcium (Hetmann and Kowalczyk, 2018). A specific Ca^{+2}influxes is observed during the PTI, this molecule is sense by intracellular protein such as calmoduline that contain specific domain binding the calcium (Mitra et al., 2004). After Ca^{+2} binding, the calmoduline phosphorylate and activate signaling protein involved in the activation of the PTI(Peng et al., 2018).

Production of reactive oxygen species (ROS) is observed during the PTI; these molecules participate on the defense response by direct effect on the microbes through their antimicrobial properties (Bigeard et al., 2015; Qi et al., 2017). ROS also display indirect effect on the pathogens by modulation of the signaling pathway via the modification of the redox status of the cell (Pieterse et al., 2012b).

Finally, phosphorylation of the signaling protein located on the cell play a crucial role on defense activation. In the addition to the calmodulines, the mitogen-activated protein kinase (MAPK) cascade is one of the best characterized protein in the defense activation (Bigeard et al., 2015). The MAPK module contain three proteins (MAPK, MAP2K and MAP3K) that act sequentially to stimulate the downstream signaling actors, MAP3K activate MAP2K trough phosphorylation. Activated MAP2K fixe a phosphate on MAPK that’s led to its stimulation, then MAPK phosphorylated targeted immune proteins (Gao et al., 2008).

II.3. Pattern recognition receptors

The PRR can bind molecules shared by a group of microbes and/or pathogens respectively referred as Microbial and Pathogens Associated Molecular Pattern (M/PAMP). Moreover PRR can also recognize DAMPs which are host compounds released after pathogens infection as the oligogalcturnate (Hetmann and Kowalczyk, 2018). Different extracellular domain of PRR were identified, each one is specialized in the recognition of a category of molecules. The two most studied domains are:

i) Leucine Reach Repeat (LRR) domain associated to the perception of peptides or proteins

ii) Lysine Motif (LysM) domain implicated in the recognition of lectines.

Most of describes intracellular domain of the PRR share Serine/Threonine kinase activities, that’s allow them to phosphorylate targeted protein on Ser/Thr residues (Macho and Zipfel, 2014). However, certain receptors don’t display kinase activity and recruit other proteins kinases for the activation of the singling pathway (Dardick et al., 2012).

II.4. FLS2-Flg22, a model for PRR functioning

One of the more studied PRR is FLagelling Sensing 2 (Gómez-Gómez and Boller, 2000; Chinchilla et al., 2006; Robatzek et al., 2006; Robatzek and Wirthmueller, 2013). This receptors recognize the flg22 peptide of the flagelling, the component of the bacteria flagella (Chinchilla et al., 2006). The flg22 recognition by FLS2, initiate the recruitment of co-receptors, the most important is the Brassinosteroid associated kinase 1 (BAK1). Interaction between FLS2 and BAK1 leads to the trans-phosphorylation of the two receptors (Figure 2, (Sun et al., 2013)). Then, activation of defense signaling cascade were observed and led to expression of defense genes as the pathogens-related protein (PR, (Aslam et al., 2009)), production of defense compound as the phytoalexines (Bigeard et al., 2015), accumulation of ROS (Mersmann et al., 2010) and biosynthesis of plants defense hormones as the salicylic acid (Tsuda et al., 2008).

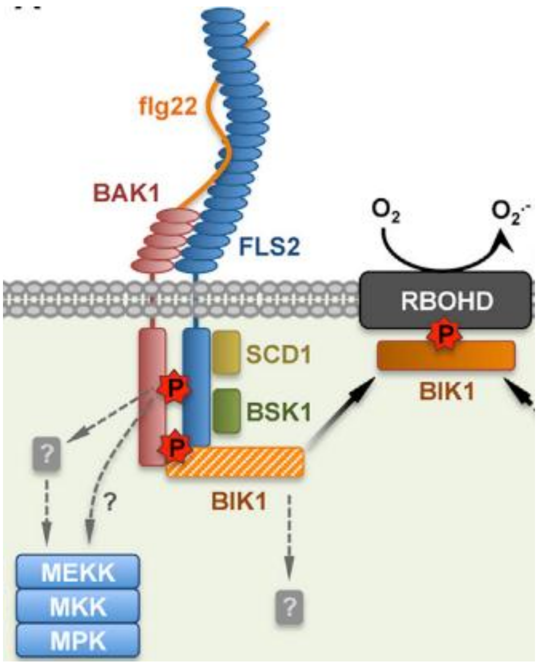

Figure 1. FLS2-flg22 model. In *Arabidopsis*, flg22 perception triggers the phosphorylation of the cytoplasmic domains of FLS2 and BAK1, as well as the RLCK BIK1. Activated BIK1 gets released from the receptor complex, leading to phosphorylation and activation of the NADPH oxidase AtRBOHD. Flg22-triggered ROS burst additionally requires the RLCK BSK1 and the endocytosis regulator SCD1. Likewise, BIK1 is required for the ROS burst triggered by the chitin-induced activation of AtCERK1. In both cases, it is unclear how PRR activation leads to the activation of MAPKs and other downstream substrates (Macho and Zipfel, 2014).

II.5. The effectors triggered immunity

Pathogens in some cases can block the PTI response: for example pathogenic bacteria can inject small peptides into the plant cells through the secretion systems, these peptides are called effectors (Figure 2, (Tsuda and Somssich, 2015b)). The secretion systems act as "molecular syringes" by piercing the host membrane to transfer effectors from the bacterial cytoplasm to the host cell cytoplasm (Abramovitch et al., 2006). Once in the cytosol, effectors recognize certain proteins associated with P/MTI signaling and inhibit their biochemical activities (Wu et al., 2014) or provoke their degradation (Jones and Dangl, 2006). In some cases effector short circuits the host immune response (Van Der Biezen and Jones, 1998) and lead to Effector Trigger Sensitivity or ETS (Figure 3).

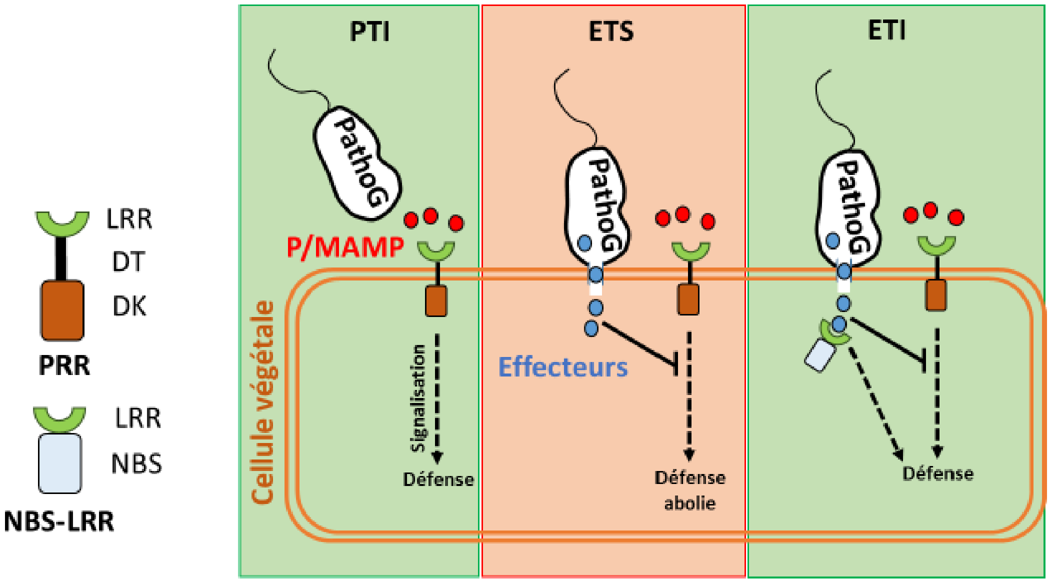

Figure 2. P / MTI suppression, effectors and resistance genes. Following the perception of the M/PAMP by PRR, an immune response is induced. Pathogens can secrete effectors in the cytoplasm of their hosts that block P/MTI signaling and lead to ETS if the plant is sensitive. Conversely, resistant plants have resistance genes which encode intracellular receptors (NBS-LRR) corresponding to the molecules injected. Following the perception of effectors by NBS-LRR, a signaling is triggered leading to immunity induced by the effectors (Effector Triggered Immunity, ETI). LRR: Repetitions Rich in Leucines, DT: Transmembrane domain, DK: Kinase domain, NBS: nucleotide binding site, PathoG: pathogens. Figure inspired by (Jones &Dangl 2006).

