Part I

Introduction

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Endophytes for a Growing World

Trevor R. Hodkinson and Brian R. Murphy

Abstract

Endophytes are any microbes that can live within plants. We divide them into three major functional groups: endosyms (endosymbionts), endopaths (pathogens) and endosympaths (those that exist in both forms along a mutualism–parasitism continuum). Within these groups, endophytologists recognise harmful pathogenic microbes and a diverse range of beneficial/commensal microbes, including bacteria and archaea, such as diazotrophs, and fungi, such as the vertically transmitted clavicipitaceous endophytes, the generally horizontally transmitted class 2 fungal endophytes, mycorrhizal fungi and dark septate endophytes. This chapter introduces the science of endophyte biology and its application for a world population that is projected to grow to over 9 billion by 2050. It explores the potential of endophytes for improved agricultural and silvicultural sustainability including: yield improvement and nutrition; biocontrol of pests and diseases; and abiotic stress resistance in the context of climate change. It outlines how bioprospectors are using endophytes as sources of novel metabolites for the pharmaceutical and biochemical industries, and describes how endophytes can be used *in vitro* to elicit the increased production of known secondary metabolites from plants.

1.1 Endophytes

The microbiome of plants is complex and dynamic, and because of this plants are increasingly being considered as holobionts commonly inhabited by endophytic microbial communities (Hardoim *et al.*, 2015; Krell *et al.*, 2019, Chapter 3). Indeed, endophytes are ubiquitous and have been found in all species of plants studied to date (Hardoim *et al.*, 2015). The microorganisms that can behave as endophytes are easy to describe taxonomically, where they can be classed as archaea, bacteria, fungi, protozoans and viruses (or taxa of lower taxonomic rank within these). However, in the absence of a really clear-cut and universally accepted definition, it is more difficult to describe what features of these microorganisms qualify them as endophytes.

In broad terms, there are two schools of thought. The first defines an endophyte as any microorganism found inside plant tissue regardless of behaviour, the second prefers a more restricted definition including only those endophytes that are beneficial or neutral for the plant. In this book, we encounter these differences either implicitly or explicitly in almost every chapter. Here, we evaluate each definition and argue that the more general first definition, of any microorganism living within a plant, is most appropriate with more specific subclasses recognised within it.

The word endophyte literally translates as 'inside plant', so if understood solely on this basis then an endophyte is any organism that can exist within plant tissue; this would include beneficial symbionts, neutralists, commensals and pathogens. It excludes epiphytes (Figure 1.1). Such a broad definition would allow for the inclusion of endophytic organisms that can change the type of interaction with the plant along a mutualism–parasitism continuum (Mandyam and Jumpponen, 2015; Fesel and Zuccaro, 2016; Collinge *et al.*, 2019, Chapter 2; Berthelot *et al.*, 2019, Chapter 7; Costa *et al.*, 2019, Chapter 12; Murphy *et al.*, 2019, Chapter 18). A broad definition also allows for sub-classification into functional types (Figure 1.2).

A more restricted definition would consider endophytes as only beneficial or near-neutral plant symbionts and this interpretation is favoured by most researchers (Schulz and Boyle, 2006; Yakti *et al.*, 2019, Chapter 6; Widiantini and Franco, 2019, Chapter 8; Beekwilder *et al.*, 2019, Chapter 9; McNees *et al.*, 2019, Chapter 10; Gupta and Chaturvedi, 2019, Chapter 14). A further refinement is favoured by some researchers who maintain that endophytes are always beneficial for plants; Meshram and Gupta (2019, Chapter 13), for example, consider that 'endophytic microorganisms asymptomatically live together with plants in mutualistic alliance'.

