Synthetic Communities of Bacterial Endophytes to Improve the Quality and Yield of Legume Crops

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Chapter

Synthetic Communities of Bacterial Endophytes to Improve the Quality and Yield of Legume Crops

Mariela I. Monteoliva, Lucio Valetti, Tania Taurian, Clara S. Crociara and María Carla Guzzo

Abstract

Plant-associated microbiomes confer fitness advantages to the plant host by growth promotion through different mechanisms including nutrient uptake, phytohormones production, resistance to pathogens, and stress tolerance. These effects of the potentially beneficial microbes have been used in a diversity of biotechnological approaches to improve crop performance applying individual bacterial cultures. However, healthy plants host a diversity of microorganisms (microbiota). Next-generation sequencing technologies have offered insights into the relative abundances of different phylogenetic groups in a community and the metabolic and physiological potential of its members. In the last decade, researchers have started to explore the possibilities to use temporal and functional combinations of those bacteria in the form of synthetic communities. In this chapter, we review the benefits of using endophytic bacteria in legumes, the available methodological approaches to study the effects of bacterial communities, and the most recent findings using synthetic communities to improve the performance of legume crops.

Keywords: sustainable agriculture, abiotic and biotic stresses protection, food security, endophytic bacteria, synthetic communities

1. Introduction

Plants constitute vast and diverse niches for endophytic organisms, and there is not a single plant species devoid of them. The most up-to-date definition for endophytes defines them as the microorganisms isolated from surface-sterilized plant tissues, which do not cause any noticeable harm to their host plants [1, 2]. The most abundant and common microbes living as endophytes are bacteria and fungi [3]. Endophytic bacteria are present in any kind of plant, from ferns and bryophytes to mono and dicotyledonous species [4]. In nature, mainly the intercellular spaces of the plant host are colonized by the endophytic bacteria [1, 5, 6]. But, endophytes have been also found in intracellular spaces of grapevine, barley, tobacco, Arabidopsis, and pine [7], suggesting that legumes may also have intracellular endophytes.

The endophytic bacterial communities make significant contributions to growth promotion and plant health in mutualistic (even symbiotic) relationships. The plant host protects the bacteria from the environment, while the endophytic community provides several benefits to the host. The benefits for the plant may include nutrient assimilation (such as nitrogen, phosphorus, or iron), growth stimulation, defense against pathogens, and/or protection against environmental stresses [8, 9]. Some of these effects might be altered when the plant is under stress [10].

The use of these natural symbionts/mutualists offers an opportunity to maximize legume crop productivity while reducing the environmental impacts of agriculture. For decades, most of the studies (and agricultural applications) have been about the effects of individual strains of bacteria, but recently with the bloom in bioinformatics and sequencing technology development, the knowledge about the plant microbiota has burst, and the potential to use and manipulate complex bacterial communities has started to be the target of a large research community.

2. Plant endophytic microbiome

In natural environments, the intracellular spaces of legumes are inhabited by numerous microorganisms, such as virus, fungi, nematodes, and bacteria. Here we focus on bacterial endophytes that benefit the plant in some way. Those bacteria colonize the host by several mechanisms, such as natural opening or injures and proliferate within the host. There is a huge taxonomic and functional diversity of endophytic bacteria, adapted to the microenvironments that the plant host provides. That diversity will be shaped by the microbial community members, the plant host, and the environmental conditions.

2.1 Colonization and distribution within the host plant

Colonization mechanisms vary with the type of interaction between the host and the bacteria and the life cycle of the microbe. Overall, most of the endophytic bacteria enter the plant through the roots. Since the microbial diversity decreases from the root to the leaves, it has been proposed that most of the microbes colonize the plant through the roots and proliferate to aboveground tissues [11] (**Figure 1**). Endophytic bacteria are usually "recruited" by plant host root exudates, such as organic acids, amino acids, and proteins [12, 13]. Once the bacteria are close to the root surface, they enter through lateral root emergence areas or other openings, caused by wounds or mechanical injuries. In the early stages, most of the endophytes are first observed in root hairs and subsequently in the root cortex [14]. However, endophytes can also colonize the leaves through the stomata, injuries in the epidermis, or introduced by vectors. In leaves, bacterial endophytes have been observed in the intercellular spaces of mesophyll, substomatal areas, and xylem tissues [15, 16].

In addition, the habit of the microbe conditions its colonization strategy. For example, obligate endophytes, which depend on the plant metabolic activity for their survival, are usually transmitted to the seed (vertical transmission) and spread inside the plant or through the action of a vector. On the contrary, most of the facultative endophytes, which have a free life in the soil and colonize the plant during some stage of their life cycle, colonize the plant through occasional wounds [17].

The colonization process itself alters host plant physiology (in a process called "niche construction" from the microbe's point of view) by defense alterations or direct shift of the host metabolism [18]. Those microenvironment changes can affect the local microbiome structure and functions, by altering relationships

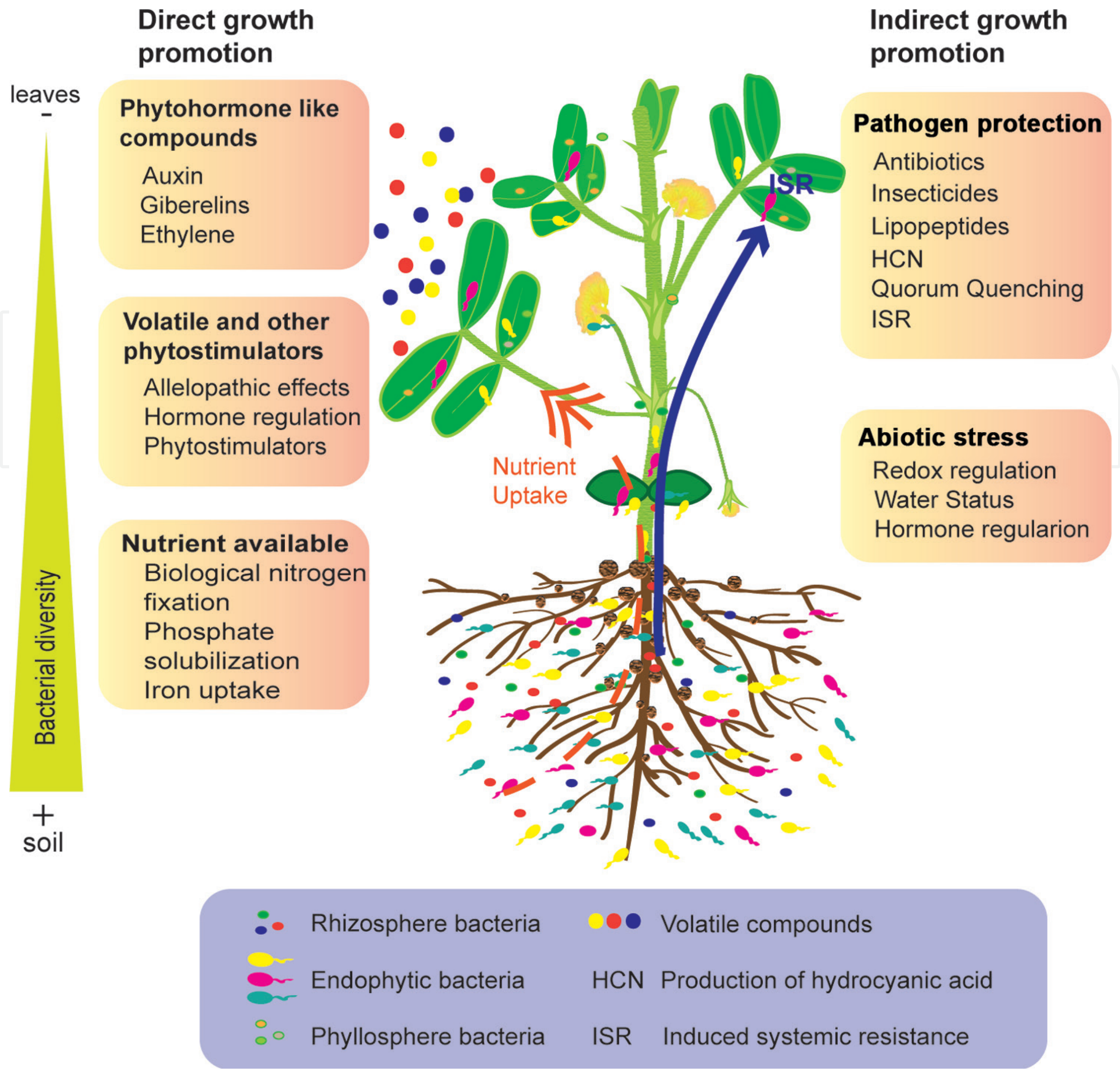

Figure 1.
Diversity gradient of bacterial endophytic microbiota and growth promotion mechanisms to legumes. Legumes are surrounded and interact with bacteria in the soil and air (epiphytic bacteria in the rhizosphere and phyllosphere) and in the inter- and intra-cellular spaces (endosphere). Those bacteria can be saprophytic, pathogenic, or beneficial for the plant. The beneficial bacteria can promote plant growth by direct and indirect mechanisms. Direct mechanisms include phytohormone, volatiles, and other compounds production and facilitation of nutrient assimilation. Indirect mechanisms include pathogen and abiotic stress protection. ISR, induced systemic resistance.

