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Current Opinion in Plant Biology

Plant and microbial features governing an endophytic lifestyle

Kathrin Wippel

Abstract

Beneficial microorganisms colonizing internal plant tissues, the endophytes, support their host through plant growth promotion, pathogen protection, and abiotic stress alleviation. Their efficient application in agriculture requires the understanding of the molecular mechanisms and environmental conditions that facilitate in planta accommodation. Accumulating evidence reveals that commensal microorganisms employ similar colonization strategies as their pathogenic counterparts. Fine-tuning of immune response, motility, and metabolic crosstalk accounts for their differentiation. For a holistic perspective, in planta experiments with microbial collections and comprehensive genome data exploration are crucial. This review describes the most recent findings on factors involved in endophytic colonization processes, focusing on bacteria and fungi, and discusses required methodological approaches to unravel their relevance within a community context.

Addresses

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Introduction

Within the plant tissue, bacteria and fungi can colonize inter- or intra-cellularly, and some are able to migrate through the entire plant via the root and shoot vasculature [1,2]. This intimate relationship between host and microbes can be beneficial for both partners (mutualistic), beneficial for the microbial partner without harming the plant (commensal), or detrimental for the plant (pathogenic), although intermediate associations certainly exist along the continuum from one to another [3]. Although microbial pathogens mostly proliferate within the host tissue, i.e., endophytically, the term endophyte is commonly used for commensal and beneficial organisms that colonize the plant without causing diseases [4]. The interaction with diverse microorganisms is crucial for plant health, development, and nutrition [5]. However, it is assumed that microbial load in host tissue should be limited, because high microbial titer is commonly associated with detrimental microbes [6]. Interestingly, commensal as well as pathogenic bacterial strains seem to be able to live within the *Arabidopsis* leaf apoplast in a multiplication-death equilibrium that avoids excessive proliferation for several weeks [7]. In addition, a harmless endophyte can turn into a pathogen depending on host developmental stage and genotype or changing environmental conditions [4,8,9]. Which type of plant-microbe association is established, is determined to a large extent by the genetic constitution and compatibility of both partners. This article aims to provide an overview of recently identified host and microbial factors and responses that are crucial for microbial endophytic lifestyles, focusing on commensal and beneficial interactions of bacteria and fungi with land plants, mainly in the root compartment, also covering more complex community interactions. Finally, an overview of current methodological approaches to uncover new mechanisms underlying endophytism is provided.

Benefits of hosting endophytic microorganisms

Among the best-described plant-microbe mutualistic interactions is the root nodule symbiosis between legumes and rhizobia under low nitrate conditions, where the bacteria live in specialized root compartments, the nodules, and fix atmospheric nitrogen for the host in return for carbon [10]. Similarly, plants engage with arbuscular mycorrhiza fungi (AMF) that allow the plant to explore a much larger volume of soil and transport phosphate through their hyphae to the intracellular arbuscules in the host root tissue [11]. These are very differentiated interactions for which the required, tightly regulated molecular crosstalk has been intensively studied and described.

Besides the microbial mutualists, commensal bacteria and fungi also exhibit plant growth-promoting (PGP) activities. These can be stimulation via hormones such as cytokinin [12], auxin [13], and gibberellic acid [14], or fertilization by mobilizing nutrients from the surroundings. For example, inaccessible soil organic and inorganic phosphate is mobilized by bacteria by acidifying the rhizosphere through the release of organic acids, or via the activity of phosphatase and phytase enzymes, the latter of which has also been shown for *Penicillium* and *Aspergillus* fungi [15]. It should be noted that the actual ecological relevance for direct plant nutrition by these mechanisms is still under debate [16]. Another example is iron uptake, since iron becomes available through chelating properties of microbial siderophores [17], or through cooperation of plant-secreted coumarins with commensal bacteria [18], although the mechanism for the latter remains to be identified. It was suggested that plants can take up microbe-derived nitrogen and carbon through microbivory by degrading the endophytes, possibly coupled to a rhizophagy cycle where bacteria enter, exit, and re-enter root cells [19]. However, the efficiency of this process seems very low and thus its significance may be weak [20].

Additionally, endophytes may grant protection against pathogens, which can be mediated through niche competition, direct antagonism, or priming of the host immune system. For example, besides the general competition for carbon sources and space [21], pathogens may face iron limitations due to siderophore activity of commensal bacteria. Secreted compounds like the bacterial 2,4-diacetylphloroglucinol (DAPG), pyoverdine, hydrogen cyanide, pyoluteorin, or pyrrolnitrin have direct antimicrobial activity in a contact-independent manner similar to antibiotics [22–24]. Moreover, colonization of the host by beneficial bacteria stimulates the host immune system which can lead to induced systemic resistance, priming the host for subsequent attack by detrimental strains [25].

