

Chapter 3

What Determines Successful Colonization and Expression of Biocontrol Traits at the Belowground Level?



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3.1 Introduction

Plant's health and development are largely influenced by the microbiota inhabiting the rhizosphere soil, the root surface (rhizoplane) and the root endosphere compartments. This compound plant-associated belowground microbiome originates from surrounding bulk soil microbial communities. It is widely accepted that the structure and composition of this microbiota is shaped and modulated by: (i) the host plant immune response; (ii) root exudate-mediated signalling and plant metabolites; and (iii) the complex trophic relationships established among the components of these communities. These interactions, primarily during the initial colonization process, are crucial for the establishment of root-associated bacterial communities that largely differ from those of the enclosing soil. It is known that the belowground microbiota is a source of plant growth-promoting microorganisms (PGPM) and biological control agents (BCA) that, once properly identified and carefully characterized, can be harnessed to improve plant fitness (Ciancio et al. 2016). Nevertheless, to reach this goal a comprehensive understanding of genetic determinants contributing to successful colonization of the target niche and effective biocontrol, as well as the wide range of factors influencing them, is needed (Cole et al. 2017). Research on biocontrol has mostly focused on two/three trophic level interactions; that is, the BCA, the pathogen and the plant host. However, since effective biocontrol is not only a consequence of the complex interactions established among the above-mentioned partners, but also with the natural pre-existing microbiota and a wide range of environmental factors, more holistic approaches are needed (Mercado-Blanco et al. 2018). One of the aims of such a global strategy is to overcome

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inconsistencies of biocontrol measures, particularly when implemented at the field level (Schreiter et al. 2018). This chapter provides a brief appraisal of traits and factors influencing successful colonization and effective biocontrol of beneficial components of the plant-associated microbiota at the belowground level (Fig. 3.1). Obviously, we do not intend an exhaustive review of this broad and complex research area. To gain insightful perspectives on matters not analyzed in detail, the reader is kindly invited to consult the available literature on topics such as: (i) the continuous search for novel BCA to confront traditional and emerging diseases (e.g. Bubici et al. 2019); (ii) specific traits defining an optimal BCA (e.g. Deketelaere et al. 2017); (iii) the complex networks and signalling pathways regulating the expression of colonization and biocontrol traits (e.g. Hartmann and Schikora 2012; Kidarsa et al. 2013); (iv) the involvement of some bacterial secretion systems in root colonization and plant-beneficial bacteria interactions like BCA displaying endophytic lifestyle (e.g. Reinhold-Hurek and Hurek 2011; Marchi et al. 2013); (v) factors affecting the belowground microbiota including beneficial components which can be harnessed for biocontrol strategies (e.g. Mercado-Blanco et al. 2018); and (vi) the impact of “-omics” in biological control research (e.g. Massart et al. 2015).