among bacterial species and within the host. Furthermore, under particular conditions, part of the response of the plant will stimulate or recruit specific endophytes, which may contribute to survival or tolerance of that condition [19, 20]. It was proved in tomato cultivars that the transplant of the rhizosphere from a resistant to a susceptible cultivar suppressed *Ralstonia solanacearum* disease symptoms. They found a highly abundant flavobacterial genome in the resistant cultivar rhizosphere, and the isolated flavobacteria suppressed disease symptoms in the susceptible cultivar in pots [21]. In legumes, it was reported that *Fusarium*-resistant common bean cultivars showed a higher abundance of Pseudomonadaceae, Bacillaceae, Solibacteraceae, and Cytophagaceae families [22], but no further inquiries have been reported.

Another aspect affecting the colonization process of the endophytic bacteria is the host defenses. Endophytes live in the same environment as many plant pathogens and share close similarities with them. Microbe- or pathogen-associated molecular patterns (MAMPs/PAMPs) are conserved and necessary for microbial survival, but plants have evolved multiple receptors to recognize them and induce the plant immune system. Then, the colonization of endophytic bacteria triggers plant defenses, and the process needs to be avoided or blocked by the beneficial endophytes to be able to colonize and proliferate within the host [2, 23, 24]. It is not well understood yet how the beneficial bacteria overcome the defenses, but a few mechanisms have been unraveled, including the blockage of MAMPs and defense signaling [25]. The beneficial bacteria *Bacillus subtilis* avoid a strong defensive response in the host by blocking the detection of their own flagellin by the secretion of the flagellin-binding peptide subtilomycin [25, 26]. Another mechanism is the secretion of bacterial antioxidant enzymes, such as superoxide dismutases and glutathione-S-transferases to detoxify the reactive oxygen species that signals the plant defense [27]. An alternative mechanism is the suppression of salicylic acid (SA)-mediated defense signaling. *Sinorhizobium fredii* HH103 with defective type III secretion system (T3SS) is unable to suppress SA-dependent defenses and subsequently fails to promote nodulation on the host [28], indicating that the suppression of the SA-dependent defense is critical for endophyte colonization. Some of those mechanisms have not been reported in legumes, but if those bacteria are colonizing legumes, similar mechanisms might be in action.

The establishment of the endophytic bacterial community in the legume host is a complex and dynamic process that has been studied mostly in fragments and simplified systems (usually one bacterial strain in one host under one or a few conditions), and it must be further understood to take the best advantages of their potential benefits for legume agriculture.

2.2 Endophytic bacterial diversity

There is an enormous diversity of bacterial endophytes in legumes, considering that the rhizobia are also endophytes. The interaction of rhizobia and legumes has been studied for more than a century [29]. Since then, many rhizobial endophytic bacteria were isolated from different legumes, particularly root and nodule tissue. These bacteria can establish a symbiotic interaction, induce the formation of new organs in roots and stems called nodules, and fix atmospheric nitrogen. In addition, the so-called “new rhizobia” (or noncanonical rhizobial genera) of Alfa- and Beta-Proteobacteria has been reported in the last decades. They can form nodules and fix nitrogen and mainly belong to *Microvirga* spp. and *Burkholderia* spp., respectively [30]. Other non-nitrogen-fixer endophytes are present in nodules and sometimes improve nodule formation [31–33]. For instance, Hoque et al. [34] isolated rhizobia and non-rhizobia endophytes from two wild *Acacia* species from Australia, and nodules were produced by species of the genera *Rhizobium*, *Ensifer*, *Mesorhizobium*, *Burkholderia*, *Phyllobacterium*, and *Devosia*, much more than expected. In addition, rhizobial species were isolated from other plant tissues apart from nodules [3].

Overall, from a large number of bacterial genera present in legumes, the most frequent ones (excluding rhizobia) are *Agrobacterium*, *Bacillus*, *Enterobacter*, and *Pseudomonas*, followed by *Acinetobacter*, *Arthrobacter*, *Curtobacterium*, *Devosia*, *Dyella*, *Herbaspirillum*, *Klebsiella Micromonospora*, *Microbacterium*, *Mycobacterium*, *Ochrobactrum*, *Paenibacillus*, *Pantoea, Rhodopseudomonas, Serratia, Staphylococcus*, and *Sphingomonas* ([3, 9, 21], and reference therein) (**Tables 1** and **2**).

2.3 Factors affecting diversity

The composition, diversity, and abundance of the endophytic microbiome are influenced by the soil microbial pool; the plant host identity and status (genotype, development, and physiology); agricultural practices; and climate and environmental conditions (such as temperature, water supply, and nutrients) [8, 16, 71].

Legume species	**Organ**	**Treat.**	**Method**	**Most abundant bacterial**	**Functions**	**Ref.**
Peanut *Arachis hypogaea*	Seed germs, sprout, cot.	Develop.	*16S*	*Synechococcus; Halothiobacillus, Paracoccus, Agrobacterium, Gallionella; Mycobacterium, Rhodococcus, Burkholderia, Erwinia, Hyphomonas, Devosia*	N.D.	[35, 36]
	Root	Monocrop vs. crop rotation	MG, MT	*Bordetella, Burkholderia, Ktedonobacter, Ktedonobacter racemifer, Opitutus terrae, Thermomicrobium roseum, Chloroflexus aggregans, Thermosediminibacter oceani, Dehalogenimonas lykanthroporepellens*	N, S, P metabolisms, oxidative stress resistance, antibiotics, siderophores, IAA synthesis genes	[37]
Chickpea *Cicer arietinum*	Roots, nodule	BT-transgene	*16S*	*Calothrix, Rickettsia, Mesorhizobium, Methylobacillus, Arthrobacter, Bacillus, Streptomyces, Saccharopolyspora, Rhodococcus, Ramlibacter, Propionivibrio, Janthinobacterium, Kaistobacter, Sphingomonas, Ammoniphilus, Rubrobacter. Actinocatenispora, Pseudaminobacter, Burkholderia Shinella.*	N.D.	[38]
Rosewood *Dalbergia odorífera*	Nodule	Seedlings, rhizobial inoculation	*16S*	*Bradyrhizobium, Chloroplast norank, Lactococcus, Mycobacterium, Bacillus, Rhizobium, Mesorhizobium, Burkholderia*	N.D.	[39]
Soybean *Glycine max*	Nodule	Salty soils	*16S*	*Ensifer, Enterobacter, Stenotrophomonas, Chryseobacterium*	N.D.	[40]
	Root	Soil type	*16S*	*Klebsiella, Pseudomonas, Stenotrophomonas, Rhizobium, Acinetobacter, Chryseobacterium, Acidovorax, Achromobacter, Agrobacterium, Burkholderia*	IAA, BNF, P solubilization, ACC-DA	[41]
		Strigolactone-related genes	*16S*	*Microbacteriaceae, Rhizobiaceae, Bdellovibrionaceae*	N.S.	[42]
	Root, nodule, soil	Develop., soil type	*16S*	Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes	N.D.	[43]
		Develop.	*16S*	*Bacillus, Bradyrhizobium, Rhizobium*	N.D.	[6]