In the context of abiotic stress, adverse environmental conditions such as drought and salt stress were shown to be alleviated by bacteria via ACC deaminase activity to decrease stress-induced ethylene levels [26,27]. The beneficial fungus *Trichoderma harzianum* conferred salt tolerance in two barley cultivars with different levels of salt sensitivity, by differentially modifying root lipid and polar metabolite profiles in either host [28].

These beneficial activities are often demonstrated for individual strains under laboratory conditions. In natural environments, the presence of other microorganisms, variable abiotic factors, and soil properties may affect these functions. On the other hand, microbial functions may only become evident in a community context and may be more robust and stable as part of the entire microbiota [29,30].

The role of the host immune system

Both pathogens and endophytes possess microbe-associated molecular patterns (MAMPs), such as the bacterial flagellin peptide flg22 or the fungal cell wall component chitin and are thus recognized by the plant's pattern recognition receptors. However, non-pathogenic microbes are thought to trigger only a weak immune response [31], although beneficial interaction may indeed require a certain immune induction. This was shown for a *Bacillus velezensis* strain which only colonizes efficiently and produces auxin when the *Arabidopsis* reactive oxygen species (ROS) signaling is initiated [32]. Metabolomics analysis of *Brachypodium distachyon* roots revealed an initial defense activation by beneficial *Azospirillum brasilense* before triggering phosphorus deficiency stress alleviation [33]. In a more holistic context, the tryptophan metabolism as part of the immune response was shown to be responsible for controlling fungal load to maintain the beneficial effects of a bacteria-oomycetes-fungi root microbiome [34]. Strikingly, a comprehensive screen of flagellin peptides revealed that commensal bacteria display a large diversity of flg22 epitopes, many of which are immune-evasive and -modulating, linking to host colonization success [35]. To avoid *Arabidopsis* defense activation completely, *Bacillus subtilis* BSn5 produces subtilomycin that binds the bacterial flagellin, thus reducing the flg22-induced immune response and allowing colonization [36]. Similarly, the LysM domain-containing effector Tal6 of the endophytic fungus *Trichoderma atroviride* sequesters chitin-derived GlcNAc and binds to chitin for protection against plant-produced chitinases, thus evading host immune response [37]. A part of the immune system can be suppressed through acidification of the rhizosphere by bacteria secreting gluconic acid (*Pseudomonas capeferrum* WCS358), or through amino acid synthesis to avoid accumulation of alkaline precursors (*P. simiae* WCS417), although pH-independent mechanisms likely exist [38].