In order to avoid ETS, plants have used intracellular receptors (DeYoung and Innes, 2006). These receptors are called NBS-LRR receptors, which recognize effectors and lead to a reactivation of the immune response (Figure 2, (Sekhwal et al., 2015). An NBS-LRR recognizes a single effector (Jones and Dangl, 2006)and generally leads to more intense host response, as the activation of programmed cell death (PCD) of infected cells (Bigeard et al., 2015). This response aims to suppress locally the pathogen, if the pathogenesis is stopped it is referred to as Effector Trigger Immunity (ETI) (Figure 3, (JDG and JL, 2006)). The gene encoding the effector is the *Avirulence* gene or *Avr* and the gene encoding the NBS-LRR is *resistance* gene or *R* (Flor, 1971).

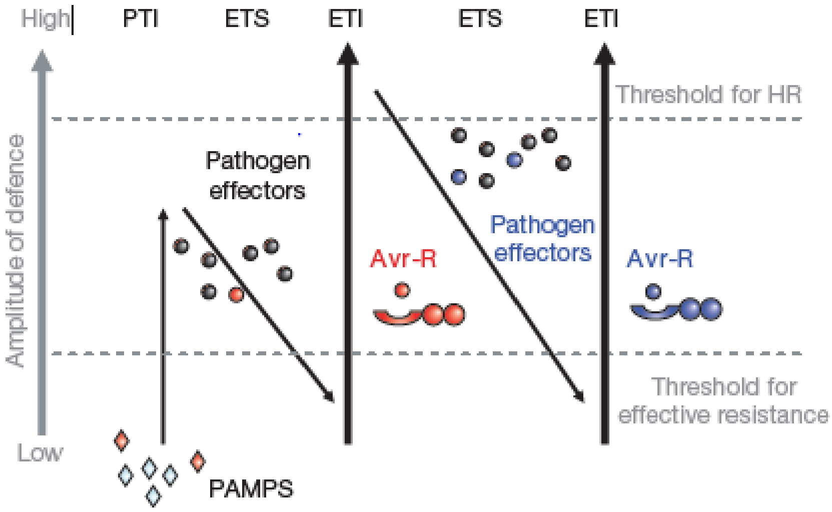

Figure 3. The zigzag model illustrates the quantitative output of the plant immune system. In this scheme, the ultimate amplitude of disease resistance or susceptibility is proportional to [PTI – ETS- ETI]. In phase 1, plants detect microbial/pathogen-associated molecular patterns (MAMPs/PAMPs, red diamonds) via PRRs to trigger PAMP-triggered immunity (PTI) with intermediate amplitude of defense activation. In phase 2, successful pathogens deliver effectors that interfere with PTI, or otherwise enable pathogen nutrition and dispersal, resulting in effector-triggered susceptibility (ETS) leading to defense shut down. In phase 3, one effector (indicated in red) is recognized by an NB-LRR protein, activating effector triggered immunity (ETI), an amplified version of PTI that often passes a threshold for induction of hypersensitive cell death (HR). In phase 4, pathogen isolates are selected that have lost the red effector, and perhaps gained new effectors through horizontal gene flow (in blue)—these can help pathogens to suppress ETI (Jones and Dangl, 2006).

II.5.1. Signaling events associated to the ETI

PTI and ETI diverge by the magnitude and the duration of immune responses which are most important during the ETI than the PTI. However, PRR and NBS-LRR activate similar downstream molecular events such as MAPK activation, oxidative burst, ion influx, and increased biosynthesis of plant defense hormones, indicating a conservation of the immune signaling between PTI and ETI (Bigeard et al., 2015). Moreover, activation of ETI by the NBS-LRR which are able to move to the nucleus appears to be more directly associated with transcriptional regulation of defense gene expression (Lopes Fischer et al., 2020).

II.5.2. NBS-LRR

NBS-LRRs are intracellular receptors encoded by resistance genes (R genes) recognizing the effectors. These receptors are classified into two broad categories, the first being TIR-NBS-LRR receptors that possess a Toll/Interleukin-like Receptor (TIR) domain present in immune receptors in animals where they participate in the activation of immune signaling (Mchale et al., 2006). The precise function of the TIR domain is currently unknown. TIR-NBS-LRRs possess, in addition to the TIR domain, a nucleotide binding site (NBS) and a LRR domain for effector perception. The second category of intracellular receptors is the CC-NBS-LRR receptors that don’t possess a TIR domain. The latter gives way to a coiled coil (CC (DeYoung and Innes, 2006) domain of unknown function. Once the end effector is linked to the NBS-LRR, an immune signaling is activated which leads to the ETI. The Theory suggests that an ETI is only possible if the effector proteins are recognized by the corresponding receptors (Jones and Dangl, 2006).

Plants microbiote represent all microbes able to colonize the plants rhizoplan (the plants surface) and endospher (the inner plant tissues, (Liu et al., 2020)). The microbiote can protect their host from various dangers as the nutrient deprivation or biotic and abiotic stresses. The plants have acquired mechanisms to shape their microbiote and to attract beneficial microbes (Teixeira et al., 2021).

I. Introduction to the plant microbiote

The plants are sounded by thousands of microorganisms (bacteria, fungi, oomycetes…) influencing the plants negatively or positively. The microbiote correspond to microbes living at the surface (epiphytes) or in the plant tissues (endophytes) (Bulgarelli et al., 2013). The microbiote play a crucial role in the plant adaptations to the environment by modulation of the plants physiology, amelioration of the nutrition, or by inhibiting pathogens (Ma et al., 2018).

II. Composition of the microbiotes

The microhabitat of the epiphyte microbe is called the rhizoplane, whereas the inner root cells is called the endosphere. Bacteria, archaea, fungi, and oomycetes are identified in the rhizoplane and to the endosphere (Trivedi et al., 2020). The composition of the rhizospher and the endospher are controlled by the plants and influence by environmental factors as the edaphic factors such as the soils pH and availability and stoichiometry of nutrients including phosphorus (P), nitrogen (N), and carbon (C). The dynamic of the microbiome assembly is very dynamic during the first stages of the plants growth (Trivedi et al., 2020), underling the importance of the recruitment of the beneficial microbes during the beginning of the plant life.

A various range of bacteria and fungi species are able to colonize the different part of the plants (Bulgarelli et al., 2013; Trivedi et al., 2020). The figures 4 from (Trivedi et al., 2020) show the global diversity of bacteria and fungi in the different plants part. Bacterial show more diversity than fungi diversity in the soil and in the different plants compartments.