Legume species	**Organ**	**Treat.**	**Method**	**Most abundant bacterial**	**Functions**	**Ref.**
Alfalfa *Medicago sativa*	Nodule	Synthetic community	*16S*	*Brevibacillus, Paenibacillus, Pantoea, Pseudomonas*	Antibiotics	[44]
		—	*16S, nodC, nodA, nifH genes*	*Sinorhizobium, Rhizobium, Bacillus Shinella, Pseudomonas, Variovorax, Novosphingobium, Methylibium, Bradyrhizobium, Mycobacterium*	N.D.	[45]
Medicago truncatula	Leaf, nodule, root	Genotype, soil	*16S*, MG	*Pseudomonas, Niastella, cyanobacteria Phormidium. Thioalkalibacter, Neorhizobium, Ohtaekwangia, Nodules: Ensifer, Rhizobium, Bradyrhizobium, Rhizobacter, Shewanella*	N.D.	[46]
Pea *Pisum sativum*	Root, nodule	Develop.	*16S*	*Rizhobium Mezorizhobium, Pseudomonas*	BNF	[47]
Black mung bean *Vigna mungo*	Nodule	—	Full-length *16S*	Ferrmicutes. *B. subtilis*, *Paenibacillus taichungensis*	P solubilization, IAA, siderophore, ammonia, HCN, ACC-DA	[48]
			18S, 16S	*Candida glabrata, C. tropicalis*	IAA, ACC-DA, siderophores, ammonia, polyamines synthesis	[49]
Mung bean *V. radiata*	Nodule	—	*16S*	*Bacillus aryabhattai, Bacillus megaterium and B. cereus*	IAA	[50]
Cowpea *Vigna unguiculata*	Nodule	—	*16S*	*Rhizobium, Paraburkholderia, Enterobacter, Strenotrophomonas Pseudomonas*	BNF	[51]
Red clover Trifolium pratense	Root	—	*16S*	*Rhizobia, Pantoea, Sphingomonas, Novosphingobium, Pelomonas*	N.D.	[52]
Lens culinaris, *P. sativum (plus canola and wheat)*	Root	Species, soil type	*16S*	*Pseudomonas, Arthrobacter,* unclassified genera of *Enterobacteriaceae, Comamonadaceae*	N.D.	[53]
A. hypogaea, G. max, V. radiata, V. unguiculata, V. mungo	Nodule	—	*16S*	*Enterobacter cloacae, E. ludwigii, Chryseobacterium indologenes, Klebsiella pneumoniae, Klebsiella variicola, Pseudomonas aeruginosa.*	BNF, P solubilization, siderophores, IAA, ACC deaminase (*nifH* gene)	[54]

Legume species	Organ	Treat.	Method	Most abundant bacterial	Functions	Ref.
Vicia villosa, T. repens, T. pretense, M. sativa	Seed	—	*16S*, MG	*Acinetobacter, Sphingomonas, Lactobacillus, Bacillus, Pantoea, Salmonella*	Energy, amino acid and carbohydrate metabolisms, cell growth and death programs, transport, genes	[55]

ACC, 1-aminocyclopropane-1-carboxylate; ACC-DA, ACC deaminase activity; IAA, indole-acetic acid; BNF, biological nitrogen fixation; Develop., developmental stages; MG, meta-genomics; MT, meta-transcriptomics; N.D. not determined; N.S., not significant; Treat, treatment or factor affecting microbiome.

Table 1.
Culture-independent studies of the endophytic bacterial microbiome in legume crops.

Legume species	Organ	Treat.	Met.	Most abundant bacterial	Function	Ref.
Peanut *Arachis hypogaea*	Nodule	Genotype	*16S*	*Rhizobium phaseoli, Bacillus tequilensis, B. altitidinus, B. tequilensis, B. siamensis, B. subtilis, Pantoea dispersa, Paenibacillus illinoisensis, Kosakonia oryzendophytica, Rhizobium mayense, P. dispersa*	IAA; ACC-DA; P, Zn, and Si solubilization, siderophore	[56]
	Seed	—	*16S*	*Pseudomonas* spp.	IAA, P solubilization, siderophores, cellulase, protease, control of *S. rolfsii*	[57]
Chickpea *Cicer arietinum*	Root	Soil type	*16S*	*Enterobacteriaceae, Pseudomonadaceae, Xanthomonadaceae, Bacillus, Stenotrophomonas, Pseudomonas, Enterobacter*	N.D.	[58]
	Root, nodule	—	*16S*	*Mcrobiospora, Streptomyces, Micromonospora, Actinomadura*	N.D.	[59]
		—	*16S*	*Enterobacter, Rhizobium, Stenotrophomonas, Pseudomonas, Burkholderia, Bacillus, Brevibacillus*	IAA, siderophores	[60]
Soybean *Glycine max*	Nodule	Antifungal activity	*16S*	*Enterobacter, Acinetobacter, Pseudomonas, Ochrobactrum, Bacillus*	BNF, IAA, siderophore	[61]
	Leaf, stem, root	RR-transgene	*16S*	Enterobacter ludwigii and Variovorax paradoxus	IAA, P solubilization	[62]
	Leaf, stem, root, nodule	—	*16S* *nifH*	Pseudomonas aeruginosa and Bradirizhobium	IAA, P and Zn solubilization, siderophore, ACC-DA, cell wall degrading enzymes, pathogenicity	[63]

Legume species	**Organ**	**Treat.**	**Met.**	**Most abundant bacterial**	**Function**	**Ref.**
Lentil *Lens culinaris*	Nodule	—	*16S*	*Pseudomonas stutzer, Lysinibacillus pakistanensis*,	N.D.	[64]
Common bean *Phaseolus vulgaris*	Roots	—	*16S*	*Bacillus velezensis Bacillus amyloliquefaciens Bacillus halotolerans,Bacillus mojavensis,Bacillus methylotrophicus, Bacillus subtilus Pseudomonas frederiksbergensis Pseudomonas lini, Agrobacterium fabrum Glutamicibacter halophytocola.*	IAA, P solubilization, siderophores, HCN, xylanase chitinase, lipopeptide genes, antifungal activity	[65]
Cowpea *Vinga unguiculata*	Nodule	—	*16S*	*Rhizobium, Paraburkholderia Enterobacter, Strenotrophomonas* and *Pseudomonas*	BNF	[51]
C. arietinum, Pisum sativum	Nodule, root	—	*16S*, RFLP	*Pantoea agglomerans, Bacillus cereus,B. sonorensis, B. subtilis, Pseudomonas chlororaphis, Ornithinibacillus* sp., *Ochromobacterium* sp.,	IAA, P solubilization, siderophores, ammonia, organic acids, HCN, biocontrol	[66]
Crotalaria spp., *Indigofera* spp. *Erythrina brucei*	Nodule	Genotype	*16S*	*Achromobacter, Agrobacterium, Burkholderia, Cronobacter, Enterobacter, Mesorhizobium, Novosphingobium, Pantoea, Pseudomonas, Rahnella, Rhizobium, Serratia, and Variovorax. Bacillus, Paenibacillus, Planomicrobium*, and *Rhodococcus*.	N.D.	[67]
V. mungo, V. radiata	Stem	—	16S	*Enterobacter, Bacillus, Pantoea, Pseudomonas, Acromobacter, Ocrobacterium*	BNF, IAA, P solubilization, siderophores, antifungal activity	[68]
P. sativum, *V. faba*	Nodule	—	*16S, nodC*	*Rhizobium leguminosarum, R. indigoferae, R. hidalgonense, R. sophorae, R. laguerrea, R. acidisoli, R. anhuiense,*	IAA, P solubilization, siderophores	[69]
A. hypogaea, G. max, V. radiata, V. unguiculata, V. mungo	Root nodule	-	16S *nifH*	*Enterobacter cloacae, Chryseobacterium indologenes, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterobacter ludwigiiy, Klebsiella variicola*	BNF, P solubilization, AIA, siderophores, ACC-DA	[54]

Legume species	Organ	Treat.	Met.	Most abundant bacterial	Function	Ref.
Trifolium, Lupinus, Ornithopus, Scorpiurus, Medicago, Trifolium, Vicia	Root	Field sites	16S	*Microbacterium, Chryseobacterium, Bacillus, Paenibacilus, Staphylococcus,Pantoea, Erwinia, Achromobacter, Lelliotia, Enterobacter, Acinetobacter, Janthinobacterium, Pseudomonas, Stenothrophomonas, Serratia, Rahnella*	IAA, P solubilization, siderophore, cellulase	[70]
Anthyllis, Colutea, Cytisus, Lathyrus, Lotus, Lupinus, Medicago, Melilotus, Ononis, Ornithopus, Robinia, Trifolium, Vicia, Wisteria	Nodule	Ecoregions (Belgium)	16S	*Bacillus, Paenibacillus, Arthrobacter, Microbacterium, Rhodococcus, Sphingomonas, Cohnella, Pseudomonas, Herbaspirillum, Pantoea, Corynebacterium, Chryseobacterium, Sphingomonas and Xanthomonas*	N.D.	[31]

ACC, 1-aminocyclopropane-1-carboxylate; ACC-DA, ACC deaminase activity; IAA, indole-acetic acid; BNF, biological nitrogen fixation; Develop., developmental stages; MG, meta-genomics; MT, meta-transcriptomics; N.D. not determined; N.S., not significant; Morph & Bioch., morphological and biochemical characterization, Treat, treatment or factor affecting microbiome.