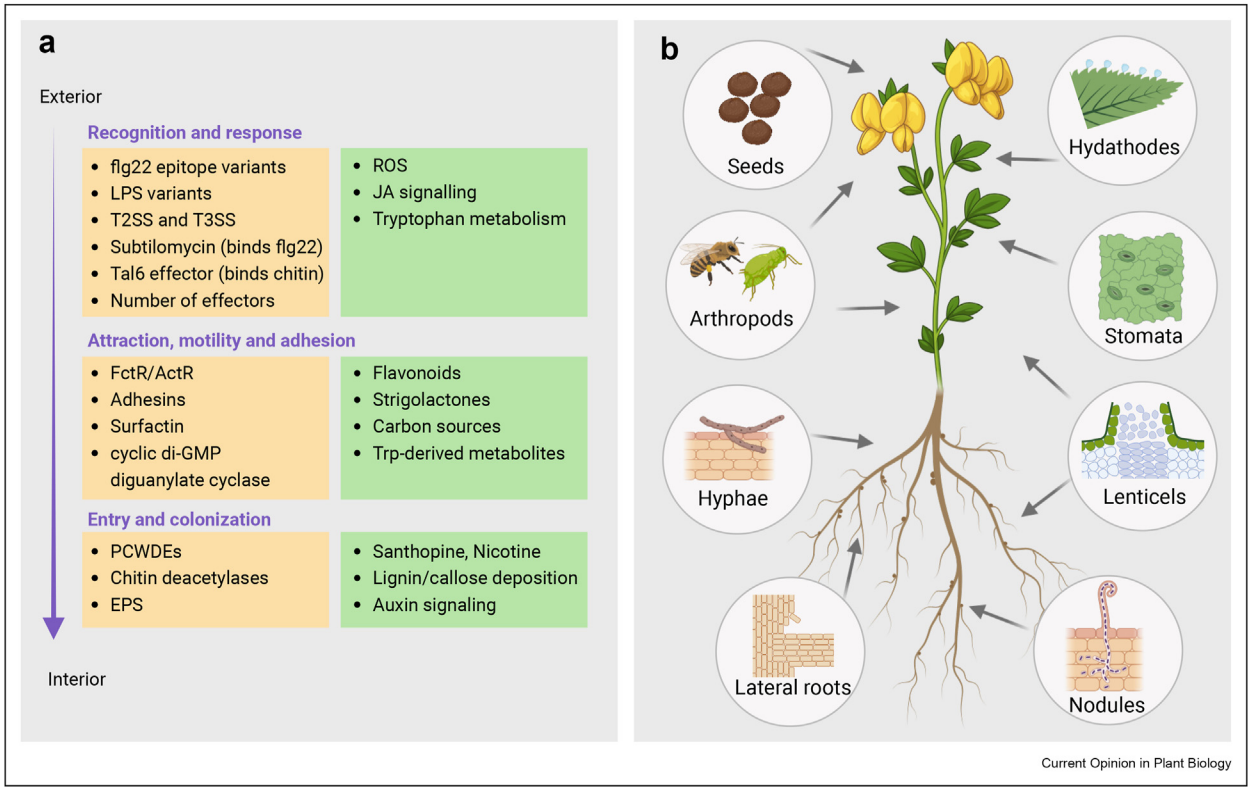
Co-cultivation of *Arabidopsis thaliana* and *Lotus japonicus* with host-adapted commensal communities also resulted in up-regulation of immunity-related transcripts in the roots [39]. Two other studies investigated bacterial commensal communities that exhibited either immune-suppressive or non-suppressive properties in *Arabidopsis*, where suppressive activity usually correlated with better root colonization [40,41]. On the other hand, a consortium of commensal *Pseudomonas* strains triggered a strong immune response in *Arabidopsis* leaves compared to marginal effects of a group of pathogenic strains [6]. With a new approach, comparison of both host and bacterial in planta transcriptome profiles of individual commensals inoculated in the *Arabidopsis* leaf apoplast

showed that the host responds in a strain-specific manner, for example with different levels of immune activation [42]. Similarly, endophytic accommodation triggered variable expression levels of genes related to energy production and nutrient uptake in these individual strains, which could be a means for the host to orchestrate colonization of diverse microbes by modifying metabolite availability [42].

Other MAMPs like lipopolysaccharides (LPS) - structural components of the bacterial membrane - can act as modulators of symbiosis efficiency through the plant immune system. For example, when the O-antigen component of the LPS was mutated in *Mesorhizobium huakuii*, the mutant strain displayed host accession-specific symbiotic phenotypes, ranging from enhanced to abolished and coupled to host immune defense gene expression [43]. To counteract defense, bacteria deliver effectors into host cells via the bacterial type III secretion system (T3SS). Accordingly, parts or all of the T3SS system is often missing in commensal bacteria, apart from mutualistic rhizobia and some non-pathogenic *Pseudomonas* and *Xanthomonas* strains [9,44]. Interestingly, the T2SS has been shown to be responsible for the immunosuppressive activity and enhanced colonization of a commensal *Dyella* bacterium on *Arabidopsis* roots [41]. In fungi, endophytic *Fusarium* species tend to possess fewer putative effector genes than pathogenic species [45], making host immune evasion more likely. Finally, plant defense hormones distinguish different microbes. In rice, endophytic *Azoarcus olearius* induced host jasmonate signaling, thought to restrict excessive proliferation, whereas it responded with salicylic acid production to a bacterial pathogen [46].

These findings show that commensals and beneficial microbes can modulate host defense to invade plant tissue (Figure 1a). However, more research on microbial communities in the context of a plant holobiont is needed to decipher how plants integrate signals from welcomed and unwelcomed colonizers [47].