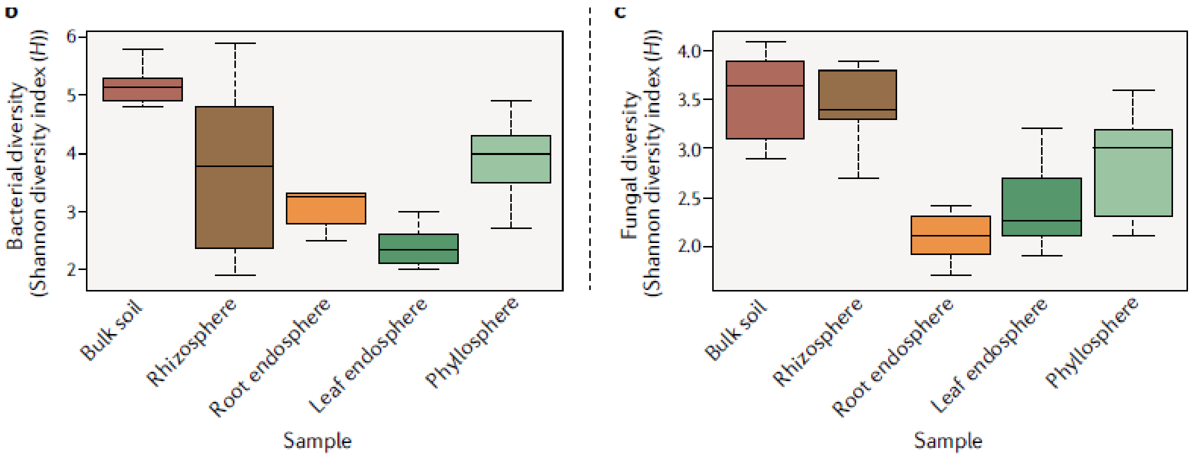

Figure 4. The diversity of bacteria and fungi change depending to the analyzed compartment. Box plots show bacterial (part b) and fungal (part c) diversity (in terms of the Shannon diversity index (H)) in the bulk soil, rhizosphere, root endosphere, leaf endosphere and phyllosphere of various plant species (Trivedi et al., 2020).

The most important genera of identified bacteria in the plants microbiote are belonging to Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Acidobacteria, Planctomycetes, Verrucomicrobia, Gemmatimonadetes and Cyanobacteria. Fungi colonizing the plant are mainly associated to Basidiomycetes and Ascomycetes (Trivedi et al., 2020).

III. Mechanisms of plants selection of beneficial microbes

The plants influence the composition of its microbiote by secretion of nutrients, secondary metabolites and microbes signaling molecules. Those occur in part by the action of a specialized root cells called border cells (Santaella et al., 2005). These cells are mainly located in the coif of the roots apex and sloughed during the roots growth. The separated border cells remain alive in the roots weeks after its separation from the plants and produce specific compounds to attract beneficial microbes (Vives-Peris et al., 2020).

Successive steps are distinguished during the microbiote assembly, the first start by the providing of preferential environment for the attraction of good microbes (secondary metabolites and nutrients), then microbes produce biofilm on the plants surfaces, finally a molecular dialogues requiring specific plant and microbe genetic background occur between the two partners for the establishment of the effective association (Stępniewska and Kuåniar, 2013).

IV. The factors influencing the composition of the plants microbiote

The bacterial group identified on the rhizospher is similar to those identified in the soil. The most important variations are observed between the rhizospher, the endospher and the phyllospher. Endospher is dominated by Proteobacteria, Firmicutes and Bacteroidets. The phyllospher compromise Proteobacteria, Bacteroidetes, Firmicutes and Actinomycetes. The vast diversity of fungi that colonize both aboveground and belowground plant tissues are mainly belonging to the phyla Ascomycota and Basidiomycota (Trivedi et al., 2020).

Fungal establishment in the rhizosphere and on the plant roots seems to be more affected by stochastic variations and to respond differently to environmental factors than is true for bacteria (Stępniewska and Kuåniar, 2013). However, the progressive reduction of the microbial density form the soil to the roots and the shoots respectively indicate that host apply a strong selection of microbes colonizing its compartments(Durán, P. et al., 2018). Interestingly the analysis of the phylogenetic composition of microbes colonizing citrus, barley, maize, sugarcane, *Arabidopsis thaliana* and rice under field conditions indicate a high level of conservation of taxa, even in different geographical location indicating a conservation of microbial selection between plants (Trivedi et al., 2020).

In the addition to the mentioned factors, virus also seems to affect the microbiote composition. Interestingly, it was observed that native phage community of the soil influence the construction of the plants microbiote (Morella et al., 2018). In the addition, protists and nematodes also contribute to microbiome diversity (Trivedi et al., 2020).

Chapter IV. The different types of beneficial bacteria

Our project was focused on beneficial bacteria and their strategies for the infecting their host plants. In this chapter we will focus our attention on the two most studied groups of beneficial bacteria: the rhizobium and the plant growth promoting rhizobacteria (PGPR).

I. The rhizobium

The term rhizobim define bacteria that are able to develop the nitrogen fixation symbiosis with the leguminous plants. This process leads to the formation of the nodules by the plants, a specialized organ hosting the bacteria (Stacey et al., 2006). The rhizobia encompasses a large diversity of bacteria including alpha and beta-proteobacteria (Udvardi and Poole, 2013).

II. Introduction to nitrogen symbiosis

Nitrogen-fixing symbiosis is one of the most recent symbioses. It is estimated that its appearance took place 60-90 million years ago (Lindström and Mousavi, 2001). Among nitrogen-fixing symbioses, actinorhizal plants are established between filamentous bacteria of the genus Frankia and actinorhizal plants comprising 220 species belonging to 9 families distributed in the orders Fagales, Rosales and Cucurbitales (Lindström and Mousavi, 2001).

The best characterized actinorhizal plants are *Casuarinasp* and *Alnus sp*. During the interaction the bacteria infect the roots of their hosts by the formation of hyphae that pass through the absorbent hairs or, by "crack-entry"; exploiting the fissures that may be located at the junction between the absorbent hairs and the other cells of the root epidermis(Froussart et al., 2016).

Bacteria cause the formation of specialized organs produced by the host, the nodule. An actinorhizal nodule presents a central vascular tissue, bordered by the zone of infection and an apical meristem responsible for the continuous growth of the nodules. A root is often formed at the end of the organ and actinorhizal nodules due to their origin and structure are considered as modified roots. Within the area of infection, the bacteria fix atmospheric nitrogen and release it to the plants. In exchange, the plants provide an ecological niche for the bacteria and carbon in organic form (Froussart et al., 2016).

The most characterized nitrogen fixing symbiosis is the interaction established between leguminous plants and the soil fixing nitrogen bacteria, the rhizobia (Benedito et al., 2008). Successive steps occur for the establishment of the interaction including: recognition, infection and nodule organogenesis and finally nitrogen fixation (Oldroyd et al., 2011).

II.1. Recognition step, the starting point for the establishment of legume symbiosis

In condition of nitrogen deficiency, legumes modify the spectrum of flavonoids they secrete. The produced compounds are perceived by the rhizobia. In response to this, bacteria produce Nod factors that are detected by the plants. How the signal is perceived is not totally understood, but Lysine Motif (LysM) Receptor-Like Kinase (RLK) is clearly involved. Some mutants in LysM-RLK receptors are altered in nodulation (Madsen et al., 2003; Broghammer et al., 2012). Another receptor has been cloned and shows a very high affinity for Nod factors, but the corresponding mutant does not have a nodulation phenotype (Fliegmann et al., 2013). Whatever the initial mechanism of perception, in response to Nod factors, a symbiotic signaling pathway is activated. It involves calcium influx, ROS production, activation of protein kinases and transcription factors. This signaling leads to the expression of symbiotic genes coding proteins called nodulines (Fedorova et al., 2002). Signaling involving nod factors seems to be shared by the vast majority of nodulating legume species. However, some rhizobia are able to induce nodulation of their hosts in the absence of Nod factors (Courtois et al., 2016). In addition, soybean mutants with altered perception of Nod factors can be nodulated under certain circumstances (Yasuda et al., 2016a).