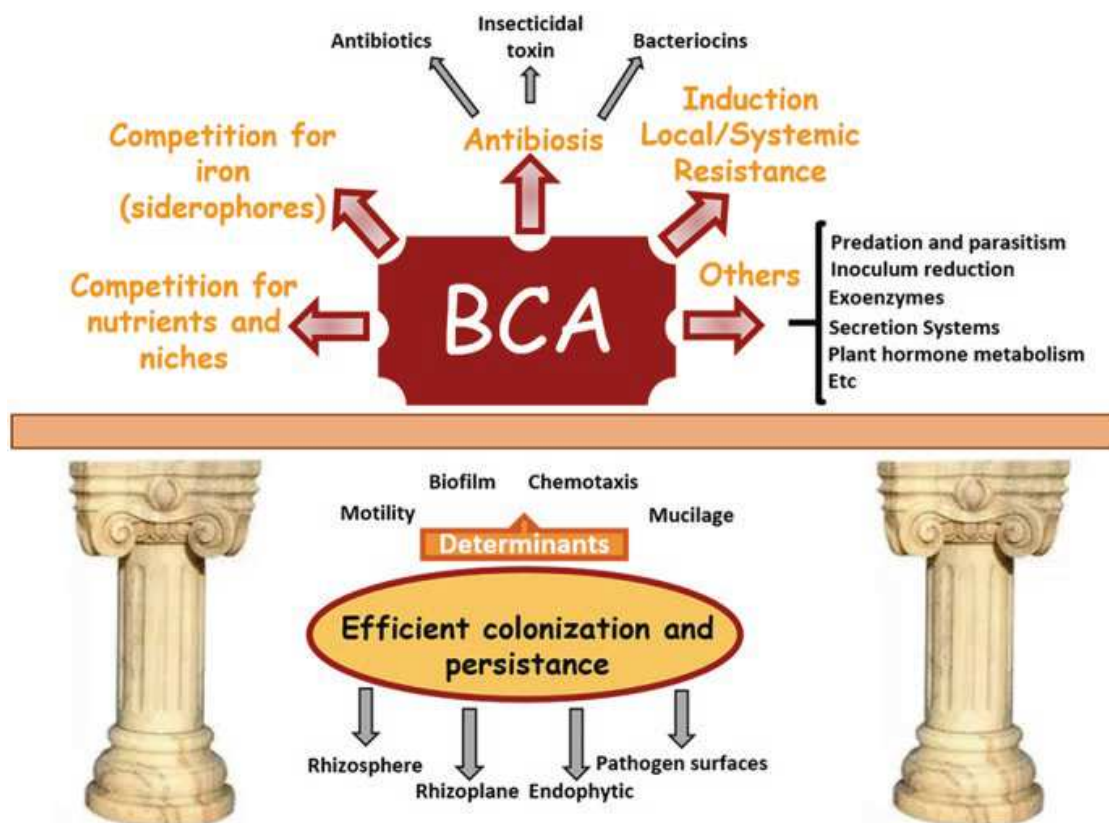


Fig. 3.1 Scheme summarizing determinants and mechanisms involved in the colonization and persistence of a model biocontrol agent (BCA). Successful biological control exerted by a BCA primarily relies on the efficient colonization of (and endurance in/on) target niches (e.g. different compartments of the plant root). A BCA does not necessarily have all these mechanisms. Modes of action against soil-borne pathogens can operate individually, simultaneously and/or sequentially in time and space

3.2 A First Requisite Towards Successful Biocontrol: Efficient and Enduring Colonization of the Target Niche

Effectiveness of any given BCA primarily relies on the successful colonization (and subsequent persistence on/in) of the ecological niche or microhabitat where its beneficial effect is expected. It is worth mentioning that successful colonization of the rhizosphere, or any other niche at the belowground level, is a consequence of a delicate equilibrium between biotic (i.e. the host plant, the introduced BCA, the rhizosphere and endosphere microbiome) and abiotic (e.g. soil type and structure, temperature, pH, nutrients and water availability, composition of root exudates, etc.) factors, which are dynamically interacting (Shaikh et al. 2018).

Plant roots are obviously the main entrance of either soilborne pathogens, by passive (breakages and injuries produced by nematodes, entomofauna, agronomic practices, etc.) or active (e.g. pathogen enzymatic activities) mechanisms. Therefore, successful root colonization by a BCA is key for effective biocontrol. Indeed, this is the organ where both types of microorganisms (phytopathogens and BCA) will mostly confront each other, deploying an “arm race” to compete for space and reliable nutrient sources.

3.2.1 Brief Overview on Determinants Involved in Root Colonization

Several important microbial traits offer a selective advantage for root colonization, allowing microorganisms to attach, thrive and compete with others on this unique environment. Among these traits, motility plays a crucial role enabling the detection and approach to the root surface. In the case of bacteria, flagella and pili are crucial for their movement towards the plant roots through the complex soil matrix. Motility is stimulated by chemotactic responses and influenced by factors such as the host plant, the soil type, etc. It is well known that flagella-defective mutants lose the capability to colonize root tips efficiently, and that non-motile or reduced motility mutants have serious difficulties for competitive root colonization (De Weger et al. 1987; Harshey 2003; Gao et al. 2016), although this issue is controversial for some authors (Scher et al. 1988). Undoubtedly, other factors are involved in bacterial motility along the root such as the host plant, the soil type and its water flow, or the root growth. Moreover, the specific root zone and the bacterium under study strongly determine the conclusions reached in this type of studies (see, for instance, Mercado-Blanco and Bakker 2007 and references therein). Functional flagella are needed for bacteria to migrate downwards along the growing root thereby reaching more profound regions such as the root tip. Motility is also required during early stages of biofilm formation and for surface attachment. Bacterial biofilms pose advantages during root colonization, providing a much better protected niche. They also play important roles in the adaptation to live on roots and in the spatial distribution of

bacteria in this organ (Gao et al. 2016; Pandin et al. 2017; Tovi et al. 2019 and references therein).

Plant root exudates are also important determinants during the colonization process of the rhizosphere and the rhizoplane (Zhou et al. 2016). Microorganisms (harmful, beneficial or neutral) are attracted by specific components of root exudates and mucilages, reacting chemotactically to them thereby favouring colonization and multiplication in both compartments. Therefore, BCA have to be very competitive in responding to these chemical signals to colonize specific root spots faster and better than other components of the soil microbiota. The colonization process can be influenced by differences in root exudate composition. Besides, the effects of root exudates can be positive or negative, and the balance between attractive or repulsive compounds determines microbial colonization (Bais et al. 2006). The composition of root exudates is shaped by factors like the host genotype (even at the cultivar level), the plant phenology, or the exposure to stress. It may also vary along the root length, so that the composition and structure of the associated communities can differ depending on the root region under survey. The overall picture is that the root exudation process is heterogeneous in time and space, provoking that specific root sites are much better colonized than others by soil microorganisms what obviously include those ones showing biocontrol activity. Differential responses to root exudates and mucilage may explain the spatial and temporal differences frequently observed during microbial colonization of root systems (Scharf et al. 2016). Root exudation also plays an important role in biocontrol exerted by fungal BCA. For instance, it has been reported that root exudates from tomato plants act as chemoattractants of *Trichoderma harzianum*, supporting a better growth of this BCA. Interestingly, some of these chemoattractants did not result in an enhanced chemotropism of the pathogen (*Fusarium oxysporum* f. sp. *lycopersici*) indicating they selectively attract the BCA (Lombardi et al. 2018).

