Chapter 43
The Minimal Rhizosphere Microbiome

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Abstract The rhizosphere provides a home to numerous (micro) organisms that in turn may affect plant growth, development, and tolerance to abiotic and biotic stresses. How plants shape the rhizosphere microbiome has been subject of many past and present studies with the ultimate goal to identify plant genetic traits that select and support beneficial microorganisms. Novel 'omics technologies have provided more in-depth knowledge of the diversity and functioning of the rhizosphere microbiome and significant advances are being made to uncover mechanisms, genes and metabolites involved in the multitrophic interactions in the rhizosphere. To better understand this intriguing complexity, both reductionists' and systems ecology approaches are needed to identify the biotic and abiotic factors involved in microbiome assembly. Here, different strategies are discussed to re-shape the rhizosphere microbiome in favour of microbial consortia that promote root development and plant growth, and that prevent the proliferation of pests and diseases.

43.1 Introduction

Currently more than one third of the crop yields worldwide are lost due to abiotic and biotic stress factors, such as drought, salinity, pests and diseases. Future increases in crop yields will have to be achieved on sub-optimal soils with reduced input of fertilizers and pesticides ('*more with less*'). These challenges have increased the awareness of the importance of the plant microbiome (i.e. the collective communities of microorganisms on and in plants, their genomes and interactions) for improved and sustainable agricultural practices. Plants are colonized by an astounding number of microorganisms that can have profound effects on seed germination, plant growth and development, nutrition, diseases and productivity. In this context, plants can be viewed as superorganisms that rely in part on their microbiome for specific functions

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and traits. In return, plants deposit a substantial part of their photosynthetically fixed carbon into their direct surroundings (spermosphere, rhizosphere, phyllosphere), thereby feeding the microbial community and influencing their activities and composition (Mendes et al. 2013). For many plant-associated microorganisms, however, there is still little knowledge of their impact on plant growth and health. Hence, deciphering the plant microbiome is critical to identify beneficial microorganisms that can be used as an integral component of future agriculture and horticulture.

43.2   The Rhizosphere

The rhizosphere is the narrow zone surrounding and influenced by plant roots via the release of so-called rhizodeposits (i.e. exudates, border cells, mucilage) (Lynch 1990). The nutrients, trace elements, volatile organic compounds and other metabolites deposited by plant roots attract many (micro)organisms such as bacteria, archaea, fungi, nematodes and protozoa, making the rhizosphere a hot spot of microbial activity and interactions (Raaijmakers et al. 2009; Buée et al. 2009; Mendes et al. 2013). Following the terminology used for microorganisms colonizing the human body, the collective communities of microorganisms on and also inside plant root tissue, their genomes and interactions are now referred to as the rhizosphere microbiome. Over the past five decades, numerous studies have shown that specific members of the rhizosphere microbiome can affect plant growth and development, plant nutrition and stress tolerance (Berendsen et al. 2012; Mendes et al. 2013; Philippot et al. 2013). In this context, Cook et al. (1995) postulated that plants may '*cry for help*' by selectively stimulating microorganisms that protect them from invading pathogens. Rhizosphere microorganisms that have been well studied for their beneficial effects on plant growth and health are the nitrogen-fixing bacteria, mycorrhizal fungi, plant growth-promoting rhizobacteria (PGPR), and saprophytic and mycoparasitic fungi (Mendes et al. 2013). For the vast majority of rhizosphere (micro)organisms, however, there is still little to no understanding of their metabolic potential and functions. This lack of knowledge has led to numerous studies to catalogue microbial communities in the rhizosphere of different plant species, to elucidate which microbes are active during plant development and to unravel which functions and biosynthetic pathways are displayed in time and space (Mendes et al. 2013; Philippot et al. 2013).