II.2. Infection and nodule organogenesis

After recognition step, the rhizobia infect legume roots by forming infection thread (Figure 3) that penetrate roots trough the absorbent hairs, which bend on contact with the symbionte and form a structure called the shepherd's crook. Some rhizobia use another pathway and infect the roots by 'crack-entry' using the fissures at the base of the lateral roots (Arrighi et al., 2012). Parallel to infection, a reactivation of cortical cell division is observed, a led to formation of nodule primordium (Benedito et al., 2008). Finally the primordium is infected by the rhizobia, which is released by the infection thread inside the host cell (Jones et al., 2007).

Infection and cortical cell division lead to the formation of a new roots organ, the nodule (Jones et al., 2009; Oldroyd et al., 2011). Based on nodule shape, we can distinguish two nodules types:

i) The determinate nodules that are mainly observed in tropical and subtropical species as soybean (*Glycine max*), pongamia (*Pongamiapinnata*), bean (*Phaseolus vulgaris*), vigna (*VignaSavi*) as well as some temperate climate species as *Lotus japonicus* (Glyan, 2018). The determinate nodules are spherical and do not form a permanent meristem. The central zone in this nodule type is occupied by infected and uninfected host cells where atmospheric nitrogen fixation occurs (Franssen et al., 1992).

ii) Indeterminate nodules are observed for example in *Medicago truncatula*. Their shape is elongated because a persistence of an apical meristem (called Zone I, i. g ZI) that allows the growth of the organ. Indeterminate nodule present different zones in addition to the meristem, the infection zone or Zone II (ZII) in which the rhizobia are internalized in the plant cells and were the bacteroids (the intracellular form of rhizobia) differentiation occur .After the ZII, a nitrogen fixation zone or Zone III (ZIII) in which the differentiated bacteroids fix atmospheric nitrogen (N_2) for the benefit of the plant (Berrabah et al., 2018). Finally a senescence zone or Zone IV (ZIV) appears when the organ becomes old, or the plants were stressed or an organic form of nitrogen, as the nitrate is added to the growth media(Pérez Guerra et al., 2010). Moreover, the senescence zone may appear prematurely in case of metabolic imbalance, such as in the case of non-effective interaction. In the ZIV the bacteroids as well as the host cell are degraded and the cellular compounds are recycled thanks to the activities of some cysteine proteinases (Puppo et al., 2005). These genes are characteristic markers of nodule senescence (Van de Velde et al., 2006; Pérez Guerra et al., 2010; Pierre et al., 2014).

II.3. The nitrogen fixation

The bacteroids express the *nif* genes cluster, which corresponds to genes encoding the different part of the nitrogenase (Udvardi and Poole, 2013). This enzyme is responsible for the convention of atmospheric nitrogen into organic form thanks to the next equation:

$$N_2 + 8e^- + 8H^+ + 16MgATP = 2NH_3^+ + H2^+\ 16MgADP + 16Pi$$

Oxygen impact negatively the nitrogenase stability, in the aim to protect this enzyme from degradation, leguminous plants product the leghemoglobin protein in the nodule (Ott et al., 2005). This protein provides a pinkish color to the symbiotic organ and regulates the O_2 diffusion in the nodule (Garrocho-villegas et al., 2007).

III. Plant growth promoting rhizobacteria

Another important group of bacteria establishing a beneficial association with the plants are the plant growth promoting rhizobacteria (PGPR) that's include a wide range of taxa (describe in the chapter III) able to colonize the plant tissues and enhance the host growth and protection.

P. fluorescens WCS365 is one of the best characterized PGPR, this strain is able to multiply on the seed coat and colonize gradually the roots tissues (Simons et al., 1997). Genetic analysis trails reveal that several genes are required for the effective infection of the plants tissues by *P. fluorescens WCS365* as the gens implicated in the motility, the adhesion to the root; the multiplication rate, the synthesis of amino acids, uracil, and vitamin B1, the presence of the O-antigenic side chain of lipopolysaccharide, the two-component ColR/ColS sensory system; fine-tuning of the putrescine uptake system (the mutant had an impaired pot operon), the site-specific recombinase Sss or XerC; the nuo operon (the mutant had a defective NADH: ubiquinone oxidoreductase), the secB gene involved in a protein secretion pathway and the type three secretion system (TTSS, (Liu et al., 2018). Together these observations indicate that colonization of the plants tissues by PGPR is a complex and multigenic process.

Most of the described PGPRs in the literature a live are able to colonize plants tissues. In the next chapter we will discuss more deeply plants endophytes, including the PGPRs and their effects.

Chapter V. Endophyte microbes

I. Introduction to endophytic microbes

Endophyte defined microbes able to colonized plant tissues without induction of negative effects (Stępniewska and Kuåniar, 2013). They are found in many mono and dicotyledonous plants and they can be isolated from various plants tissues (roots, stems, leaves, inflorescences of weeds, fruit plants, (Stępniewska and Kuåniar, 2013; Trivedi et al., 2020). The wide distribution of endophytes in the plants is explained by the fact that the variations in the outside environment put the plant metabolism out of homeostasis, which creates necessity for the plant to harbor some advanced genetic and metabolic mechanisms within its cellular system (Gill and Tuteja, 2010). Herein, the importance of microbes, especially the endophytes, increases immensely.

Actually, the most studied endophytes are fungi and bacteria (Stępniewska and Kuåniar, 2013). Most of the endophytes fellow pathogenic behaviors and it’s proposed that at least some of them are derived from pathogenic strains (Lata et al., 2018). Based on the nature of pathogenicity, endophytes may be of three types: (i) pathogens of another host that are nonpathogenic in their endophytic relationship; (ii) nonpathogenic microbes; and (iii) pathogens that have been rendered nonpathogenic but still capable of colonization by selection methods or genetic alteration (Backman and Sikora, 2008).

The relationship between host and endophytes is considered as a symbiosis with beneficiates exchanges (Arun et al., 2012). At present, research on endophytes mainly focuses on two aspects: the exploitation of valuable bioactive molecules produced by endophytes and the exploration of the possibility of endophytes as bio-control agents (Yan et al., 2019).

II. Plant tissues colonization by endophytes

Some studies reported a higher level of endophytes colonizing the phylloplane compared to other tissues. The high nutrient content, including carbon, nitrogen, and phosphorus in the leaf tissue, may have contributed to the high microbial diversity in the phylloplane (Raimi and Adeleke, 2021).

It’s proposed that fungi and bacteria in plant roots followed a stochastic assembly process for fungi and a deterministic process for bacterial (Powell et al., 2015). The main factors that may regulate microbial colonization include the plant genotype, the growth stage, the physiological status, the type of plant tissues, some soil environmental conditions (humidity and temperature), as well as some agricultural practices (Stępniewska and Kuåniar, 2013).

Moreover, the colonization of plants tissues start early during the development process and it's greatly enhanced during senescence stages. High similarity between microbes found internally in root tissue with those found in the rhizosphere has been indicated that this compartment may be one of the main sources of endophytic colonization (Bulgarelli et al., 2013; Yan et al., 2019). Endophyte microbes typically use two transmission patterns: vertical transmission from maternal plants into progeny seed is the primary mechanism with which the offspring is infected. The endophytes enter into newly formed plants during seed germination or can be introduced into the seed during its formation (for example into inflorescence during the fecundation stage, (Compant et al., 2011)). Several endophytes in aboveground tissues are horizontally transmitted via spores and/or hyphal fragmentation, by biotic (herbivores or insects) or abiotic dispersion agents (wind or rain) from plant to plant (Yan et al., 2019). Moreover the plants can acquire endophytes from by *de novo* infection of roots or aerial parts by microbes.