Figure 1



a. Microbial (orange boxes) and plant (green boxes) features discussed in this article as determinants for successful endophytic colonization of commensal or mutualistic organisms. T2SS/T3SS, type II/III secretion system; LPS; lipopolysaccharides; ROS, reactive oxygen species; JA, jasmonate; FctR/ActR, transcriptional motility regulators; PCWDEs, plant cell wall-degrading enzymes; EPS, exopolysaccharide. **b.** Ports of microbial entry. Schematic depicting different ways and tissues by which microorganisms can enter plant tissue, including seeds (movement of microbes internally to the flower tissue, or externally from the aerial environment), arthropods (vector-mediated entry into flower, leaf, or stem tissue), hydathodes (passive entry), stomata (passive entry or active opening), lenticels (in stems or roots), lateral roots (through tissue cracks at the emergence sites), root nodules (via hitch-hiking along with symbionts), and fungal hyphae, shown representatively for the model plant *Lotus japonicus*.

The role of plant metabolites, microbial motility, and attachment

Plant-derived chemical compounds play an important role in the attraction and maintenance of associated microorganisms, including specialized metabolites that can not only repel pathogens but also control colonization of endophytes [48]. For example, balanced colonization and PGP activity of *Colletotrichum tofieldiae* on *Arabidopsis* were dependent on metabolites of the tryptophane pathway, as shown with corresponding plant mutants [49]. In tobacco, the specialized metabolites santhopine and nicotine are both responsible for the endophytic enrichment of the bacterial genus *Arthrobacter*, which catabolizes these compounds [50].

Indispensable microbial properties for endophytic colonization are motility and the ability for attachment. Flagellum activity, swarming, swimming, adhesion, and biofilm formation are essential for rhizobacteria to move towards the host plant root. For example, the production of *Bacillus velezensis* surfactin is stimulated by root exudates and this lipopeptide is essential for swarming and biofilm formation, which enhances early tomato root colonization and priming of host immunity [51]. In another *B. velezensis* strain, several mutants defective in individual adhesin genes were strongly affected in adhesion to cucumber roots, resulting in impaired colonization [52]. Deleting the gene for the bacterial motility regulator ActR in *Azorhizobium caulinodans* led to loss of swimming ability and impaired biofilm formation, which reduced colonization and nodulation capacities on the host plant *Sesbania rostrata* [53]. Another study showed that cyclic-di-GMP diguanylate cyclase, which is important for biofilm formation, is also required for internal colonization of *Azospirillum baldaniorum* in wheat roots [54].

To move more efficiently through the soil, especially through dry patches where flagellum and cilial movement are impaired, bacteria may hitchhike on fungal hyphae, the so-called fungal highways [55]. This association requires a give-and-take strategy. For example, in return for transport along its mycelia, *Bacillus subtilis* provides the fungus *Aspergillus nidulans* with the vitamin thiamine, promoting mycelial growth and thus further dispersal of the bacteria [56]. Beneficial AM fungus *Rhizophagus irregularis* attracts and feeds *Rahnella aquatilis* with fungal exudates which allows the bacterium to proliferate and move along the water film around the hyphae [57]. On the other hand, after hitchhiking hyphae of the fungal pathogen *Fusarium oxysporum* to reach the host plant, *Rahnella* can even prevent fungal growth and infection of tomato seedlings through gluconic acid-induced acidification of the rhizosphere, which counteracts virulence-associated alkalinization [58]. During peanut-rhizobium root nodule symbiosis, nodulation capacity was enhanced due to the modification of bacterial growth and chemotaxis in response to root exudates from plants co-cultivated with the beneficial fungus *Phomopsis liquidambaris* [59].

These studies highlight the importance of extending binary host-microbe interaction assays to at least tripartite or more complex interactions that better mimic the natural soil microbiota.