Table 2.
Culture-dependent studies of the endophytic bacterial microbiota in legume crops.

Comparisons among plant species (canola, wheat, pea, and lentil) in different locations and soil types pointed to the genotype influence as the highest effect determining endophyte diversity ([72] in **Table 1**). However, when considering close *Medicago* genotypes (intraspecies comparison), the host genotype effect was not significant (1% of contribution to the total variance), but both soil and plant genotypes were significant for the root microbiota diversity [53]. In the case of the leaf microbiome, the soil reduces its relative importance, since some bacteria colonize it from underground organs, but others enter through stomata or vectors [46]. Broadly, the soil limits the available microbial pool, while the host genotype is a relevant barrier for colonization. Agricultural practices could directly affect the microbiome by chemical applications or through changes in the host physiology. The effects of biotic and abiotic factors shaping the endophytic bacteria communities in plants were reviewed by Papik et al. [73]. In addition, the actual diversity could be masked by the method used to describe it (such as culture-dependent or -independent, see Section 2.4) [16].

2.4 How to study microbiome diversity

Natural communities of endophytic bacteria are conventionally studied using culture-dependent and -independent methods [74]. Culture-dependent methods imply the extraction of the microbes and their growth in synthetic media. Those strategies allow to isolate the microbe and further study them *in vitro* and in manipulative experiments, but they strongly underestimate the number of bacteria (and the diversity of the community), as cultivable bacteria usually represent only 0.001–1% of the actual bacteria in a sample [16, 75]. Recently, Hartman et al. [52] isolated 200 bacteria strains that represent ~20% of the most abundant genera in *Trifolium* roots, which was one-quarter of the ~3500 detected OTUs in a manageable effort to increase the cultivated endophytic bacteria from a legume (**Table 1**).

On the other side, culture-independent methods mostly rely on the extraction of bacterial genetic material from plant tissues. The genomic DNA can then be analyzed using a range of molecular fingerprinting techniques such as Amplified rDNA Restriction Analysis, Gradient Gel Electrophoresis, and Terminal Restriction Fragment Length Polymorphism (RFLP) [16]. In recent years, DNA fingerprinting techniques have been set aside by more advanced molecular techniques. Those new methods involve DNA extraction from the entire bacterial population to sequence a specific phylogenetic marker, such as the 16S rRNA gene, or the whole genome [76]. In addition, using RNA instead of DNA, it is possible to detect active functional diversity, which provides information about the transcriptionally active functions, as well as the massive analysis of proteins (peptides) or metabolites (by high throughput analysis of "omics"). The latter two do not provide taxonomic information but a functional one.

The sequence-based methods allow a deeper analysis of the endophytic diversity than traditional fingerprinting, although some of the species with low abundance might be still missed. To minimize those losses, it is important to sequence with high depth and carry out rarefaction analysis (to check that the OTU versus the diversity or richness reaches the plateau). Other technical considerations for sequencing analysis are discussed in detail by Lucaciu et al. [77].

The bacterial diversity of the microbiome can be described taxonomically and functionally by different approaches. The most traditional strategy is the taxonomic description of the diversity, which identifies the species present in the microbiome and quantifies their abundance by genome or specific gene sequencing. From that data, researchers have started to uncover what is known as the "core microbiome" [78], which is defined as the group of species present in one plant across different genotypes, environments, developmental stages, etc. Depending on the scale of the analysis, a higher or lower number of species are shared among them. For instance, if dicot and monocot species are compared, the number of shared species will be lower than if two cultivars of the same species are compared in the same environment. A core endophytic microbiome of roots of red clover (*Trifolium pratense*) includes 70% of Rhizobia, and it was dominated by the genera *Pantoea*, *Sphingomonas*, *Novosphingobium*, and *Pelomonas* [52] (**Table 1**). *Glycine* spp. nodules showed a majority of *Ensifer* genera, followed by *Enterobacter*, *Stenotrophomonas*, and *Chryseobacterium* (>0.5%), and some nonrhizobial bacteria only in soybean (*Glycine max*), including *Enterobacter cloacae* (3.62%), *Stenotrophomonas* sp. CanR-75 (2.79%), and *Stenotrophomonas maltophilia* (2.41%) [40] (**Table 1**). Overall, little is known about the core endophytic microbiome in legumes, although some core rhizospheric microbiomes have been described (e.g., [79]).

In addition to the core microbiome, the "keystone" species have been described [80]. Keystones are highly connected species that largely change the structure and function of the microbiome when removed. They may be predicted by co-occurrence networks (by correlation analysis) and are defined as those whose abundance highly correlates with most of the other species [81]. Those correlations can be positive or negative (i.e., two species are always together or the presence of one excludes the other), and the interaction between each other may be indirect (for instance, mediated by a change in the host) [82]. It has been predicted that when the keystone species is missing, the abundance and proportion of the community change, and occasionally, one species may extremely proliferate over the others. Knowing which are the keystone species for one host is critical to effectively design any agricultural management strategy to protect a healthy microbial community and improve the fitness of the crop.

A second strategy to characterize the microbiome is the functional description, based on the metabolic functions present in the microorganisms. According to the previous model (with a core microbiome and keystone species), the communities in the microbiome are built to occupy functional niches [81]. This means that one species might be (at least partially) replaced by another one, which provides the same function to the community and/or the host. Those key functions of a particular species are given by a set of genes that allow the microbe to effectively interact and benefit the rest of the microbial community and the plant host under specific conditions. These functional traits can be screened and studied by any "omic" analysis and then grouped by the presence of specific metabolic functions (see [83, 85] in **Table 1**). For instance, the most important genes differentially detected in the rhizosphere of pea (*Pisum sativum*) under different tillage and fertilization treatments were genes coding ABC transporters and secretion systems, transcription factors, peptidases, methane metabolism, quorum sensing, and bacterial motility proteins [85]. To understand which services the microbial community provides and may favor the host plant, the functional analysis may be more useful than a taxonomic-only approach. However, both are necessary and provide valuable information about the microbiomes.

3. Benefits of endophytic microbiota to the host plant

Once within the plant, endophytes might provide several benefits. We grouped them into three different kinds: direct growth promotion, protection against pathogens, and protection against abiotic stress (**Figure 1**).

Direct promotion occurs when endophytes stimulate shoot and/or root growth by increasing the availability of limiting nutrients or producing compounds that directly stimulate growth. On the other hand, indirect promotion occurs when the endophytes can protect the plant against diseases, pests, or environmental stress, indirectly improving the host performance [86]. The molecular mechanisms and pathways are not exclusive for each direct or indirect growth promotion effect. A single endophytic bacterial strain may have more than one of these plant-growth-promoting traits (e.g. [37, 41, 48, [49, 55] in **Table 1**, and [56, 57, 63, 65, 66, 68] in **Table 2**).

3.1 Increase of nutrient availability

The main mineral nutrients required for plant growth are nitrogen, phosphorus, and iron. There are numerous plant-growth-promoting microorganisms able to increase their availability, and some mechanisms have been determined.

3.1.1 Biological nitrogen fixation (BNF)

Nitrogen is crucial for plant growth and health. Approximately 30–50% of the N in crop fields results from BNF by soil microorganisms. The ability to fix atmospheric nitrogen (N_2) is present in various bacterial species that are either free-living or endophytically associated with plant roots. BNF is the most and long-term studied plant-growth-promoting effect of soil microorganisms in legumes [87, 88]. Other plant growth promoter bacteria genera, different from rhizobia, are also able to enhance the acquisition of N by legumes. Anzuay et al. [89] and Taurian et al. [90] observed that endophytic bacteria belonging to *Serratia, Acinetobacter, Bacillus*, and *Enterococcus* enhanced peanut (*Arachis hypogaea*) N content. Dey et al. [91] reported that the increase in the number of nodules in plants inoculated with growth promoter bacteria could be attributed to the enhancement of root growth and root length. This enhancement provides more sites for nodulation by rhizobial strains in the soil. Furthermore, since BFN is a highly demanding ATP process, phosphorus is a critical nutrient for legumes.