Plants control the epiphyte and the endophyte microbial community through multiple processes, one of the most studied is the secretion of root exudates by the host able to attract beneficial microbes for colonization of roots surface and inner tissues (Vives-Peris et al., 2020). The exudates comprise high molecule diversity, including low-molecular weight compounds (e.g., sugars, amino acids, organic acids, nucleotides, peptides, inorganics, and hormones) and high-molecular-weight compounds (e.g., polysaccharides and proteins, (Yan et al., 2019)). In the addition, a second layer of control occur during which the plants select the microbes penetrating their tissues thanks to the activation of host cell signaling after a recognition process (Yu et al., 2019). In order to penetrate the cuticle and cell walls, microbes produce cell wall-degrading enzymes such as cutinase, pectinase, cellulase, hemicellulase, protease and lignin-peroxidases to enter into the plant (de Vries and Visser, 2001). This proteinase was abundant within fungal membrane vesicles and in the plant and/or fungal cell walls at the time of infection while absent in fungal pure culture. In case of root endophyte, *Piriformospora indica* interferes with the host cell death program to form a mutualistic symbiosis with plants (Deshmukh et al., 2006). The already established plant endophytes may transmit from one generation to another generation via plant seeds. The rhizospheric bacteria may enter and establish as root endophytes through emergence of lateral roots or root hair cells, primary and lateral root cracks, and diverse tissue wounds occurring as a result of plant growth (Lata et al., 2018).

Both abiotic factors and host genotype interact to control microbial communities. These variations act directly on a small number of closely linked taxa and exert a strong effect on communities, via microbe–microbe interactions, transmission of the effects to the microbial community, and causing changes of plant microbial community structures (Gill and Tuteja, 2010). Moreover, the microbial metabolic pathways of colonization may play an important role as determinants of endophyte diversity. For example, the rate of motile bacteria isolated from the interior part of roots was approximately five folds higher than that of bacteria in the soil tightly adhering to the roots. It has been proved that the ability of soil bacteria to approach plant roots is induced by chemotaxis and the efficiency in microcolony formation. These are the key factors that determine the success of bacteria to become endophytic. Metagenomic analyses of bacterial microbiota in plants have shown that the phylogenetic and taxonomic composition of such microbial communities is limited to few bacterial phyla, including actinobacteria. Endophytic bacteria show a tremendous diversity not only in plant hosts, but also in bacterial taxa (Stępniewska and Kuåniar, 2013).

III. Effect of endophytes on plants growth promotion

Endophytes can enhance plant growth through multiple processes; actually two of them are largely harvested:

i) Improvement of the plant nutrition through providing essential nutrients inaccessible or in a poor concentration into the soil. As an example microbes thanks to the action of specific group of enzymes, can solubilize phosphate and potassium trapped in the soil into chemical complexes (Stępniewska and Kuåniar, 2013). As an example, *Pseudomonas sp.* can mediate phosphate solubilization in rice and wheat by producing gibberellic acid (Abadi and Sepehri, 2016). In the addition, certain endophyte can convert atmospheric nitrogen (N_2) into organic form (NH_3^+) used by the plants (Arun et al., 2012). Whereas others can provide micronutrient as the iron by the production of specific proteins like siderophores that bind iron (Trivedi et al., 2020). Finally endophytes have the ability to decompose organic components, including lignin, cellulose and hemicelluloses, which facilitate in nutrient cycling (He et al., 2012).

ii) The second process for plant growth stimulation is associated to the ability of endophytes to modulate hormone concentration by excretion of certain growth or stress hormones as auxins, cytokinines, gibberellins and abscisic acid (Ma et al., 2018). Microbes can also manipulate the concentration of hormone into the plants by degrading their precursors, for example several strain produce ACC (Acide 1-aminocyclopropane-1-carboxylique) desaminase enzyme which is able to degrade the ethylene precursor, the ACC (Ratchasima, 2015).

IV. Plant protection from stresses by endophytes

Endophytes improve plant responses to stress by multiple ways, as biosynthesis of antistress biochemicals (Schulz et al., 2002). Endophytes can also stimulate plants immunity and enhance the plant tolerance to the stress through activation of specific signaling pathways. Certain MAMPs (Microbial Associated Molecular Pattern) like the flg22 peptides and ß-glucan that are found in respectively the bacterial flagella and the fungal cell wall can trigger the plant immune system (Yu et al., 2019). Those conduct to the increase of the immunity level and thus reduce the impact of biotic and abiotic stress on infected plants (Lata et al., 2018). Moreover, endophytes can also induce the overproduction of antimicrobial or immunological compounds by plants (Yan et al., 2019). *Trichoderma hamatum UoM 13* can induce the overproduction of endogenous salicylic acid and the overexpression of defense enzymes and PR (Siddaiah et al., 2017).

In addition, endophytes can ensure symptomless survival in plant tissues in two ways: one is that the microbes produce toxic metabolites to counter those of the host and mediate a host endogenous defense response by influencing phytohormone concentrations (Zamioudis and Pieterse, 2012); another way is that plants detoxify constitutive defense metabolites and secrete lytic enzymes. Moreover, endophytes participate in the regulation of redox stat of inoculated plants, by production of ROS scavengers including glutathione, ascorbate and tocopherol, and the enzymes, superoxide dismutases (SOD), catalases (CAT), ascorbate or thiol-dependent peroxidases (APX), glutathione reductases (GR), dehydroascorbate reductases (DHAR) and mono-dehydroascorbate reductases (MDHAR). Finally the balanced antagonism hypothesis posits that a balance exists between plant defensive responses in response to endophytes and the toxic effect of endophytes on plants, that’s could conduct to a fine tuning of the plant response to the stress (Lata et al., 2018).

Endophytes play a great role in the amelioration of plant adaptation to abiotic stresses (drought, salinity, pollutes…etc, (Lata et al., 2018)). Endophyte-associated plants (panic grass, rice, tomato and dune grass) have been reported to use significantly less water, increased biomass than in non-symbiotic plants. These microbes enhance resistance to drought by the reduction of leaf conductance and a slowdown of the transpiration stream (Malinowski and Beleskey, 2000). Endophyte manipulates secondary signals as phytohormones (e.g. ABA, ethylene), ROS and intracellular second messengers (e.g. phospholipids) to enhance plant resistance (Zhang and Davies, 1991). Root-derived ABA secreted by endophytes can ascend with transpiration flow to regulate stomata aperture in leaves under drought. The ability of grass species *Dichanthelium lanuginosum* to survive soil temperatures ranging between 38 and 65°C in Yellowstone National Park was directly linked to an association with the fungus *Curvularia protuberata* and its mycovirus (Redman et al., 2002). Another great example of amelioration of plants stress responses by endophytes is the inoculation of the barley by *P. indica* that's induces salt tolerance by increasing the levels of antioxidants (Baltruschat et al., 2008).

V. Bio-control

Another important process of plant protection by endophyte against invaders is antagonisms occurring by production of antimicrobial compounds by endophyte against pathogens (Backman and Sikora, 2008). An example of this mechanism is *Paraconiothyrium SSM001*, which acts as a fungicide against host pathogens by producing taxol, which suppresses the mitosis of other fungi. *In vitro*, co-culture of SSM001 with other fungi from its host plant (*Alternaria* or both *Alternaria* and *Phomopsis*) can stimulate taxol biosynthesis via SSM001, either directly or via their metabolites. In plants, pathogens and their diffusible chemicals, such as chloromethane and chitin, can induce the expression of genes related to the taxol biosynthesis and the release of taxol by SSM001. Yew tree can interact with SSM001, during pathogens attack, since Yew tree perceives pathogen entry or branch cracks, SSM001 can migrate to the perceived points where taxol is specifically released (Soliman et al., 2015). Actually, antagonistic endophytes were shown to be mainly fungal endophytes, and they were found primarily in weed and medicinal plant samples (Stępniewska and Kuåniar, 2013; Nair and Padmavathy, 2014).