Entry and migration within plant tissue

Natural entry routes for microorganisms can be openings such as stomata, lenticels, or hydathodes, or cracks in the tissue through wounding by soil particles, pathogen attack, lateral root emergence, or abiotic stress (Figure 1b). Non-mutualistic microbes can also enter root nodules as cheaters by hijacking the entry of mutualistic strains. Even though these commensals are not able to fix nitrogen, they can still be beneficial by promoting shoot growth and nodule development, as shown for two *Serratia* strains from lentil nodules [60]. A *Pseudomonas* strain was found to co-colonize healthy, effective nodules of *Lotus burtii*, and to decrease the number of ineffective nodules on *L. japonicus* [61]. Depending on tissue type and plant species, *Enterobacter* sp. SA187 can utilize different entry mechanisms such as passive entry, enzymatic cell wall degradation, or active stomata opening [62]. In general, to proceed into deeper-lying cell layers, plant cell wall-degrading enzymes (PCWDEs) such as cellulases, cutinases, pectinases, and lignin peroxidases are important for colonization by many fungal and bacterial species [8,63,64]. Accordingly, *Arabidopsis* can restrict beneficial *Fusarium* localization to xylem vessels of lateral roots and elongation zone of the primary root compared to pathogenic ones via induced lignin and callose deposition [65], likely preventing fungal overgrowth while keeping beneficial activities. In line with this, this strain possesses fewer PCWDEs than its pathogenic relatives tested in this study [65]. Another interesting example is the beneficial fungus *Epichloë festucae*, which converts its cell wall chitin to chitosan with the help of chitin deacetylases in order to switch from epiphytic to endophytic lifestyle in *Lolium perenne* leaf blades [66]. Furthermore, exopolysaccharides (EPS) are important membrane components of both bacterial and fungal species. EPS outside of fungal cell walls was shown to be crucial for colonization of host plant cells based on a dual function of fungal cell wall modification and plant ROS detoxification with the help of a beta-glucan, which is released through plant hydrolase activity on the EPS [67]. Recently, a plant receptor kinase has been identified that binds these glucans and facilitates AMF symbiosis in *L. japonicus* [68]. Pectinase, cellulase, and ligninolytic activities have also been described for bacterial endophytes of the medicinal plant *Alkanna tinctoria*, and may be linked to the induction of the pharmaceutically important antimicrobials alkannin and shikonin [69].

Microorganisms can be transmitted directly into the plant interior via arthropod vectors. Besides various examples for vector-derived pathogens [70], there has recently been stronger recognition for the significance of insect-mediated exchange of commensal or beneficial microbes between plants. For example, strawberry flowers were shown to function as hubs from which pollinator bees transfer *Streptomyces* strain SP6C4 from one plant to another, so that the beneficial bacterium can exert its antagonism towards fungal and bacterial pathogens on both the plant and the insect [71]. SP6C4 was also able to move from the rhizosphere or from flower tissue to the plant vasculature [71]. Another study found that beneficial sap-sucking mirids acquire and transfer two PGP strains, *Paraburkholderia* and *Enterobacter*, from and between tomato plants, and bacteria migrated from shoots to roots [72].

Once in the plant's interior compartments, microbes may migrate to different organs. The endophytes can subsequently be vertically transferred from the seeds to the next plant generation and influence plant health and microbiota [73]. In rice, seed endophytic communities were shown to be stable over two generations, and shape the root microbiome of rice plants [74]. In maize, protection against *Fusarium* was reported for bacterial seed isolates, alongside of seedling growth promotion [75]. *Sphingomonas melonis* was found to be stably transmitted over three generations of rice seeds, and to confer resistance to detrimental bacterium *Burkholderia plantarii* by interfering with the production of its virulence factor tropolone through anthranilic acid [76].

These studies highlight the versatility of microbial entry modes into host tissue, and that movement between plant organs and generations is possible. Especially regarding stable, field-applicable bioagents, it is important to investigate microbial colonization, heritability, and soil legacy with sophisticated methods.

Methodological approaches for the identification of colonization factors

Characterizing microbial growth, chemotaxis, carbon preferences, and plant beneficial traits of commensals isolated from the inner plant tissues is relevant to understand their individual functions. However, behavior and mechanisms may differ in planta. Culture-independent approaches offer the opportunity to identify host and microbial features involved in host-microbe associations in a more holistic, yet potentially more targeted, manner, better capturing the state of host interaction. With more economic high-throughput sequencing platforms and large public databases being available, comparative omics analyses can provide such comprehensive insights (Table 1). For example, a metagenome analysis of 74 *Micromonospora* strains derived from marine, soil, and plant-associated environments was used to identify both potential plant growth-promoting and host colonization features [77], while comparative genomics between *Arthrobacter* strains from tobacco roots and soil revealed genes for plant-specialized metabolites that are crucial for plant root colonization [50]. Comparative analysis of 41 fungal genomes of natural *Arabidopsis* isolates showed that PCWDEs are key contributors to endophytic root colonization [63]. Moreover, specific enzyme families are linked to either endophytic (xylanases, lytic polysaccharide monooxygenases) or detrimental (pectate lyases) behavior. Accordingly, overexpression of a pectate lyase in an initially non-detrimental strain resulted in aggressive colonization and negative impact on host fitness [63]. Omics approaches may also be used to predict plant-microbial interactions, as in a recent study that compiled genomes from pathogenic and non-pathogenic *Xanthomonas* strains and employed a machine learning approach to predict plant-associated and pathogenicity factors for diverse strains [44]. Despite the big value of such genomics analyses, they do not consider if genetic elements are indeed expressed under relevant conditions. It is therefore important to validate the results obtained from in silico analyses by in planta experiments and orthogonal assays.