One thing common to most definitions is that the effect of an endophyte on a plant is environmentally and genetically dependent. If we accept this proviso, then a broader definition would again seem to be favoured. Here, the endophytic microorganism will be detrimental or beneficial for the plant under different conditions. This understanding will be familiar to plant pathologists, many of whom consider that there is no such thing as a pathogen, rather merely a pathosystem where all parts of the necessary causal factors of disease need to be in place – the pathogen, a compatible host and suitable environmental conditions (Fang *et al.*, 2013; Collinge *et al.*, 2019, Chapter 2). For example, a host with some degree of resistance, but not

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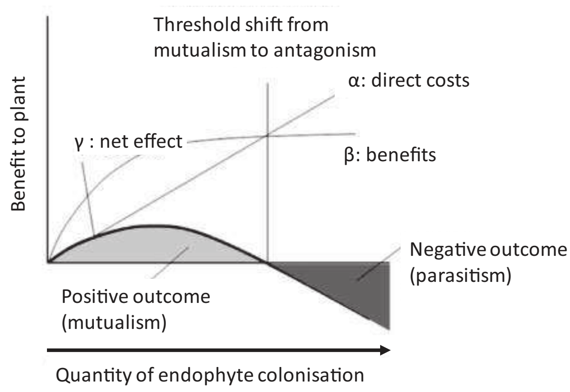

Figure 1.1 Mutualism–parasitism continuum. The dose–response relations in plant–endophyte interaction are shown. Endophyte colonisation has direct metabolic energy costs that should be correlated with their abundance within the host plant (α). Benefits to the plant in contrast are likely to follow a saturation curve (β). The net benefit (γ) follows an optimum curve with positive values at low to intermediate levels indicating mutualistic endophyte densities (light shading). However, negative values indicating antagonistic densities are found at high infection densities. Adapted from Partida-Martínez and Heil (2011) with permission.

immune, will result in an overall lower level of disease. This disease pathosystem model could just as well be applied to endophytes.

Those who favour the more restricted definition of an endophyte as always being beneficial or neutral for the plant would see the environmental and genetic factors in the system as making the endophyte either more or less beneficial for the plant. This definition therefore allows no room for the endophyte to behave as a pathogen and/or to have a detrimental effect on important plant growth parameters. While, in general, many endophytes do not have adverse effects on plants under any circumstances, this is not always the case. Murphy *et al.* (2014b) showed that even an almost universally beneficial endophyte such as the model organism *Serendipita indica* (syn. *Piriformospora indica*) can have negative effects on barley grain yield under severe nitrogen limitation and cold growing conditions. In this and other similar cases, the endophyte may behave as a competitor with the plant for important nutrient resources.

A definition of endophytes that caters for both of these perspectives would necessarily have to be somewhat more complex than either. Endophytes were first described in 1809 by the German botanist Heinrich Friedrich Link, who considered them to be fungal plant parasites. Since then, definitions of endophytes have increased in proportion to the growing number of endophyte studies: Wennstrom (1994) was one of the first to point out how the endophyte definition has been misused and he argued the case for new definitions focusing on particular endophyte behaviours. During the last 25–30 years, nearly all studies that feature endophytes have included a definition of the term, some being highly cited (Wilson *et al.*, 1992; Wilson, 1995; White *et al.*, 1993; Clement *et al.*, 1997; Schulz *et al.*, 1999; Jumpponen, 2001; Stone *et al.*, 2004; Schulz and Boyle, 2006; Rodriguez *et al.*, 2009). Most of these authors describe and define what an endophyte is from a generally plant-beneficial perspective. However, we are now beginning to see definitions that more accurately address the temporal and dynamic nature of the plant–endophyte relationship; for example, from Murphy *et al.* (2014a):

> Endophytes are a class of plant-associated microorganisms (bacteria, fungi and unicellular eukaryotes) that live at least part of their life cycle inter- or intracellularly inside plants, usually without inducing pathogenic symptoms. This can include competent, facultative, obligate, opportunistic and passenger endophytes (a passenger endophyte enters the plant by accident in the absence of selective forces maintaining it in the internal tissue of the plant).

Some also see the plant phenotype as being extended by its microbial inhabitants (Partida-Martínez and Heil, 2011), just like the human phenotype is extended by its own microbiome.