Endophytes participate into plants responses against herbivore by production of toxic compounds against herbivores and thus protect plants from animal ingestion. For example several fungi can produce bioactive alkaloids, while root-colonizing pseudomonads, may directly act against plant-feeding insects by producing volatile organic compounds (VOCs) that have insecticidal properties (Yan et al., 2019).

V. Other effects

In addition to describe effects, endophytes displays a various range of other benefits applied to agriculture as well as to the industry and the medicine (Schulz et al., 2002; Nair and Padmavathy, 2014). Actually a total of 157 different bioactive compounds from both endophytic fungal and bacterial genera were identified. The variation observed in the synthesis of bioactive compounds amongst endophytes varied with host type, endophyte species, and cultivation medium (Raimi and Adeleke, 2021).

V.1. Bioremediation

Endophytes can degrade several pollutes *in* or *ex planta*, that's include heavy metals, hydrocarbons, volatiles compounds (carbon dioxide, methane, nitrous oxide, and halogenated compounds) and other contaminant as phenols, toxic dyes, pesticides…etc (Stępniewska and Kuåniar, 2013). A promising area of exploitation of endophytic bacteria for phytoremediation of contaminated environments has been described. Large numbers of bacterial strains isolated from grapevine (*Vitis vinifera L.*) plants were resistant to lead, mercury, nickel, zinc, and manganese (Altalhi, 2009).

Biodegradation of pollutes is associated generally with microbial growth and metabolism; microbes catabolize pollutes and used it for their growth. In phytoremediation processes, selected or engineered microorganisms have been recently used in order to enhance phytoremediation (Stępniewska and Kuåniar, 2013). Numerous studies have demonstrated that endophytic microorganisms can accelerate these processes efficiently by interacting closely with their host plants (Abhilash et al., 2012). These microorganisms reside inside both specific plant tissues and the root cortex or the xylem (Stępniewska and Kuåniar, 2013). Endophytes can also colonize dead and hollow hyaline cells of the plant genus Sphagnum which can be used for assimilation of methane, fixation of nitrogen, bioremediation of pollutants (e.g., pesticides, herbicides, insecticides, petrochemicals, polychlorobiphenyls, phenols/chlorophenols), and biotransformation of organic substances, for example propylene to epoxypropane and production of chiral alcohols. The methanotrophic endophytes inhabiting *Sphagnum spp*. can act as natural methane filter that can reduce CH_4 and CO_2 emission from peat lands by up to 50 % (Stępniewska and Kuåniar, 2013).

Several studies related to heavy metal decontamination (direct or indirect) by endophytes have been published. As example the bacterium *Bacillus sp.* reduced cadmium to approximately 94 % in the presence of industrially used metabolic inhibitors N,N′-dicyclohexylcarbodiimide (specific ATPase inhibitor, DCC) or 2,4-dinitrophenol (DNP). Similarly, inoculation with endophytic bacteria, *Serratia nematodiphila LRE07*, alleviated growth inhibition in *Solanum nigrum L.* in the presence of cadmium (Wan et al., 2012). Endophyte can enhance plant growth in the presence of heavy metal by manipulation of the hormones, alteration in the levels of 1-aminocyclopropane- 1-carboxylate (ACC) by *Pseudomonas* and *Gigaspora* can alter the tolerance of heavy metals directly through the manipulation of plant ethylene levels (Stępniewska and Kuåniar, 2013).

Interestingly, recombinant endophytic bacteria are easier in application than genetic plants because their strains can successfully colonize multiple plants. That's underlying the importance of using these microbes in the area of the bioremediation (Stępniewska and Kuåniar, 2013).

V.1. Medical applications

Endophytes display a high potential for identification of bioactive compounds used in medical treatment of various disease: antifungal, anticancer, antioxidant, anti-plasmodial, anti-hypertensive, anti-sleeping sickness, and antistroke. Streptomyces is the largest antibiotic-producing genus against clinical microorganisms (fungi and bacteria) and parasites. They also produce other clinically important bioactive compounds such as immunosuppressant. Several metabolites produced by endophytic are also found in their host plants. It has been suggested that the genes that govern these antimicrobial metabolites might originate from the host plants though horizontal gene transfer (HGT). The production or release of antimicrobial metabolites by beneficial endophytic fungi can be stimulated by elicitors, such as pathogen-derived chemicals or other residents within their host plants (Raimi and Adeleke, 2021).

Endophytes exhibit high antimicrobial activity against different human pathogenic bacteria. As important Gram-positive and Gram-negative bacteria, including *B. subtilis*, *E. coli*, *K. pneumonia*, *Listeria monocytogenes*, *P. aeruginosa*, *S. aureus*, *S. paratyphi*, *S. pneumoniae*, and *Vibrio cholera* (Raimi and Adeleke, 2021). Antibacterial activity of endophytes is usually attributed to the bioactive compound content of their crude extracts, as revealed in 28 *in vitro* trials (Zilla et al., 2013; Egan et al., 2016). One of the studies also demonstrated that surfactin, iturin, and fengycin derivatives from *Bacillus sp.* are responsible for its unique antimicrobial property (Jasim et al., 2016). Moreover the endophytic *D. phaseolorum* isolated from *Paullinia cupana* plant produced two major compounds: 3-HPA and DEHP, which exhibited high anti-bactericidal activity against multi-resistant strains of *P. aeruginosa* (De Azevedo Silva et al., 2018). About 15% of the studies that researched the antibacterial properties of endophytes revealed *Fusarium spp.* as the most common species with high potent antibacterial compounds (Raimi and Adeleke, 2021). Antibiotics, such as ampicillin, chloramphenicol, ketoconazole, fluconazole, streptomycin, miconazole, and rifampicin, have been reported in *Aspergillus clavatonanicus MJ31* (Mishra et al., 2017).

Due to the regular exchange of genetic materials between the endophytes and the host plants, the endophytes have developed the ability to produce metabolic compounds similar to that of the host plant. The various extracts and bioactive compounds such as alkaloids and polyketides. Secondary metabolites that may have an influence on antifungal and antibacterial properties, plant hormones, or their precursors such as plant growth factors, vitamins B12 and B1, and bio-protectants (Raimi and Adeleke, 2021).

VI. Streptomyces as promising tools for bio-solution development

Streptomyces spp. include many saprophytes, some of them becoming beneficial plant endosymbionts, but also include a few plant pathogens. These filamentous bacteria and their sporulating nature allow them to survive during unfavorable environmental conditions. Therefore, they appear to compete more efficiently against many other microorganisms present in the rhizosoil. Streptomycetes are aerobic able to produce also vegetative hyphae that eventually form a complex mycelium and are able to grow and colonize different substrates . Streptomycetes produce various lytic enzymes during their metabolic processes. Such enzymes are able to degrade insoluble organic polymers, such as chitin and cellulose, breaking them to substituent sugars for binding and uptake by multiple ABC transporters (Vurukonda et al., 2018).

The most studied actinomycetes are species from the genus Frankia, a nitrogen-fixing bacterium of non-leguminous plants (Benson and Silvester, 1993), and a few species of the genus Streptomyces that are phytopathogens (Raimi and Adeleke, 2021). Mundt and Hinckle (Mundt and Hinkle, 1976) were able to isolate different species of Streptomyces and Nocardia from 27 different plant species, finding these actinobacteria present as endophytes in different plant tissues such as seeds and ovules. Sardi et al. (Sardi et al., 1992) isolated and observed, through direct microscope examination, endophytic actinomycetes from the roots of 28 plant species from Northwestern Italy, finding actinomycetes belonging to the genus Streptomyces and other common genera, namely *Streptoverticillium*, *Nocardia*, *Micromonospora,* and *Streptosporangium* (Raimi and Adeleke, 2021).