On the other hand, to identify differential host responses according to the colonization patterns and of microbes with different lifestyles, cell type-specific expression of host genes using TRAP (translating ribosome affinity purification) can be analyzed [78]. However, this method requires knowledge of cell type-specific markers and availability of corresponding transgenic plant lines.

The significance of combining experimental data with bioinformatic analyses was also demonstrated in a recent study where bacterial substrate preference determined in the laboratory was integrated with genome-based models of metabolic pathways [79]. This allowed the authors to predict with high confidence which strains compete or cooperate based on the niche overlap, which was transferable to community colonization dynamics on *Arabidopsis* leaves [79]. If an analogous approach proves successful in the root compartment, where additional organic matter for consumption is present in the rhizosphere, or with fungi, remains to be shown.

Another way to investigate colonization niches and dynamics and decipher the corresponding genetic features on the microbial side is to use forward genetic screens with barcoded insertion mutant libraries coupled to high-throughput sequencing, such as TnSeq, BarSeq, and INSeq [80,81]. To track colonization patterns of multiple commensal species of similar taxonomy or mutant strains of the same species in microbiota reconstitution experiments, new methods are being developed, where members of microbial collections are individually barcode-

Table 1

Methodologies for the identification of colonization features.

Feature	Method	Required resource/material	Challenges	Limitations	Representative reference	Examples for functional comparisons
Microbial	Comparative genomics of multiple microbes	Annotated microbial whole genome sequences	Available genomes only from cultured microorganisms	Actual in vivo expression/activity unknown	[63]	• Beneficial vs. pathogenic • Rhizospheric vs. endophytic
Microbial	Forward genetic mutant screen	(Barcoded) mutant library of strain of interest	Genetic tractability of certain taxa	Only one or few strains	[81]	• Beneficial vs. pathogenic • Rhizospheric vs. endophytic
Microbial	Tracking and quantification of multiple individually barcoded strains	Tagged single (mutant) strains	Genetic tractability of certain taxa	Only one or few focal strains	[82]	• Competetiveness (within different communities)
Microbial, host	In planta (meta) transcriptomics	Annotated microbial whole genome sequences	Low ratio of microbial to plant RNA, low sensitivity	Transcript assignment to specific organisms not always possible	[42]	• Different host species • Host exposure to stress
Host	Comparative transcriptomics within one or across host species	Annotated plant genome sequence(s)	Qualitative differences in plant genome annotation	Direct comparison only possible for homologous genes	[39,42]	• Beneficial vs. pathogenic • Rhizospheric vs. endophytic • Different microbial communities
Host	Comparative metabolomics within one or across host species	Plant metabolite profile under conditions of interest	Identification of different metabolites of similar mass	Differentiation between plant and microbial metabolites	[33]	• Beneficial vs. pathogenic • Rhizospheric vs. endophytic • Different microbial communities

tagged [82]. These technologies, however, are restricted to culturable bacterial and fungal strains.

In the next step, the integration of complex data sets from such studies has the potential to reveal novel networks between hosts and their inhabiting microorganisms, including molecular factors involved in their interaction.

Conclusions

The current state of the art underlines the idea that i) there are shared strategies between endophytes and pathogens for entering host tissue, ii) beneficial microbes also elicit a plant immune response, and iii) the outcome of this response is modulated by additional, endophyte-specific factors. Viewed from the host perspective, the plant is surveilling and managing the behavior of colonizing microorganisms, which sit along a continuum between beneficial and detrimental strains. Omics techniques are being employed to discover further host and microbial factors that are crucial for endophytic host plant colonization by comparing the genetic makeup either of pathogens vs. mutualists, or of microbes inhabiting different host compartments. Currently, there is a need for robust in planta microbial metatranscriptomic studies, which are still technologically challenging, and for tracking and quantifying individual microbial strains within communities to explore community dynamics. Host specificity and priority effects will play a significant role in this context. Subsequently, different analyses should be integrated to, e.g., link microbial occurrence or abundance with function. Beyond these approaches to identify genetic factors, spatiotemporal imaging of host tissue colonization and proliferation of microbes will help to understand endophytic microbial lifestyles. Finally, the knowledge gained from these studies needs to be translated to agricultural settings, where the effective usage of beneficial strains and consortia is challenged by application strategies and variations of stability in the field.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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