Another complication with the endophyte definition occurs with fungi that form mycorrhizae. Under the broad definition, mycorrhizal fungi can be considered endophytes but a distinction is usually made in the literature. The plant–endophyte relationship is often said to differ from mycorrhizal symbiosis by lacking cellular interfaces where specialised structures develop (e.g. arbuscules) and synchronised development between the plant and the fungal associate (Brundrett, 2006; Berthelot *et al.*, 2019, Chapter 7). As with the term mycorrhizal fungus, the term ‘endophyte’ is now so firmly entrenched in the literature that it will remain as the word of choice for these types or microorganisms. However, there is a strong case to be made for using this term as a very general description for microorganisms that inhabit plant tissue without resorting to some of the unwieldy definitions discussed above. It is in the nature of any relatively new research field for the terminology and descriptive language to evolve along with new discoveries and insights. Thus, in the spirit of this evolution of language (or linguemes), we could differentiate between the different endophyte lifestyles and variability of behaviour, by suggesting three major subcategories of endophytes (Figure 1.2):

- Endopathogenic microbes (**endopaths**): always detrimental/pathogenic
- Endosymbiotic microbes (**endosyms**): always beneficial or neutral
- Endosympathetic microbes (**endosympaths**): fluctuate between these two different behaviours depending on other factors (facultative endopaths/ facultative endosymbionts).

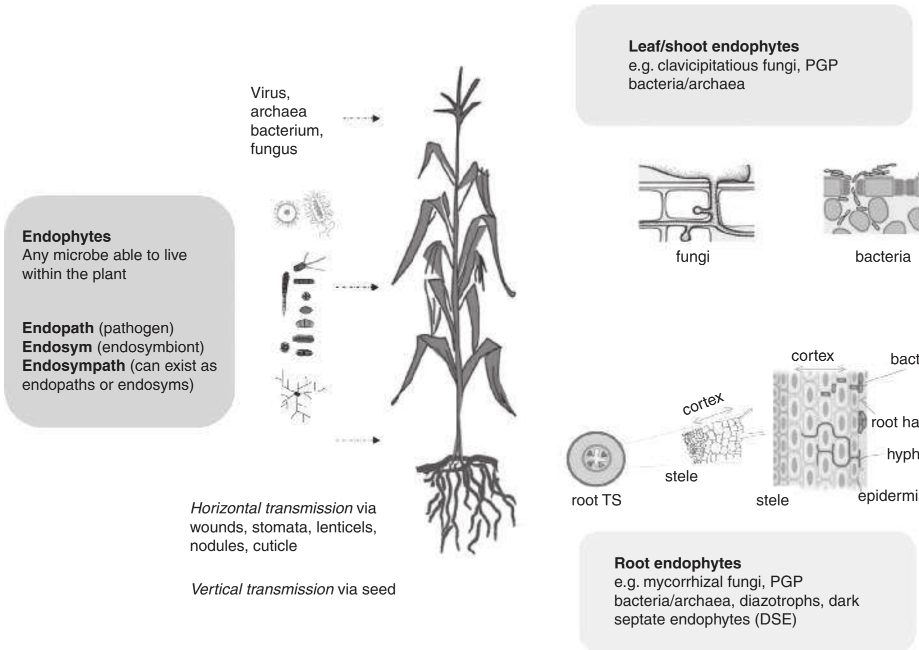

Figure 1.2 Subgroups of endophytes. Endophytes are any microbe that can live within a plant. Subclasses of endophytes can be defined in a hierarchical manner. Endopaths are pathogens, endosymbionts (endosyms) are always mutualistic and endosympaths can exist as both endosymbionts and endopaths. Within endosymbionts, major groups of beneficial microbes can be recognised including plant growth-promoting (PGP) and diazatrophic bacteria/archaea, class 1 (clavicipitatious) fungal endophytes, class 2 fungal endophytes, mycorrhizal fungi and dark septate endophytes (DSEs). They can also be divided by tissue type such as shoot or root. (A black and white version of this figure will appear in some formats. For the colour version, please refer to the plate section.)
Drawn from images in D'Arcy *et al.* (2001), Melotto *et al.* (2008), Hirsch and Mauchline (2012), Gelorini *et al.* (2012), Pickett *et al.* (2014) and Sarah Fogg with permission (www.sarahfogg.co.uk).

The etymology of the word 'sympaths' would also neatly suggest that the endophyte is 'sympathetic' to the environmental conditions (and also either symbionts or pathogens existing on the mutualism–parasitism continuum). These suggested new terms would also have the added advantage that they would be equally applicable to all life forms, not just plants (e.g. human, bird endosympaths). Whatever definition we use for endophytic microorganisms, the dynamic nature of this relatively new field of research will be reflected for some time to come in the equally dynamic and evolving terminology used to describe them. To some researchers it is sufficient for an endophyte to simply be 'what endophytologists study'.