To go beyond '*collecting stamps*', several meta-'omics' approaches (transcriptomics, proteomics, metabolomics) have been and are still being developed to identify gene transcripts, proteins and metabolites in the rhizosphere. For example, Wang et al. (2011) adopted a metaproteomics approach to unravel interactions between plants and rhizosphere microorganisms in different cropping systems. They found, among others, that approximately half of the bacterial groups classified by proteomic analysis were not found in the DNA-based metagenomic analyses of the rhizosphere bacterial community and *vice versa* (Wang et al. 2011), emphasizing the need to improve the resolution and sensitivity of these approaches. Also, stable isotope probing (Prosser et al. 2006) has provided new opportunities to identify microorganisms that

are metabolically active in the rhizosphere. These and other technologies revealed that also fungi make up a significant part of the rhizosphere microbial biomass, especially during flowering and senescence (Hannula et al. 2010). Hence, top-down approaches such as metagenomics and bottom-up approaches targeting individual microbial species or strains should be integrated to provide a comprehensive coverage and understanding of the microbial community and their activities as a whole (see also Zengler and Palsson 2012; Mendes et al. 2013).

43.3   Shaping the Rhizosphere Microbiome

Several species and strains of rhizobacterial and fungal genera, including *Bacillus*, *Pseudomonas*, *Collimonas, Trichoderma, Piriformospora* and nonpathogenic *Fusarium oxysporum,* have been shown to promote plant growth and to protect plants from stress by different mechanisms (Lugtenberg and Kamilova 2009; Raaijmakers et al. 2009; Raaijmakers and Mazzola 2012; Chap. 3) . These include biofertilization (Chaps 23, 24 and 25), stimulation of root growth (Chap. 26), antibiosis (Chap. 18), induced systemic resistance (Chap. 14), parasitism and rhizoremediation (Chap. 29). These mechanisms are well documented for rhizobacteria belonging to the Proteobacteria and Firmicutes, i.e. *Pseudomonas* (Chap. 18) and *Bacillus* (Chap. 40), as well as for the mycoparasitic fungi *Trichoderma* (Chap. 36) and *Gliocladium*. Hence, there is a major interest to develop strategies that re-shape the rhizosphere microbiome in favour of microorganisms that promote root development and plant growth, and that prevent the proliferation of pests and diseases.

The first and most obvious strategy to re-direct the microbial composition and activities in the rhizosphere is changing the quality and/or quantity of root exudates via plant breeding or via genetic modification. This form of '*rhizosphere engineering*' requires detailed knowledge of the exudate composition (spatial, temporal) and their effects on microbial growth and activity (Bakker et al. 2012). Although our understanding of exudate chemistry and microbial interactions in the rhizosphere has improved considerably, there are, to my knowledge, no specific breeding programs yet that evaluate plant lines for their broad interaction with the rhizosphere microbiome. More than a decade ago, Smith et al. (1999) investigated the genetic basis in plants for interactions with beneficial rhizobacteria. They discovered substantial variation among recombinant inbred lines of tomato and identified loci that were associated with growth of and disease suppression by a beneficial *Bacillus cereus*. Rudrappa et al. (2008) further showed that plants can stimulate, via malic acid, the protective effects of a beneficial *Bacillus subtilus* strain in the rhizosphere. Similarly, Neal et al. (2012) showed that a beneficial *Pseudomonas putida* strain was attracted to 2,4-dihydroxy-7-methoxy-1,4-benzoxazine-3-one (DIMBOA), the allelopathic compound that is exuded in relatively high quantities from roots of young maize seedlings. These and other studies exemplify that specific phenotypic traits and genetic variation in host plant species can be exploited to enhance beneficial associations of plants with rhizosphere microorganisms. To date, however, our knowledge

of root exudation *in situ* is still too limited to provide specific targets that can be used in plant breeding programs.

The second strategy to re-direct the rhizosphere microbiome is to introduce selected beneficial microorganisms at high densities in soil, onto seeds or other planting materials (Mendes et al. 2013). Over the past decades, many bacterial and fungal strains with different beneficial traits have been studied for their ability to boost plant performance and to control pests and diseases. Although there are several successful cases (e.g. *Agrobacterium radiobacter, Bacillus subtilis*), many of the promising microbes tested to date were less effective in disease control than their chemical counterpart and therefore not commercially attractive enough for product development and implementation in practice. The observed inconsistency in performance of various promising microbial agents has been attributed to various reasons, including poor establishment on/in seed or plant tissue, poor survival or lack of expression of the desired microbial trait/activity at the right time and place.