Streptomyces are able to produce various compounds as siderophores and solubilizing phosphate, they are known also to produce various enzymes—including amylase, chitinase, cellulase, invertase, lipase, keratinase, peroxidase, pectinase, protease, phytase, and xylanase—which make the complex nutrients into simple mineral forms (Raimi and Adeleke, 2021). Several metabolites with antibiotic nature produced by pseudomonads have been studied and characterized so far, e.g., the cyclic lipopeptide amphysin, 2,4-diacetylphloroglucinol (DAPG), oomycin A, the aromatic polyketide pyoluteorin, pyrrolnitrin, the antibacterial compound tropolone (DÉFAGO, 1993; De Souza et al., 2003). Other bacterial genera, such as *Bacillus*, *Streptomyces*, *Stenotrophomonas spp*., produce the macrolide oligomycin A, kanosamine, the linear aminopolyol zwittermicin A, and xanthobactin. They also synthesize several enzymes that are able to disrupt fungal cell walls(Raimi and Adeleke, 2021).

Treating seeds with endophytic *Streptomyces spp*. and *Micromonospora spp*. prior to sowing, *A. thaliana* was protected from infection by *Erwinia carotovora* and *Fusarium oxysporum* (Raimi and Adeleke, 2021). Streptomycetes were observed antagonizing pathogens by inducing the expression of defense pathways in the plant (Maumus and Quesneville, 2014). Production of chitinolytic enzymes and siderophores (iron-chelating compounds) is a known additional mode of action for fungal growth inhibition by endophytic actinobacteria. Over 90% of chitinolytic microorganisms are actinomycetes (Raimi and Adeleke, 2021).

Streptomyces spp. have the capacity to produce cellulolytic enzymes and various secondary metabolites, which directly act on herbivorous insects and show toxic activity on phytopathogens and/or insect pests. A set of different molecules from Streptomyces spp. that act against insect pests have been found and characterized; these are, for instance, flavensomycin, antimycin A, piericidins, macrotetralides and prasinons. *Streptomyces avermitilis*, a common soil inhabitant mainly act on the insect peripheral nervous system by targeting the-aminobutyric acid (GABA, (Raimi and Adeleke, 2021)).

I. Plant response to beneficial microbes

The role of the plant immune system in detecting and controlling pathogenic microorganism has been well described. In contrast, much less is known about plant immunity in the context of the beneficial microbes and commensals that inhabit plants. Recent research indicates that, just like pathogens, commensals in the plant microbiome can suppress or evade host immune responses (Yu et al., 2019). Moreover, the plant immune system has an active role in microbiome assembly and controls microbial homeostasis in response to environmental variation. Its propose that the plant immune system can shapes the microbiome, and that the microbiome expands plant immunity and acts as an additional layer of defense against pathogenic organisms(Teixeira et al., 2019).

II. The crucial role of the immunity during legume symbiosis

The control of the immunity plays a central role for the nodule formation. During the first steps of the interaction a repression of defense genes is observed in the roots (Maunoury et al., 2010). However activation of defense response can lead to arrest of the symbiotic interaction (Jones et al., 2009). Different mechanisms have been demonstrated for the control of the immunity during the symbiosis since the first step of the interaction, to the formatted nodules.

Two phases of immunity control can be distinguished during legume-rhizobium symbiosis: the first occur from recognition to nodule initiation, we referred to this phase as the early phase. The second phase occur form nodule initiation to formatted nodules characterized by the presence of tissues zonation, this is called latest phases.

II.1. Control of the immunity during early phases of the association

Rhizobia can stimulate immune responses in host and non-host plants (Baier et al., 1999; Tesfaye et al., 2006; Oa et al., 2010; Lopez-Gomez et al., 2012). This is probably due to the presence of MTI-inducing MAMPs. However, it should be noted that to date no rhizobium MAMPs have been shown to activate an MTI-type response in its host. Symbionte have implemented different strategies to avoid or block defensive reactions (Gourion et al., 2015). This seems to be the case in *Sinorhizobium meliloti* where the part of the flagellin responsible for stimulating immune responses (flg22 peptide) was diverge during the evolution of the bacteria (Benjamin et al.; Lopez-Gomez et al., 2012). In addition, exo- and/or lipopolysacharides in some rhizobia could reduce the expression of defense genes (Gourion et al., 2015).

Some rhizobia possess effectors that could block MTI (Nelson and Sadowsky, 2015). On the other hand, plants acquired resistance genes that code for effector receptors. The interaction between the two components would in some cases induce defense response and the rejection of the symbionte (Zamioudis and Pieterse, 2012).

Hormones also play an important role in the control of defenses during infection: ethylene and the salicylic acid (SA) are two defense hormone produced by the hosts in response to pathogens (Teixeira et al., 2019). These molecules could control spatially and temporally the infection by the rhizobia (Liu and Murray, 2016). For example, the application of exogenous SA reduces the number of nodules (Martínez-Abarca et al., 1998) and conversely, the reduction of the level of endogenous SA by overexpression of the *nahG* gene, which codes an SA-degrading enzyme, increase in the number of nodules (Stacey et al., 2006). Some rhizobia interfere with the ethylene by producing ACC deaminases (Echeverria et al., 2010).

II.2. Control of the immunity during the latest phase of nodule formation

When the nodule is already formed, a repression of the immunity inside the organ steels to be required for correct nodule functioning (Benezech et al., 2020). Alteration of immunity repression leads to formation of non-functional nodules as it’s observed in some fix- mutants (Kang et al., 2016). These plants produce non-functional nodules that enable to produce organic nitrogen for the plant benefit. Certain mutants of *M. truncatula* such as *dnf2* (*dosest not fixe nitrogen 2* (Bourcy et al., 2012)), *symCRK* (symbiotic cysteine reach kinase (Berrabah et al., 2014)), *rsd* (*regulator of symbiosoms differentiation* (Sinharoy et al., 2013)) and *nad1*(*nodule with activated defenses* (Wang et al., 2016)) produce necrotic nodule with exacerbate defense characterized by accumulation of defense compounds and expression of defense genes that led to bacteroids suppression(Berrabah et al., 2018). The corresponding genes encode respectively phospholipase C-like protein, cysteine reach receptors like kinase, C_2H_2 transcriptional factors and a protein with unknown function.

The defense observed in necrotic nodules leads to the suppression of the bacteroids that conduct to the inability of nitrogen fixation by the rhizobia (Berrabah et al., 2015). It was recently showed that the ethylene control intracellular accommodation of the rhizobia, it speculated that *SymCRK* repress defenses at least through repression of the ethylene pathways. However the symbiotic genes *DNF2*, *SymCRK*, *RSD* and probably *NAD1* control the immunity respectively at different stage during nodule formation (Kang et al., 2016).

III. Immunity management during plants-PGPRs interaction

Actually, little information is available in the literature about the regulation of the immune response against PGPR during the interaction with their host. However, the presence of MAMPs in the PGPR indicates that these microbes as any other bacteria are able to stimulate defense responses as MTI (Yu et al., 2019). That is demonstrating by activation of the MAPK casades, the ROS production and the defense genes expression after inoculation of plants with PGPR (Zamioudis and Pieterse, 2012).

PGPR as the rhizobia have adapted to their host by modification of the canonical structure of their MAMP to reduce immune stimulation (Yu et al., 2019). The grapevine FLS2 receptor differentially recognizes flg22 epitopes derived from beneficial *B. phytofirmans*, initiating significantly reduced immune responses compared with the immune responses induced by the flg22 epitopes derived from the pathogenic bacteria *P. aeruginosa* and *Xanthomonas campestris* (Trdá et al., 2014). Interestingly, like rhizobia that use exopolyschararides to MAMPs recognition by the host (Gourion et al., 2015), PGPR also use this strategy. As an example certain PGPR strains share *AprA* genes which encode an extracellular alkaline protease able to degrade flagellin monomers to reduce the stimulation of the FLS2 receptor (Yu et al., 2019).