In addition to mucilage, root exudates, chemotaxis and bacterial flagella, quorum sensing (i.e. regulation of gene expression of processes like antibiotic production in response to fluctuations in cell-population density; see, for instance, Hartmann and Schikora 2012), and production of specific compounds/enzymes (e.g. lipopolysaccharides, fimbriae, NADH dehydrogenase I, vitamin B1 or amino acids) are relevant factors influencing the root colonization process by BCA. Moreover, cell surface proteins (e.g. type IV pili, agglutinin, and outer membrane proteins) and site-specific recombinases involved in phase variation, among others, can be related to colonization as well. This indicates that BCA may employ different mechanisms, either alone or in combination, in order to successfully colonize plant roots (Mercado-Blanco and Bakker 2007; Compant et al. 2010; Garge and Nerurkar 2017).

3.2.2 *BCA Able to Colonize the Root Interior: Endophytes*

Plants are holobionts in which the host and its associated microbiota cooperate to shape an extraordinary ecosystem (Hassani et al. 2018). Some fungi and bacteria colonizing the bulk soil, the rhizosphere and/or the rhizoplane are also able to colonize the internal plant tissues. Endophytes are not only neutral, commensal and/or beneficial microorganisms that can be isolated from asymptomatic plant tissues, but also dormant saprobes and pathogens during their latent phase of their life cycle. These microorganisms engage an intimate association within their hosts, establishing a microbial community in the plant endosphere, which can vary among different plant compartments (Compant et al. 2016; Brader et al. 2017). Endophytes have evolved to: (i) be adapted to niches (i.e. inner plant tissues) providing a specific source of nutrients and (ii) evade the host plant defence responses deployed against colonization by microbes. Hence, plants seem to use similar defence/immune responses to interact with both pathogenic and non-pathogenic endophytes. However, the outcome is different depending on the establishment of a negative (pathogens) or positive (beneficial endophytes, endosymbionts) interaction. Some endophytes seem to contribute to plant health, fitness and development, displaying beneficial traits with great agro-biotechnological potential. They present advantages over microorganisms only present in the root exterior and traditionally used in biocontrol strategies since, as mentioned above, they are already adapted to the plant interior (Mercado-Blanco and Lugtenberg 2014). For endophytes that are seed transmitted, the advantage is even greater since from an agro-biotechnological point of view, its commercialization does not need complicated formulation approaches (Verma and White 2019). Strategies of colonization and mechanisms involved represent an important aspect of this particular BCA-plant interaction. Similar to the uneven surface root colonization usually observed, the endophytic plant microbiome is not uniformly distributed, constituting a dynamic entity (composition, abundance, distribution and functionality) that may change over time influenced by the plant physiological state and growth phase, and/or diverse biological, physical-chemical and environmental factors. Yet, the underlying mechanisms of endophytic colonization by BCA are poorly understood. Penetration to the plant interior could operate through active or passive (at root tips, cracks at lateral root emergence sites, injuries caused by pathogens, nematodes or insects) means. Thus, all rhizosphere microorganisms can potentially enter and live in the root interior as endophytes at some stage of their life. Overall, colonization traits mentioned above for BCA acting at the rhizosphere/rhizoplane level can also be operative during endophytic colonization of the root inner tissues: secretion of cell-wall degrading enzymes, lipopolysaccharides, flagella, pili, twitching motility, etc. Genes that are important for colonization include those involved in chemotaxis, flagella and pili formation, and in various metabolic pathways and transport systems (see, for instance, Hardoim et al. 2015; Santoyo et al. 2016 and references therein). Several non-pathogenic endophytic strains of the fungal genera *Colletotrichum*, *Cladosporium*, *Fusarium*, *Pestalotiopsis* and *Trichoderma* spp. constitute an attractive option for management

of some plant diseases. These fungi usually display different biocontrol mechanisms, including mycoparasitism, production of lytic enzymes and/or antibiotics and induction of plant defences (De Silva et al. 2019). The genus *Trichoderma* is the most commonly used fungal BCA, showing a wide range of biocontrol mechanisms (see Chap. 7). Interestingly, some *Trichoderma* spp. strains displaying endophytic lifestyle have been shown to be effective as plant growth promoters and BCA (Toghueo et al. 2016). An introduced BCA (either or not displaying endophytic lifestyle) must interact not only with the host but also with the native endophytic microbiome. However, our knowledge on the effects that the introduction of BCA causes on the indigenous endophytic microbial communities and *vice versa* is almost null. Apparently, transient changes are observed in fungal and bacterial populations inhabiting the roots after BCA application, although a broad range of plant defence responses are triggered (Bankhead et al. 2004; Gómez-Lama Cabanás et al. 2014). Despite all advantages endophytes have, relatively few of them have so far been successfully commercialized (De Silva et al. 2019).