3.1.2 Phosphate solubilization and mineralization

Even in phosphorus-rich soils (such as phosphate-fertilized soils), most of this element is in insoluble forms, and only a small proportion (~0.1%) is available to plants [92]. The solubilization of phosphates in the rhizosphere is one of the most common modes of action of growth promoter microbes that enhance nutrient availability to plants [93]. Phosphate-mineralizing and phosphate-solubilizing bacteria (PMB/PSB) secrete phosphatases and organic acids to convert insoluble phosphates (organic and inorganic) into soluble monobasic and dibasic ions [93]. Among legume endophytes, there are several phosphate-solubilizing bacteria able to promote plant growth, and some studies demonstrated that plant growth promotion was directly correlated with the increase of P in the plant tissues [89]. Soybean and peanut endophytes solubilize mineral phosphate [90]. In addition, several studies described endophytic bacteria with phosphate-solubilizing/-mineralizing ability that increase legume growth [89, 90, 94, 95]. The inoculation of pea with phosphate-solubilizing *Pseudomonas* spp. isolated from this legume, enhanced the plant biomass [96]. *Pantoea* spp. isolated from root nodules of peanut showed a strong phosphate-solubilizing activity [97]. However, the inoculation of phosphate-solubilizing bacteria isolated from peanuts did not promote growth when they were inoculated in the rapeseed culture [98]. These results point to a specific plant-bacteria interaction that directly affects the ability to promote growth or the efficiency of the mechanism.

The main phosphate-solubilizing mechanism in Gram-negative bacteria involves the bacterial PQQ cofactor, described as essential in P nutrition and plant growth. Mutation in the *pqqH* gene from *Pseudomonas fluorescens* caused the loss of the phosphate-solubilizing phenotype and plant growth promotion ability on tomato plants [99]. In legumes, Ahmed and Shahab [100] observed that a non-producing-PQQ bacteria (which lost the phosphate solubilization ability) showed a decrease in the growth promotion of bean plants. On the contrary, Ludueña et al. [101] determined that in the non-producing PQQ strain *Serratia* sp. promoted the growth of peanut at a similar level to the wild type, indicating that PQQ is not essential for growth promotion.

3.1.3 Iron uptake

Iron is essential for all living organisms, and its bioavailability in the soil is limited. Siderophores are small molecular compounds, secreted by microbes, which chelate iron in the soil and generate soluble complexes that can be absorbed by plants [97]. Microbial siderophores' secretion directly stimulates plant growth by increasing the availability of iron in the soil surrounding the roots [102]. Plants lacking soil bacteria suffered from iron deficiency [103]. Therefore, this mechanism helps plants to thrive in low-iron soils. The inoculation of black mung bean (*Vigna radiata*) with the siderophore-producing endophyte, *Pseudomonas* sp. GRP3, reduced iron deficiency and chlorotic symptoms and increased the content of chlorophyll a and b [104]. Furthermore, since diazotrophic organisms require Fe^{+2} and Mo^{+2} factors for the functioning and synthesis of nitrogenase, iron solubilization by microbes also improved nitrogen fixation in legumes [105]. Native peanut isolates produce siderophores together with other plant-growth-promoting traits, increasing peanut growth and performance [106].

3.2 Phytostimulators

Endophytic bacteria directly promote plant growth by the production of phytohormones, such as auxin or cytokinin, or by lowering the plant ethylene (ET) levels. By these mechanisms, bacterial endophytes can also accelerate seedling emergence and promote plant establishment under adverse conditions.

3.2.1 Phytohormone-like molecule production

The production of phytohormones-like compounds is considered an important trait of endophytes that positively affects the growth and development of many plants including legumes [8, 10, 107]. Thus, changes in plant growth frequently reflect alterations in phytohormone levels induced by endophytes [3]. But, even when production of these compounds by growth promoter microbes has been demonstrated, that effect cannot be unequivocally attributed to them.

The five main phytohormones produced by bacteria are auxins, cytokinin, gibberellins, ET, and abscisic acid (ABA). It has been postulated that genes encoding biosynthesis of the auxins, cytokinin, and gibberellins are often present in the metagenome of plant endophytic bacterial communities [108]; however, it has not been yet explored in legumes using any omics approach (ET and ABA are discussed in Section 3.4.3).

Among these growth regulators, auxins are the most studied. These compounds affect plant growth by inducing cell enlargement and division, root development, apical dominance, increase growth rate, photo- and geo-tropism [109]. The production of auxin-like compounds increases seed production and germination along with increased shoot growth and tillering. Within these compounds, indole-acetic acid (IAA) is the most frequent and indeed most studied phytohormone in growth promoter bacteria. IAA produced by endophytic bacteria is one of the most relevant and studied effector molecules in growth promotion, pathogen defense, and plant-microbe interactions [104]. For instance, rhizobia from soybean, pea, and faba bean nodules not only fix nitrogen and produce siderophores, but also auxins (see Refs. [54, 110] in **Tables 1** and **2**, and [61]). IAA can be synthesized directly by plant-associated microbes, and ~ 80% of the rhizosphere bacteria may produce IAA [69, 111]. For instance, it could be produced by *Alcaligenes, Azospirillum, Pseudomonas, Pantoea, Rhizobium*, and *Enterobacter* in the presence of L-tryptophan as a precursor, although there are other pathways and a variety of auxins, such as indole-3-butyric acid (IBA), indole-3-pyruvic acid (IPA), or tryptophol (TOL), which are also produced by growth promoter bacteria [112].

Cytokinins are another group of growth-stimulating phytohormones that are responsible for cell division, plant senescence, seed germination, flower and fruit development, and apical dormancy [113, 114]. Although cytokinins are produced by several growth promoter microbes, few studies have demonstrated their beneficial effects.

Gibberellins are involved in many developmental processes in plants, such as flowering regulation, seed germination, stem and leaf elongation [114], but also the promotion of nodule organogenesis and the negative regulation of the rhizobial infection and root system development [115].

Several bacteria produce and regulate the production of more than one phytohormone, such as the rhizobacteria *Bacillus aryabhattai*, which produces ABA, IAA, cytokinin, and gibberellic acids *in vitro* and promotes soybean growth [116]. Thus, inoculation with endophytic bacteria may benefit legumes via the production or suppression of some phytohormones.

3.2.2 Volatile compounds and other phytostimulators

Some growth promoters' bacteria can regulate plant growth by releasing volatile compounds [86]. For instance, *B. subtilis, Bacillus amyloliquefaciens*, and *E. cloacae* promote plant growth in legumes by releasing volatiles, such as 2,3-butanediol and acetoin [117, 118], while the mutants of *B. amyloliquefaciens* IN937a and *B. subtilis* GB03, blocked in their biosynthesis, did not promote Arabidopsis growth [118]. Studies on growth promotion by *Chryseobacterium* rhizoplane in mung bean indicate that 2,3-butanediol is the molecule causing growth stimulation [119]. Growth promotion mechanisms of volatiles in plants were reviewed by Sharifi and Ryu [120].

Other nonvolatile molecules such as bacterial cell components or secreted compounds have been proposed to be plant growth stimulators. The endophyte *Serratia proteamaculans* was able to promote soybean growth by the production of a lipo-chitooligosaccharide [121]. And the PQQ peptide, previously mentioned to be associated with P solubilization, has also shown growth promotion [99], antifungal activity, and the ability to induce systemic resistance [86]. The role of PQQ in plant-microbe interaction has been reviewed by Carreño-Lopez et al. [122].

Lastly, endophytes can generate allelopathic effects inhibiting the growth of neighboring plants or protecting the host plant from allelopathic effects from adjacent plants [123]. For example, endophytic bacteria of red clover seem to be responsible for the negative allelopathic effects observed over maize, reducing seedling emergence and height [124]. Additionally, some weeds have negative allelopathic effects on legumes, mediated by their endophytic bacteria, which inhibit nodulation [125].

Overall, there is a body of evidence that suggests that enhancing or regulating phytohormone or other phytostimulators via endophytic microorganisms is a viable strategy to increased crop production in agriculture [108], and because of these attributes, endophytes have gained ground in the area of agricultural sustainability.

3.3 Protection against pathogens

Among the major factors restraining agriculture are crop diseases and pests, while one important driver of plant health is the structure and dynamics of the plant-associated microbial communities [126]. In recent years, a deeper understanding of the endophytic microbiome and its potential has been achieved to become a fundamental tool in phytosanitary management and reduce the damage of plant diseases.

Endophytes can decrease the harmful effects of pathogens by different mechanisms, including direct and indirect mechanisms [104]. Direct inhibition of pathogens is mainly mediated by the synthesis of inhibitory allelochemicals such as antibiotics, hydrogen cyanide, iron-chelating siderophores [127], secretion of lytic enzymes, or quorum quenching (QQ) by degrading pathogens autoinducer signals [128]. Indirect biocontrol mainly includes the induction of the plant systemic resistance that inhibits the proliferation of a broad spectrum of phytopathogens [129].