43.4 Reconstructing a 'Minimal Rhizosphere Microbiome'

To date, there has been a strong emphasis on '*one-microbe-at-a-time*' applications, whereas many ecosystem functions, including nutrient cycling and disease suppression, are generally driven by the (sequential) activity of microbial consortia. Furthermore, several microorganisms only exhibit a specific activity when they are part of a consortium (Garbeva and de Boer 2009; Garbeva et al. 2011). Hence, the use of assemblages of different rhizosphere microorganisms with complementary or synergistic traits may provide a much more effective and consistent effect. This concept of so-called 'reconstructed microbiomes' or 'synthetic communities' (De Roy et al. 2013; Grosskopf and Soyer 2014) is gaining momentum not only in plant-microbe interactions but also in the fields of probiotics and natural product discovery. However, to find and select the right players and microbial composition of a rhizosphere consortium for a specific function (e.g. disease suppression) is still a puzzle and requires more fundamental understanding of the temporal and spatial dynamics of the rhizosphere microbiome, the chemistry, the underlying communication and beneficial activities.

Natural disease suppressive soils (Chap. 38) provide a very good 'model system' to unravel and design the optimal microbial consortium to protect plants from infection by soil-borne pathogens. Studies by Kyselkova et al. (2009); Mendes et al. (2011) and Rosenzweig et al. (2012) on soils suppressive to different fungal and bacterial plant pathogens pinpointed multiple bacterial genera that were more abundant in the suppressive than in the corresponding disease conducive soils. Although the potential role of the identified bacterial communities in disease suppressiveness was addressed for only a few genera, these studies do provide a framework to reconstruct microbial consortia for disease control. Clearly, there is a need for a community systems approach to resolve the interplay between individual community members, the host plant and the soil environment (Zengler and Palsson 2012). In this context, Kinkel

et al. (2011) proposed a co-evolutionary framework for inducing or managing natural disease suppressiveness of soils. They also argued that control of different plant pathogens on different crops most likely requires a different subset of microorganisms (Kinkel et al. 2011). Ideally, the ultimate goal is to design a so-called '*minimal rhizosphere microbiome*' that is effective against multiple soil-borne pathogens in different agro-ecosystems. Based on the concept of the minimal genome (Moya et al. 2009; Juhas et al. 2011), the minimal rhizosphere microbiome is defined here as the minimal set of microorganisms, microbial traits and genomes that are needed to effectively and consistently execute a specific function in the rhizosphere, e.g. protection of plant roots against fungal infections or rhizoremediation of toxic compounds (Mendes et al. 2013).

For controlling plant diseases, designing a separate minimal rhizosphere microbiome for each of the major pathogen groups (bacteria, fungi, nematodes, oomycetes) may be feasible. This assumption is based on the fact that various studies have pointed to common players and mechanisms in different soils that are naturally suppressive to specific fungal pathogens. For example, *Pseudomonas* species have been shown to contribute to suppressiveness of soils to either Fusarium wilt disease or to take-all disease of wheat (Weller et al. 2002). Furthermore, the onset of natural disease suppressiveness of soils follows a similar pattern for different fungal pathogens (Weller et al. 2002), suggesting that similar cues, mechanisms and microbes play a role in the transition of a soil from a conducive to a suppressive state. An in-depth understanding of the shifts in community composition and microbial activities during this transition will be required to select the right microbiome members. Selection and assembly of minimal rhizosphere microbiomes should be based on functional traits and genes rather than on taxonomic classification only (Burke et al. 2011; Boon et al. 2013). Using a modelling approach, Scheuring and Yu (2012) suggested three easy steps to assemble a beneficial microbiome. In their models, the first step is that the new host's microbiome starts with a higher proportion of beneficials either by vertical transmission or by a higher immigration rate. The second step involves a high resource supply from the host to the beneficials, which in turn (third step) fuels intense interference competition via antibiotic production leading to competitive dominance of the beneficial microbes. Although Scheuring and Yu (2012) focused primarily on antibiosis as a key function of a beneficial microbiome, their models are highly instrumental to identify major processes that drive assembly of a beneficial microbiome. Whether these models could also be used for other important traits of beneficial rhizosphere microbiomes, such as parasitism, induced resistance and resource competition, remains to be determined.

In conclusion, the rhizosphere is a diverse and dynamic habitat with multiple microorganisms that affect plant growth, development and tolerance to abiotic and biotic stresses. To better understand the multitrophic interactions in the rhizosphere, both reductionists' and systems biology/ecology approaches are needed to resolve the underlying mechanisms involved in microbiome assembly and activity.

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