1.2 Endophytes for a Growing World

1.2.1 The Role of Endophytes in Meeting Global Sustainable Development Goals

The rapidly growing world population is projected to reach over 9 billion by 2050 raising serious concerns over food security (FAO *et al.*, 2017). The number of chronically undernourished people in the world is currently estimated to be 815 million (IFPRI, 2018). Environmental damage including pollution, overuse of water, habitat loss, land-use change, biodiversity loss and climate change is also on the rise (Hodkinson *et al.*, 2011). It will be important to maintain high crop yields and productivity while improving sustainability. This is not a trivial challenge for several reasons. Approximately 85% of global water consumption is presently used for agricultural irrigation, of which 15–35% is unsustainable (Rosegrant *et al.*, 2009). Food systems contribute about one-fifth of all greenhouse gas emissions and agriculture is a primary cause of biodiversity loss (Arndt *et al.*, 2016). Furthermore, fertiliser production and application changes both nitrogen and phosphorous cycles, risking expensive, potentially irreversible environmental damage (Rockström *et al.*, 2009).

These problems have been recognised at a policy level by several international initiatives, such as the Strategic Plan for Biodiversity (Convention on Biological Diversity; COP, 2010), the Paris Agreement on Climate Change (2016), the G20 Agriculture Ministers' Action Plan 2017 for protecting water resources, the Global Challenges of the Consultative Group on International Agricultural Research (CGIAR, 2016), and the Sustainable Development Goals (SDGs; including 17 goals and 169 targets) of the United Nations 2030 Agenda for Sustainable Development (United Nations, 2015). Seven of the United Nations SDGs are particularly relevant to the endophyte research community:

- Goal 2: Achieve food security and improved nutrition and promote sustainable agriculture
- Goal 3: Ensure healthy lives and promote well-being for all at all ages
- Goal 6: Ensure access to water and sanitation for all
- Goal 7: Ensure access to affordable, reliable, sustainable and modern energy for all
- Goal 12: Ensure sustainable consumption and production patterns
- Goal 13: Take urgent action to combat climate change and its impacts
- Goal 15: Sustainably manage forests, combat desertification, halt and reverse land degradation, halt biodiversity loss.

There is also a pressing need to discover and produce new and useful biomolecules to provide assistance and relief in all aspects of human life especially those that involve global health (United Nations, 2015). Medicine needs to constantly adapt and cope with new challenges presented by the growing and ageing world population, including increased urbanisation, globalisation and travel (United Nations, 2015). Molecules are needed to tackle drug resistance in bacteria, combat viruses and fight fungal infections (Cannon and Simmons, 2017; Meshram and Gupta, 2019, Chapter 12; Gupta and Chaturvedi, 2019, Chapter 13). Endophytes are proving to be a novel source of metabolites for the pharmaceutical and biochemical industries providing biologically active compounds such as antibiotics, antioxidants, anticancer agents, immunosuppressive compounds, insecticides, plant growth-promoting (PGP) agents and volatile antimicrobial agents representing a wide range of organic molecules including terpenoids, peptides, carbohydrates, aromatics and hydrocarbons (Strobel, 2018; McNees *et al.*, 2019, Chapter 10). Endophytes can also be elicitors and enhancers of secondary metabolite production in plants as outlined in Gupta and Chaturvedi (2019, Chapter 13).

1.2.2 Endophytes in Sustainable Crop Production

Agricultural productivity has improved in pace with population growth particularly since the Green Revolution (Godfray *et al.*, 2010). However, current agricultural and silvicultural production systems need to become more environmentally, economically and socially sustainable to provide ecosystem services and help address problems associated with maintaining productivity including pollution, erosion, soil fertility, greenhouse gas emissions, pests/disease and reduced agroecosystem biodiversity, in line with the United Nations SDGs outlined above. There is a need to minimise chemical applications, better manage water, enhance carbon sequestration, maximise biodiversity and increase resilience to pests and diseases. There is also a need to make sustainable use of biomass and bioenergy crops (Hodkinson *et al.*, 2015; Beekwilder *et al.*, 2019, Chapter 9). To do this, crop management regimes need to manipulate the plant microbial communities, including their endophytes, to best effect (Busby *et al.*, 2017).