3.3.1 Antibiosis

Most endophytes have been reported to produce secondary metabolites, and some of them exhibit antibacterial and antifungal properties, which help to inhibit the growth of phytopathogenic microorganisms [44]. Many metabolites with antimicrobial properties synthesized by endophytes have been described so far, such as flavonoids, peptides, quinones, alkaloids, phenols, steroids, terpenoids, and polyketides. Antimicrobial properties of bacterial metabolites were recently reviewed [130]. Hansen et al. [131] studied the microbiome of alfalfa (*Medicago sativa*) nodules and identified two families of molecules produced by *Brevibacillus brevis in planta*, such as antibacterial thyrozidines, and a new set of gramicidin-like molecules, britacidins. They conclude that, in addition to nitrogen fixation, it is likely that legume root nodules are also a source of active antimicrobial production.

3.3.2 Lipopeptides

Lipopeptides are low-molecular-weight cyclic peptides attached to a hydrophobic fatty acid. These molecules are classified into three families: surfactin, iturin, and fengycin. Iturins and fengycins show strong antifungal activities while surfactins exhibit strong antibacterial activity. Antimicrobial lipopeptides can form toroidal-like pores on cell membranes leading to membrane permeation and/or disintegration and protect plants directly suppressing the growth of pathogens or inducing systemic resistance [132]. Recently, 263 different lipopeptides were synthesized by 11 microbial genera, with *Bacillus* being the most abundant [133].

The common bean root microbiome was used to search potential biocontrol agents of *Fusarium* sp., *Macrophomina* sp., and *Alternaria* sp. fungi, causal agents of root rot disease [65]. Biocontrol assays conducted under controlled conditions demonstrated that *B. amyloliquefaciens, B. halotolerans, Bacillus velezensis, Agrobacterium fabrum,* and *Pseudomonas lini* displayed the highest protective effect, and lipopeptide biosynthetic genes encoding surfactin, iturin, bacillomycin, and fengycin were present. These bacteria can produce at least one or more lipopeptides that may be involved in biocontrol activity.

3.3.3 Lytic enzymes

During plant colonization, endophytes produce numerous enzymes, which successively aid the hydrolysis of the plant cell wall. There are numerous types of enzymes such as chitinases, cellulases, hemicellulases, and 1,3-glucanases [70, 134]. These enzymes are also capable of degrading fungal (and oomycetal) cell walls hyphae, spores, and sporangia, thus contributing to the protection of the plant. The isolate *Pseudomonas* spp. EGN 1 was the most promising bioagent for the management of the stem rot (*Sclerotium rolfsii*) in groundnut, mediated by an important protease and cellulase production [57]. While, Brigido et al. [135] evaluated the diversity and functionality of the endophytic bacterial strains in the roots of native legumes from two different sites in Portugal, finding 15 isolates with a high cellulase production.

3.3.4 Hydrogen cyanide

A few bacterial species are known to produce and excrete hydrogen cyanide, a potent inhibitor of cytochrome c oxidase and several other metalloenzymes [136]. The host plant is unaffected by the bacteria or the hydrogen cyanide produced by it. For this reason, hydrogen-cyanide-producing bacteria have an application as biological control agent. Zaghloul et al. [137] isolated a total of 167 endophytic bacterial from roots, nodules, leaves, and stems of faba bean (*Vicia faba*), pea, fenugreek (*Trigonella foenumgracum*), lupine (*Lupinus* spp.), common bean (*Phaseolus vulgaris*), and rice (*Oryza sativa*) at flowering stage. About 82% of the isolates showed positive results of hydrogen cyanide production. In another recent investigation, ~20 endophytic bacteria isolated from roots and nodules of chickpea (*Cicer arietinum*) and pea showed HCN production [66].

3.3.5 Siderophores

As previously mentioned, siderophores chelate iron in the soil making it more available for plants. Furthermore, by tightly binding the iron, siderophores reduce its bioavailability for plant pathogens and facilitate the death of the phytopathogens [138]. Some of the siderophores are known to be produced by endophytes, such as hydroxymate, phenolate, and/or catecholate types, confer biocontrol activities [139]. Also, the role of siderophores as part of the protective effect of the induced systemic resistance has been described in many studies. The production of siderophores is very common among *Pseudomonas, Frankia, Streptomyces* sp. Several researchers described endophytic bacteria producing siderophores isolated from different legumes as peanut, faba bean, soybeans, chickpea, pea, and bean [65, 66]. Bahroun et al., [140] demonstrate that *Rahnella aquatilis* B16C, *Pseudomonas yamanorum* B12, and *P. fluorescens* B8P isolated from faba bean nodules suppressed *Fusarium solani* root rot in three faba bean cultivars in greenhouse. The three strains were able to produce siderophores and significantly reduced the disease severity. Zhao et al. [54] obtained 276 isolates from root nodules of soybean, six of which showed antagonistic to the pathogenic fungus *Phytophthora sojae* 01. The isolates were identified as *Enterobacter, Acinetobacter, Pseudomonas*, *Ochrobactrum*, and *Bacillus* genera. The high correlation of siderophores production and the fungal inhibition of nodule endophytic bacteria in that study supported the idea that the ferrous absorption by endophytic bacteria may be a viable inhibitory mechanism.

3.3.6 Quorum quenching

The regulation of gene expression in response to fluctuations in cell-population density is known as “quorum sensing.” Many important bacterial processes are regulated by it. Quorum sensing regulates gene expression depending on the accumulation of a signal molecule in the environment. The signal, called autoinducer, allows the bacteria to perceive the existing population density and jointly executed responses. Gram-negative bacteria use acyl-homoserine lactone (AHL) as an autoinducer, whereas Gram-positive bacteria utilize modified peptides [141]. The bacterial quorum sensing controls a wide variety of physiological processes such as virulence, extracellular polymeric substances (EPS) production, mobility, and biofilm formation among others, which are essential for the establishment of a pathogen in the host plant [142].

Often endophytic bacteria can disrupt quorum sensing. This ability to interfere with bacterial cell-to-cell communication was collectively called “quorum quenching” and can be crucial to prevent the plant colonization by pathogenic bacteria that use quorum sensing to coordinate virulence [143]. Several chemicals and enzymes have been identified that target the key components of bacterial quorum-sensing systems in the recent years (such as [33]). The mechanisms of quorum quenching may be the inhibition of the signal synthesis or detection, signal enzymatic degradation (by enzymes such as AHL acylase, AHL lactonase, and oxidoreductases), or synthesis of structural analogs of the signal [144]. Lopes et al. [145] reported antimicrobial activity against *Pseudomonas syringae* pv. tabaci or *Hafnia alvei* 071 in endophytic bacteria isolated from common bean. The isolates *Microbacterium testaceum* BAC1065, BAC1100, and BAC2153, *Bacillus thuringiensis* BAC3151, and *Rhodococcus erythropolis* BAC2162 exhibited a greater ability to inhibit the response of AHL reporter.

3.3.7 Insecticides

Some metabolites with insecticidal action have been described. The famous *B. thuringiensis* produces crystalline inclusion bodies consisting of delta-endotoxins (also referred to as Cry proteins) during sporulation. These proteins, which are formed by variable-molecular-weight polypeptides (27–140 kDa), are highly toxic for a broad range of pest insects [146]. *P. fluorescens* strains exhibited a protective effect against aphids and some herbivorous beetles and termites [147]. The bacterium *Lysinibacillus sphaericus* (former *Bacillus sphaericus*) produces sphaericolysin, which is toxic for *Spodoptera litura* [148].

3.3.8 Induction of systemic response

Induced systemic resistance (ISR) is a term used for the resistance stimulated by chemicals agents or signals (elicitors) produced by beneficial microorganisms [149], whereby the plant's innate defenses are potentiated against subsequent biotic challenges. In this way, the endophytes enhance the plant defenses against many pathogens [129]. The plant hormones jasmonic acid (JA) and ET are responsible for the regulation of the group of interrelated signaling pathways required to activate ISR. The main routes by which microbes regulate ISR in plants include: (i) phytohormones, (ii) pathogen-associated molecular patterns (PAMPs)/microbe-associated molecular patterns (MAMPs), and (iii) several elicitors (volatile organic compounds, siderophores, phytases, miRNAs, among others) [150]. Bacterial endophyte-mediated ISR has a broad spectrum of effectiveness. It was demonstrated that *Acinetobacter*, *Azospirillum*, *Rhizobium*, *Pseudomonas*, and *Bacillus* are beneficial inducers of systemic resistance in both leguminous and nonleguminous plants [151]. Dey et al. [91] described an endophytic isolate *Klebsiella pneumoniae* HR1 from the root nodules of black mung bean (*Vigna mungo*) capable of reducing the occurrence of *Macrophomina phaseolina*, which is the causal agent of the root rot disease in *Vigna*. The lowest percentage of disease incidence (18.2%) was observed when *K. pneumoniae* was applied in dual mode (seed bacterization + soil drench application). The increased activities of peroxidase (PR9), chitinase (PR3), and β-1,3-glucanase (PR2) in leaves indicated that *K. pneumoniae* HR1 induces a systemic response.