The PGPR strains display several copies of the genes encoding components of the secretion system III (T3SS) in their genome (Yu et al., 2019). T3SS is implicated in the injection of effectors into the cytoplasm of the host cell. Into the cytoplasm, the effectors interfere with specific proteins and short-circuit the activation of the immune signaling (Genin, 2010). Reduction of the ROS production and inactivation of MAPK cascades are two main process targeted by effectors during rhizobia (Gourion et al., 2015; Ge et al., 2016; Yasuda et al., 2016b; López-baena and Giraud, 2017), as well as PGPR interaction with the host (Yu et al., 2019). Recently it was show that the secretion system II (T2SS) is a robust suppressor of the MTI signaling during the interaction between *A. thaliana* and bacteria strain representative of the root microbiota (Teixeira et al., 2021).

Hormones may also participate actively in the host responses to PGPRs, certain rhizobacteria are able to enhance SA and JA in their host (Pieterse et al., 2012b). Moreover, PGPR have evolved to escape from the action of hormone by acquiring genes able to manipulate hormone synthesis or action (Ma et al., 2018).

I. Scope of the master 2 project

Actually the control of the plants immunity during PGPR and endophyte infection is poorly studied. By contrast, this process is more investigated in other model bacteria as the rhizobia that are using various strategies to overcome defense activation during the symbiosis (Gourion et al., 2015).

In our project we investigate the evolutionary conservation of certain key genes of immune manipulation between rhizobia, that's are well characterized in the control of host immunity and PGPRs. For that we analyze the phylogenetic conservation of selected genes between different species of rhizobium and published PGPRs strains.

II. Evolutionary trajectory of the secretion systems II and III genes has diverged from the ribosome 16S gene

In the aim to analyze the potential conservation or the divergence of genes involved in the control of the immunity we focus our attention on the secretion system II (TIISS) and III (TIIISS) that have been described as key bacterial genes for the control of host defenses (Genin, 2010; Russell et al., 2014). Phylogenetic analysis were made on protein sequences of TIISS and TIIISS from described PGPRs and rhizobial strains. To analyses the level of the phylogenetic divergence, we compared the results obtained for each secretion system with the phylogenies of a typical gene used for strain identification and classification, the genes encoding the ribosome subunit 16. This gene is considered as good markers of the phylogenetic divergences between bacteria (Head et al., 1993; LaMontagne et al., 2002; Rajendhran and Gunasekaran, 2011).

For studied strains, we downloaded the DNA and the protein sequences for respectively the ribosome 16S and the secretion systems from the NCBI database (https://www.ncbi.nlm.nih.gov/). Phylogenic analyses were realized in the MEGAX software (https://www.megasoftware.net/) with the recommended method as describe in (Tamura et al., 2011). The figure 5 shows the obtained results.

Figure 5. Phylogenetic analysis of ribosomal 16S, TIISS and TIIISS. A: Phylogenetic analysis of the 16S ribosomal sequence of PGPR and rhizobia. Neighbor joining analysis was made on DNA sequence after a clusteral W alignment. B & C show respectively Neighbor joining tree analysis of the TIISS and the TIIISS from PGPR and rhizobia. Muscle alignment was realized. Green: rhizobia and red: PGPR. For the secretion, certain bacterial strain displays various genes copies.

Analysis of the ribosomal 16S sequence reveals the presence of two separated clusters (Fig.5A): the first cluster contain only rhizobia (green) and the second show only PGPRs. Interestingly analysis of the TIISS (Fig.5B) and the TIIISS (Fig.5C) reveal multiple clusters with interchange of clusters position between rhizobia and PGPRs. The TIISS display three clusters of rhizobia sequences and two cluster containing sequence from the PGPR. By contrast the TIIISS show two cluster of sequences obtained from rhizobial strain and two other from PGPR.

Together these results indicate that the evolutionary trajectory of the two analyzed genes TIISS and TIIISS has diverged from the evolution of the 16S ribosome. These new finding indicate that at least the tested secretion system are potentially subjected to an evolutionary selection different than those observed for the ribosome 16S.

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Abstract

Plants interact with various microbes; some of them are pathogens, whereas others are benefic and make a symbiosis with the plants. The fine tuning of the immunity is crucial for the plants to adapt its response according to the nature of the microbes (pathogen or symbionte). Rhizobia and Plant Growth Promoting Rhizobacteria are two important groups of bacteria, making symbiotic interaction with the plants. These microorganisms use different strategies to shut down the immune plants responses and to make effective symbiosis. Here we have analyzed the different strategies describe in the literature and used by beneficial bacteria to evade from the immune responses during symbiosis. Moreover, we have investigated the conservation in selected rhizobia and PGPR strain of certain key genes used by the bacteria to shut down the plant immunity (the secretion system II and III). Our preliminary results indicate that the studied genes have evolved differently than the gene encoding the ribosome subunit 16, which is used as a key marker for the analysis of the evolution of the bacteria.

Résumé

Les plantes interagissent avec divers microorganismes; certains d'entre eux sont pathogènes, tandis que d'autres sont bénéfiques et établissent une symbiose avec les végétaux. Le control de l'immunité est crucial afin que les plantes adaptent leur réponse en fonction de la nature des microorganismes auxquels elles font fasses (pathogènes ou symbiotes). Les rhizobiums et les rhizobactéries qui favorisant la croissance des plantes (PGPRs) sont deux groupes importants de bactéries qui créent des interactions symbiotiques avec les plantes. Ces microorganismes utilisent différentes stratégies afin de bloquer les réponses immunitaires des plantes et créer une symbiose efficace. Dans notre travail nous analysons les différentes stratégies décrites dans la littérature et utilisées par les bactéries bénéfiques pour échapper aux réponses immunitaires au cours de la symbiose. De plus, nous avons étudié la conservation dans des souches de rhizobia et de PGPRs certains gènes clés utilisés par les bactéries pour bloquer les réponses immunitaires chez l’hôte (le système de sécrétion II et III). Nos résultats préliminaires indiquent que les gènes étudiés ont évolués différemment du gène codant la sous-unité 16 du ribosome, qui est utilisé comme marqueur clé de l'analyse de l'évolution des bactéries.

ملخص:

تتفاعل النباتات مع الميكروبات المختلفة؛ بعضها من مسببات الأمراض، في حين أن البعض الآخر مفيد ويتعايش مع النباتات. يعد الضبط الدقيق للمناعة أمرًا ضروريًا للنباتات لتكييف استجابتها وفقًا لطبيعة الميكروبات (الممرض أو المتعايش). بكتيريا الجذور والبكتيريا الجذرية المعززة لنمو النبات هما مجموعتان مهمتان من البكتيريا لها علاقات تعايشية مع النباتات. تستخدم هذه الكائنات الحية الدقيقة استراتيجيات مختلفة لإيقاف استجابات النباتات المناعية ولخلق تعايش فعال. لهذا قمنا بتحليل الاستراتيجيات المختلفة الموضحة في المؤلفات والتي تستخدمها البكتيريا المفيدة للتهرب من الاستجابات المناعية أثناء التعايش. علاوة على ذلك، قمنا بالتحقيق في قدر المحافظة على جينات رئيسية معينة تستخدمها البكتيريا للايقاف مناعة النبات (نظام الإفراز الثاني والثالث) في سلالات مختارة من بكتيريا الجذور والبكتيريا الجذرية المعززة لنمو النبات. تشير نتائجنا الأولية إلى أن الجينات المدروسة تطورت بشكل مختلف عن الجين المشفر للوحدة الفرعية للريبوسوم 16، والتي تُستخدم كعلامة رئيسية لتحليل تطور البكتيريا.