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Review

Impact of Bacterial–Fungal Interactions on the Colonization of the Endosphere

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Research on different endophyte taxa and the related scientific disciplines have largely developed separately, and comprehensive community-level studies on bacterial and fungal interactions and their importance are lacking. Here, we discuss the transmission modes of bacteria and fungi and the nature of their interactions in the endosphere at both the molecular and physiological level. Mixed-community biofilms in the endosphere may have a role in protecting endophytes against encountered stresses, such as from plant defense systems. However, transmission from static (in biofilms) to free-living (planktonic) forms may be crucial for the exploration of new habitable spaces in plants. Important features previously recognized as plant–microbe interactions or antagonism in endophyte genomes and metagenomes are proposed to have essential roles in the modulation of endophyte communities.

Trends

No general consensus can be provided on the precise ecological boundaries of different types of endophyte or on the establishment of their interactions with plants.

Traditionally, understanding of bacterial and fungal endophytes developed in separate research fields and both taxa have rarely been studied in one single integrated approach.

Interactions between plants and individual populations of endophytes are key in most studies and, with the exception of antagonism against phytopathogens, no other interactions between members of the endophytic communities have been taken into account.

The inner tissues of plants are hostile environments to most microorganisms due to competition with the host plant and with other microbes for nutrients and available space.

Defining Endophyte Communities and the Endosphere

Endophyte communities are mixed communities wherein a broad spectrum of taxonomically diverse species asymptomatically interact with each other and with the host plant. Given the ubiquitous nature of microbial communities, interspecies interactions must occur. However, research on different endophyte taxa and the related scientific disciplines have largely developed separately, and comprehensive community-level studies on these interactions and their importance are lacking. The main questions addressed in this review are which types of interaction are relevant for the **endosphere** and what the consequences of these interactions on plant growth and production are.

We apply Wilson's [1] definition of endophytes with the notation by Chanway [2] that 'bacteria can be endophytes too' and 'endosphere' covers all plant parts, including the roots. The definition of endophytes stated in these papers was rather pragmatic, defined as those **microorganisms** (see Glossary) that spend at least part of their life cycle in plants without causing visible harm to their hosts. Pragmatism is common in attempts to define endophytes. For example, one of the most commonly accepted definitions of bacterial endophytes by Hallmann and co-authors [3] stated that bacterial endophytes are 'those bacteria that can be isolated from surface-disinfested plant tissue or extracted from within the plant, and that do not visibly harm the plant'. However, the term 'endophyte' has been controversially and sometimes confusingly used in literature because almost all microbes invading the endosphere have an asymptomatic period during their life cycle that can vary from imperceptibly short to life-long (e.g., [1,4]). The

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same species can be commonly detected as endophytic, pathogenic, or saprophytic, since exact niche borders and functional roles of organisms are not necessarily limited to the interior tissues of plants and asymptomatic life styles [1,4,5]. Many of the species can emanate on the plant surface or into the soil [1,5,6] and the lengths of latency periods vary; thus, as suggested by Wilson [1], 'the term which best fits the context of the situation should be used'.

In this review, we consider commensals, phytopathogens, and saprotrophs during their asymptomatic phase of the life cycle to be integrated components of endophyte communities. We also focus on the complexity of microbial interactions in the endosphere and their outcome with respect to their fitness and impact on plant and microbial physiologies, biochemistry, and ultrastructure.

From Individuals to Plants and Associated Microbial Communities

Here, we emphasize that, instead of individual plants, phenotypic selection often operates on the community of organisms comprising a plant and its associated microbes [4]. This expands the components of selection from individuals to a phenotypic unit comprising at least two, but often more, associated species; a fact that should be acknowledged in future studies to fully understand the performance of individual plants in plant–plant, plant–pathogen, and plant–herbivore relations [7–12]. Similar to any biotic interaction, plant–endophyte interactions are based on mutual exploitation and benefits to the partners and these interactions are only rarely symmetric. Thus, we need a more comprehensive understanding of the life-history strategies of plants and their associated microbes as well as of the physiological mechanisms driving them to understand how plants and associated microbial communities evolve and persist. Below, we use the literature on the ecology, physiology, and molecular biology of plant-associated fungi and bacteria to provide novel insights into their coevolution and ecological importance.

Reproduction and Transmission Modes of Microbes

Most plant–microbe interactions are generalized and opportunistic interactions made by fungi or bacteria that are **horizontally transmitted** from plant to plant (Table 1). They can be either free-living for most of their life cycle or closely associated hitchhikers of plants. Increasing evidence has emphasized the importance of trophic interactions to the transmission of these horizontally transmitted endophytes. For example, at least some endophytes are often transmitted by invertebrates [13]. At the other end of the spectrum of plant–microbe interactions are systemic and vertically transmitted endophytes that can form life-long symbiotic interactions with the host plant [5]. In some cases, these microbes have entirely lost their ability to spread contagiously. **Vertical transmission** alludes to how the microbe is distributed and is linked to the sexual life cycle of the microbe. Transmission (horizontal or vertical) and reproduction (clonal or sexual) modes are well recognized as key drivers of epidemiology and the evolution of virulence in parasite–host interactions, because they can determine how strongly the fitness of the partners depends on the interaction [14–16]. For example, vertically transmitted and strictly asexual systemic *Epichloë* endophytes of grasses (formerly referred as *Neotyphodium* [17]) are likely to fall nearer the mutualistic end of the interaction continuum (from antagonistic to mutualistic interactions) compared with mixed-strategy (both vertically and horizontally) or only horizontally transmitted fungal endophytes [5,18,19].

In contrast to fungi, bacteria propagate only clonally, but can acquire genetic material via their 'classical' bacterial gene transfer routes of transformation, conjugation, and transduction [20]. Vertical transmission of seed-associated and endophytic bacteria occurs via seeds and representatives of the most common bacterial phyla (i.e., Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria) have been recovered from seeds [21]. Bacteria present in seeds are clearly distinct from those living in the adjacent soil [22] and some seed-transmissible bacteria have also

Glossary

Arbuscular mycorrhizal (AM) fungi: fungi forming mutualistic relations with plants.
Biofilm: protective sheet of extracellular polymeric substances surrounding bacterial cells. In nature, biofilms contain multiple species, including eukaryotes.
Endosphere: all microbial species occupying the internal spaces of a plant.
Extracellular polymeric substances (EPS): an essential component in biofilm formation.
Herbivore-induced resistance (HIR): priming of plants for enhanced defense against herbivore attack.
Horizontal transmission: contagious transmission of a fungus by sexual or asexual spores, or of the bacterium as cells or spores (Gram-positive species).
Horizontal gene transfer: exchange of genetic material between microorganisms.
Homoserine lactone (HSL): bacterial signaling molecules.
Induced systemic resistance (ISR): priming of plants for enhanced defense against pathogens.
Microorganisms (microbes): unicellular organisms living as dependent (such as viruses and obligate endosymbiotic bacteria) or independent entities of prokaryotic and eukaryotic origin. In the context of this paper, 'microorganism' refers to both bacteria and fungi.
Polyketide synthase (PKS): secondary metabolites produced by fungi and bacteria.
Quorum sensing: cell density-dependent communication between bacterial cells by the release and sensing of small diffusible signal molecules.
Reactive oxygen species (ROS): produced by plants as defense mechanism against invading microorganisms.
Systemic acquired resistance (SAR): plant response evoked by a previous infection with a pathogen.
Vertical transmission: transmission of the systemic fungus or bacterium from plant to offspring via host seeds.