Endophytic bacteria have diverse mechanisms that could contribute, even simultaneously, to protect the plant against the attack of different pathogens, having the potential to produce a more efficient pathogen control on the fields.

3.4 Abiotic stress tolerance

Under abiotic stress conditions (such as drought, salinity, flooding, heat, chilling, or heavy metals), several metabolic responses are shared among plant species. Most of the stresses cause photosynthesis inhibition, oxidative stress, and hormone imbalances ending in reductions of shoot growth and yield impairments [10, 97, 152–154]. In addition, some of the responses are interconnected, for instance, reactive oxygen species and hormones mutually affect each other at early and late phases of abiotic stress (reviewed by [155]).

Endophytic bacteria can protect the host plant against some of those deleterious effects, by at least two different ways (alone or combined): (i) activation of host stress response systems soon after exposure to stress (named induced systemic tolerance), and (ii) biosynthesis of chemicals, which will contribute to the stress tolerance in the host [9]. Here we focus on three mechanisms by which the bacteria can protect the plant host against abiotic stress: redox status, water balance, and hormone regulation.

3.4.1 Redox status regulation

Oxidative damage (caused by reactive oxygen and nitrogen species) is a common consequence of environmental stress, which may cause damage to lipids, proteins, and overall to any subcellular component [156]. Then, the activation of the enzymatic and nonenzymatic antioxidant system is critical to tolerate adverse conditions. Several endophytic bacteria mediate a higher induction of the antioxidant system under stress. For instance, under salinity, the inoculation of peanut with the halotolerant bacteria *Brachybacterium saurashtrense* JG-06, *Brevibacterium casei* JG-08, or *Haererohalobacter* JG-11 showed lower oxidative damage, ion leakage, and K/Na ratio and higher growth, IAA, and Ca [157], while the inoculation of *B. subtilis* (alone or combined with *Mesorhizobium ciceri*) of chickpea reduced hydrogen peroxide accumulation and improved plant growth [10]. Soybean plant inoculated with *Curtobacterium* sp. SAK1 induced polyphenol oxidase activity, associated with growth protection and hormonal changes [158], while inoculated with *Pseudomonas simiae* increased catalase and peroxidase, but not polyphenol oxidase gene expression under salinity [159]. Also, soybean inoculated with *B. cereus, Pseudomonas otitidis*, and *Pseudomonas* sp. showed a reduction of hydrogen peroxide and membrane oxidative damage caused by PEG-induced drought [160]. However, if these responses are generated by the plant or bacterial enzymes remains unknown.

3.4.2 Water use efficiency regulation

Under stress, plant tissues usually modulate osmotic and water retention, by stomata activity and/or accumulation of osmotically active compounds. The latter compounds, also known as compatible solutes, include sugars (e.g., sucrose, trehalose, etc.), organic acids (e.g., malate), inorganic ions (e.g., calcium), amino acids (e.g., glycine betaine, proline) [161]. An increase in drought tolerance was detected after the inoculation of *Sphingomonas* sp. LK11 (isolated from *Tephrosia apollinea*) in soybean, by the accumulation of sugars and amino acids (glycine, glutamate, and proline) [162], and after the inoculation with *Rhizobium etli* in common bean, by the overexpression of trehalose-6-phosphate synthase [163]. Trehalose is an osmotically active compound that accumulates both in plants and microbes under stress. In particular, the role of trehalose in the tripartite symbiosis between plants, rhizobia, and arbuscular mycorrhiza under abiotic stress has been recently reviewed [164].

The optimal regulation of water use efficiency is critical to improved crop production. On one side is essential to survive dehydration stress (such as drought, salinity, heat, and chilling), but a constitutively highly efficient water use may reduce yields, by reducing CO_2 assimilation. The use of bacteria that contribute to transiently intensify stress-tolerance responses can help to improve productivity in marginal environments. In addition, if the endophytic bacteria enhance the osmo-compatible compounds in response to the stress, it is possible to increase not only the tolerance to drought, but also the tolerance to chilling, heat, and salinity stress, which share a “dehydration” component. In the latter case, we expect a partial tolerance due to the ion toxicity, not related to the reduction in water potential.

3.4.3 Hormone regulation

As it was mentioned before, endophytic bacteria can regulate hormone synthesis and degradation and synthesize some of the plant hormone-like compounds by themselves. In addition, specific hormone regulation could also protect against abiotic stress increasing growth, yield, and survival.

Abscisic acid (ABA) is the main plant hormone related to water stress. It stimulates root growth and optimizes water uptake and nutrient acquisition, regulates shoot and root hydraulic conductivity, and upregulates the antioxidant system and compatible osmolytes synthesis [161]. The inoculation of *Sphingomonas* in soybean leaves induced ABA accumulation and reduced chlorophyll degradation and growth inhibition. However, under drought, ABA levels were lower in inoculated plants. So, in this case, the initial increase of ABA might have a role in acclimation to the stress induced by the bacteria inoculation [162]. In addition, ABA may interfere with SA-, JA-, and ET-mediated plant defenses [165], which may have undesired consequences under biotic stress.

Ethylene (ET) is usually considered a plant growth inhibitor, but at low levels, it can promote growth in several plant species. At moderate levels, ET inhibits both root and shoots elongation, while at high levels, enhances senescence and organ abscission [166]. The direct precursor of ET in the plant biosynthetic pathway, 1-aminocyclopropane-1-carboxylate (ACC), is exuded from plant roots together with other amino acids. The enzyme ACC deaminase cleaves ACC into ammonia and alfa-ketobutyrate. Plant growth promoter bacteria that express the enzyme ACC deaminase utilize their products (ammonia and ketobutyrate) as nitrogen and carbon sources, respectively. Bacterial ACC deaminase is not excreted from the bacterial cytoplasm [167]; hence, the decrease of plant ET levels relies on the ability of ACC deaminase expressing bacteria to take up ACC before it is oxidized by the plant's ACC oxidase [167]. When those bacteria are present, ET production could be lowered, relieving stress-induced growth inhibition [168]. For instance, the inoculation of pea (*P. vulgaris*) plants with *Aneurinibacillus aneurinilyticus* and *Paenibacillus* sp., two strains with high ACC activity *in vitro*, increased salt and drought tolerance. The combined inoculation reduced plant ET content and increased root and shoot length and biomass, as well as chlorophyll content [169]. The inoculation of alfalfa plants with *Bacillus megaterium* NMp082, which can produce ACC deaminase activity and IAA *in vitro*, also enhanced their salt tolerance [170]. Lastly, a novel mechanism was proposed in which salt tolerance is mediated by the activation of ET signaling. The inoculation of alfalfa with the bacteria *Enterobacter* sp. SA187 (isolated from a desert plant) increases salt tolerance, and studies in Arabidopsis indicate that the bacteria activate the ET signaling pathway [171]. The different mechanisms by which microorganisms can interfere with ET signaling were reviewed by Ravanbakhsh et al. [167].

Auxins regulate many important physiological processes related to growth and development affecting photosynthesis and responses to stress [161]. Under stress, auxins stimulate root elongation and density, increasing the water and nutrient availability, although they may interfere with SA-dependent plant defenses.

The inoculation of chickpea with *Serratia* sp. in nutrient-deficient soil induced more IAA and higher yields [172], while the same plant inoculated with IAA-producing *B. subtilis* NUU4 in combination with *M. ciceri* IC53 stimulated root and shoot biomass and improved nodule formation under salt stress [173]. Soybean plants inoculated with *B. aryabhattai* strain SRB02, which produces IAA, GA, and ABA, showed higher drought tolerance through stomatal closure, and higher root and shoot rates under high temperatures [116], and the same host treated with *Sphingomonas* sp. LK11 and *Serratia marcescens* TP1 (which produced IAA *in vitro*) stimulated root and shoot growth with increased ABA and GA and reduction of JA [162]. Overall, abiotic stress protection mediated by plant hormones and crop salinity protection mediated by beneficial bacteria have been reviewed [10, 174, 175].