Endophytes can contribute to both productivity and sustainability in combination with several other approaches, including plant breeding and improved farm and soil management. The challenge to endophytologists is to widely integrate endophytes into modern agricultural practices in the most efficient and beneficial ways (Le Cocq *et al.*, 2016). To achieve this goal, there is a need to investigate the role of endophytes in the resilience of crops and forest trees to abiotic and biotic stress; understand the biodiversity and community structure of endophytes; and utilise the beneficial attributes of endophytes to improve the sustainability of agroecosystems and forestry.

The potential application of endophytes to agriculture is well documented (Murphy *et al.*, 2014a, b, 2015a, b, c, d, 2017b, 2018; Soares *et al.*, 2016). Some PGP endosyms can improve mineral nutrition and yields (Achatz *et al.*, 2010; Hubbard *et al.*, 2013; Yakti *et al.*, 2019, Chapter 5; Murphy *et al.*, 2019, Chapter 18), confer insect or pathogen resistance (Clement *et al.*, 1997; Vega *et al.*, 2009; Wiewióra *et al.*, 2015; Collinge *et al.*, 2019, Chapter 2) and improve stress tolerance (Waller *et al.*, 2005; Waqas *et al.*, 2012; Bertherlot *et al.*, 2019, Chapter 7). In forestry, the importance of mycorrhizal fungi is well documented but other endophyte groups are understudied and could play important roles in productivity, stress tolerance and the control of pests and diseases such as cork oak decline (Costa *et al.*, 2019, Chapter 12) and ash dieback disease (Lahiri *et al.*, 2019, Chapter 15).

Few of the beneficial endosyms are well characterised from a mechanistic perspective (Finkel *et al.*, 2017). There are some well-known examples, such as alkaloid production in the fungus *Epichloë* (Saikkonen *et al.*, 2016; Johnson and Caradus, 2019, Chapter 16); biological nitrogen fixation by rhizobia in legumes (Desbrosses and Stougaard, 2011); phosphate solubilisation/mobilisation by bacteria and mycorrhizal fungi (Timmusk *et al.*, 2017); hormone production, such as *Azospirillum* induced auxin synthesis in wheat (Dobbelaere *et al.*, 1999), enzymatic effects, such as *Enterobacter* 1-aminocyclopropane-1-carboxylate (ACC) deaminase mediated phytostimulation (Li *et al.*, 2000); and contribution to systemic resistance (Ryu *et al.*, 2004). However, mechanistic studies are still in their infancy and need assessment for the wide range of differing endophyte/plant systems.

Biocontrol offers a promising alternative or supplement to synthetically produced pesticides (Collinge *et al.*, 2019, Chapter 2; Krell *et al.*, 2019, Chapter 3; Barra-Bucarei *et al.*, 2019, Chapter 4; Høyer *et al.*, 2019, Chapter 5). One group of promising biocontrol agents (BCAs) are the endophytic entomopathogenic fungi and most interest has focused on the application of entomopathogenic aerial conidia from *Beauveria* spp. and *Metarhizium* spp. for crop protection. An early study by Bing and Lewis (1991) showed that application of *Beauveria bassiana* to maize and its subsequent colonisation of plant tissue suppressed the European corn borer *Ostrinia nubilalis*. Research on several other entomopathogenic fungal genera, such as *Acremonium*, *Cladosporium*, *Clonostachys*, *Isaria* and *Lecanicillium*, is taking place (reviewed in Krell *et al.*, 2019, Chapter 2 and Barra-Bucarei *et al.*, 2019, Chapter 4). Some of these fungi have demonstrated the dual ability to control both insect pests and plant pathogens (Ownley *et al.*, 2004) and others have been shown to improve other agronomic traits (Murphy *et al.*, 2015a, b).

Some microbes such as the clavicipitatious fungal endosyms of several forage grasses produce alkaloids, phenolics and terpenoids that reduce insect damage and therefore improve yield (Clement *et al.*, 1997; Rasmussen *et al.*, 2012; Żurek *et al.*, 2010; Johnson and Caradus, 2019, Chapter 16). They are often vertically transmitted through seed, and there is evidence that they have co-evolved with the plant host (Saikkonen *et al.*, 2016; Hodkinson, 2018). They colonise the plant from the seed, so are sold as endosym-containing seed (Johnson and Caradus, 2019, Chapter 16).