Some primary stresses share the responses among them, such as those that generate dehydration (water or temperature deficit) or oxidative stress (dehydration, hypoxia, ions). For example, the double inoculation of chickpea with *M. ciceri* IC53 and *B. subtilis* NUU4 reduced the infection rate of root rot caused by *Fusarium solani* in salty soils [173], although the mechanism was not determined. Then, a bacteria strain, inducing a protective mechanism against oxidative stress, can protect the crop against a diversity of stress, which generates redox imbalances. Consequently, knowing the responses that each stress triggers in the plant may allow us to predict which bacteria or group of them could protect the plant against a combination of stresses.

4. Synthetic communities of plant-associated bacteria to a more sustainable agriculture

Natural microbial communities within the plants are complex systems, with unknown functions and interrelationships among the microbial species and with the host plant. Small consortia of bacteria, with a "designed" composition, called "synthetic communities," reduce the complexity of those systems to be studied and used. The goal is to simplify the network while preserving the interactions and most of the functions, which may be lost in single plant-microbe interactions [175]. The use of synthetic communities allow us to ask questions about the performance and stability of the microbial community as well as to study conditions necessary to generate interaction patterns required to provide specific benefits. They are not only valuable as models but also as assays for biotechnological approaches [176].

4.1 How to study synthetic communities?

Manipulative experiments with synthetic bacterial communities can validate the predicted keystone species and, in general, help to find out specific effects of the resulting community under some pathogen infection or environmental condition. Those studies required *in vitro* experiments in gnotobiotic (germ-free) systems [11], where the plant is inoculated with a few or several microbial species, and the diversity is monitored across time. For instance, a gnotobiotic system was used to study the bacteria-colonizing alfalfa nodules [131]. The authors inoculate alfalfa with the four accessory bacterial members *B. brevis* Ag35, *Paenibacillus* sp. Ag47, *Pseudomonas* sp. Ag54, and *Pantoea agglomerans* Ag15, plus the nodulating strain *Sinorhizobium meliloti* RM1021. They observed that the addition of *B. brevis* neutralized the cooperation between *Pseudomonas* sp. Ag54 and *Paenibacillus* sp. Ag47, shifting the community from cooperative to competitive.

Another alternative, it is to use synthetic communities in a non-germ-free environment (more accessible and simpler to set up) to evaluate the protective or antagonist effect of a small group of species under a particular condition. Overall, only a few studies of the kind have been carried out in legumes until now. For instance, Lu et al. [177] described the diversity of nonrhizobial bacteria (32 genera) in legume nodules inoculated with *Bradyrhizobium elkanii* H255, *Rhizobium multihospitium*–like HT221, or *Burkholderia pyrrocinia* with or without the addition of N fertilization. The study suggested a vital role of that group of bacteria in N fixation in legumes.

The synthetic communities are a way to understand how microbial communities are built in the plants but also the base to a more complex (and likely more effective) phytostimulation effects, biological control of diseases, and protection against abiotic stress.

4.2 Can we manipulate the plant microbiome to improve the fitness or yield of legumes?

There are a variety of strategies to manipulate the microbiome of a plant host and could be classified according to the direct target: (i) the microbiome itself,

(ii) the plant genome, or (iii) the holobiome (plant plus microbial community) (reviewed by [39, 178]).

The microbiome (i) can be modified by the exogenous inoculation of the microbe, increasing the abundance of a single strain or a few species together. The first case is the most traditionally used, and there are thousands of examples, such as the inoculation with rhizobia. In those cases, the single strain should be compatible with the host genotype and able to overcome the competence of the native microbiome and the environmental conditions. The second case is open to unexplored scenarios, such as an infinite possibility of a higher number of strains/species combinations. This strategy is just starting to be explored, such as with non-nodulating bacterial species present in the nodules (and sometimes in the rest of the plant) that promote nodulation. For instance, the inoculation of common bean (*P. vulgaris* L.) with *Paenibacillus polymyxa* and *B. megaterium* strains showed a synergistic effect with *Rhizobium* strains on the plant growth [179]. On the contrary, the inoculation of alfalfa with different strains of the mutualistic *P. fluorescens*, showed that the increase in the community richness led to a negative complementary effect causing the loss of the protective effect against pathogens [180]. These results highlight the importance to evaluate the effects of any agricultural treatment or management on the microbial community.

The inoculation with synthetic communities has the advantage (over the use of the native microbiome) to allow the design of a community, which includes distant species (which may provide complementary benefits), or similar species, which increase the efficiency of the community (by using a wider diversity of resources) [19]. However, with the number and diversity of species, it also increases the complexity to handle the system and to commercialize the inoculants.

The plant genome (ii) could be manipulated by traditional breeding, gene editing, or transgenesis, changing the ability of the host to interact with the microbes (such as changing the exudates or volatiles). Instead of only breeding for pathogen resistance or abiotic stress tolerance, this could be a complementary alternative to select crop legumes to be more responsive to the presence of beneficial microbes [181]. For instance, modern accessions of common bean showed a lower abundance of Bacteroidetes and higher of Actinobacteria and Proteobacteria than the wild accession [79], with a gain in the diversity of rhizospheric bacterial and a stronger effect of the bean genotype [182]. In addition, Mendes et al. [183] showed that common bean breeding for *Fusarium oxysporum* resistance altered the functionality of the rhizosphere, unintentionally increasing the host protection against other pathogens. We hypothesize that a similar effect is happening in the endosphere, although it has not been explored yet. Additionally, when using this approach, it is relevant to evaluate that host defenses against pathogens are still functional.

Lastly, the holobiome (iii) could be altered through specific agricultural practices such as crop rotation, mineral, and organic fertilization, tillage practices, etc., favoring a specific community composition or function. Several studies reported the effect of agricultural management on the rhizosphere of legumes and its effect on crop performance. A meta-study showed the effect of crop rotation, intercropping, or companion planting on the rhizospheric microbial richness and diversity [184]. Those agricultural practices did not always have positive effects in richness and diversity, and legume-cereal crop rotation (relevant to reduce N fertilization) showed inconsistent results on the microbiome. A recent study showed that pea-wheat rotations showed no effect in the diversity index, but they affected the specific co-occurrence networks for each crop [185] suggesting a more complex effect of crop rotation that needs to be further studied. Certain chickpea cultivars select a more beneficial microbiome for the subsequent wheat plants, and they were associated with the antagonist species *Penicillium canescens* [186]. Red clover and potato crops in rotation shared 73% of the bacterial endophytes, and 21% of those species promoted plant growth and yield in potato bioassays [187], while 74% of the shared species showed some degree of *in vitro* antibiosis against *Rhizoctonia solani*, a pathogen of both crops. We hypothesize that changing the rhizosphere will affect the endosphere too, by changing the available microbial pool, but that effect has not been explored at legume endophytic microbiomes.

4.3 Are there collateral impacts of using synthetic communities in agriculture?

Lastly, it is important to consider alive microbes will be released to the environment and into products used or consumed by humans and animals, so the potential risks need to be considered and tested [188]. There is no internationally agreed protocol to be complimented, but recently, Vilchez et al. [189] have proposed an Environmental and Human Safety Index (EHSI) protocol to determine the safety of the bacterial strains. The protocol evaluates microbial and animal sensitivity/pathogenicity and ecotoxicity in different model organisms, and it has been validated for many well-known bacteria. In addition, on the agronomical level, little information is available on the nontarget effects on microbial communities and the resulting impact on the soil function [32].

5. Final remarks and future directions

Agricultural legume crops are usually treated with synthetic chemicals to increase growth, control diseases, and mitigate environmental stress, which has high economic, environmental, and health costs. However, there is a myriad of endophytic bacteria that colonize the plant at least in part of its life cycle that could replace or complement those chemicals with great benefits for the plants. In addition, the huge bacterial diversity could be combined to provide several benefits at the same time. For that purpose, the use of synthetic communities is critical to study how the microbial community evolves within the plant as much as their beneficial effects.

The use of synthetic bacterial communities to improve and make more sustainable legume production is still in early stages of development, but it is a promising field. Using synthetic communities has the theoretical advantage of combining strain benefits and contributing to the survival of the bacteria on the field and inside the plant while producing a package of benefits for the legume. Although it is expected to have more difficulties at the time of commercial production.

On the other hand, changes in the agricultural management with some specific purpose could be a more affordable strategy for most of the small-scale producers in low-income countries, which are the ones in more need of sustainable and accessible technologies. Additionally, the use of soil-native microorganisms could have the advantage to reduce possible adverse consequences on the environment and health.

For the moment, the knowledge about endophytic bacteria in legumes, the possibility to “design” synthetic communities for a specific goal, and to manipulate the holobiome by agricultural practices is still incipient. However, the potential benefits for current agriculture to improve yields and sustainability have a great unexplored potential in the endophytic bacterial microbiome of legume crops.

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Author contributions

LV and MM conceived and planned the overall idea of the review manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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