3.2.3 Colonization of Pathogen Surfaces by BCA

BCA can directly (i.e. physical contact) interact with phytopathogenic fungi by different mechanisms. The final outcome is to feed from these eukaryotes. This interaction includes several steps: detection of the fungal host, attachment to the fungal cells, and growth of the BCA on fungal surfaces. For instance, BCA such as *P. fluorescens* WCS365 and *P. chlororaphis* PCL1391 are able to colonize the *Fusarium oxysporum* f. sp. *radicis lycopersici* (Forl) hyphae inhibiting pathogen germination and survival. Microscopic visualization of BCA–fungal interactions showed that at least some antagonistic strains exhibit chemotaxis toward fungal exudates (e.g. fusaric acid secreted by Forl) enabling them to congregate on the fungal surface (De Weert et al. 2004). Upon contacting the fungus, the BCA may scavenge nutrients from the fungal cell wall, fuel on fungal secretions, or use metabolites released after fungal cell lysis. Evidence suggests that hyphal colonization by BCA may play an important role in biocontrol activity, particularly when compounds or enzymes that inhibit the fungal growth are involved in the biocontrol mechanism. Yet, information about genes involved in interactions with fungi is scarce. The identification of BCA genes involved in fungal colonization or acquisition of nutrients from fungi will undoubtedly contribute to design new and improved biocontrol strategies (Pliego et al. 2011 and references therein). For instance, several *P. putida* gene promoters involved in carbon catabolism, amino acid/nucleotide metabolism, and membrane transport processes are induced during the growth of this BCA upon pathogen's surface colonization (Lee and Cooksey 2000; Ahn et al. 2007).

3.3 A Concise Appraisal on Mechanisms of Biocontrol

Once the BCA has successfully colonized the target niche, effective biocontrol is a consequence of diverse modes of action that can be deployed either individually or as a combination of them. In addition, they may operate differentially in time and space. As mentioned for colonization determinants, biocontrol mechanisms are also influenced by a range of factors. Benefits provided by microbial BCA are based on either direct (i.e., antibiosis, parasitism and predation, and signal interference) or indirect (i.e., competition for nutrients and niches, production of siderophores [competition for iron], and induction of systemic resistance responses [induced systemic resistance, ISR]) effects. Direct effects lead to less pathogen's inoculum in the target site (e.g. the rhizosphere, the root interior, etc.) or to lower infection potential of the deleterious agent. Indirect mechanisms promote plant growth and/or stimulate host defence responses thereby decreasing the adverse effects of the pathogen. However, both types of mechanisms are not mutually exclusive and it is often reported that a single BCA may work through several mechanisms. These mechanisms can operate simultaneously or being triggered at different stages (spatially and temporarily) during the plant-BCA-pathogen interaction. It is worth noting that effectiveness of many biocontrol traits depends on the BCA population levels reached on/in the specific target niche, and that some of these traits (i.e. antibiotics production) depend on cell-density (Pierson et al. 1994). Competition for nutrients, microelements, and antibiosis can be operative without a direct interaction with the plant. However, disease suppression mediated by ISR is a consequence of an intimate interaction between the BCA and the host plant. Remarkably, signal transduction pathways leading to enhanced resistance in plants upon interaction with beneficial microorganisms are better known than bacterial traits responsible to trigger such defence responses (Singh et al. 2018).

3.3.1 *Antibiosis: Antibiotics and Bacteriocins*

Production of antibiotics and other toxic compounds (e.g. insecticidal toxins) by microbial BCA is one of the best-known biocontrol mechanisms of plant diseases. Among well-characterized antibiotics related to biocontrol phenazines, phloroglucinols, pyoluteorin, pyrrolnitrin, hydrogen cyanide, cyclic lipopeptides, 2-hydroxymethyl-chroman-4-one, D-gluconic acid, 2-hexyl-5-propyl resorcinol, 2,4-diacetylphloroglucinol, pyocyanine and viscosanamide have been studied in detail (Jayaprakashvel et al. 2019). Antibiotics produced by BCA play important roles in both colonization and biocontrol although this point may give raise to some controversial issues (Sarangi et al. 2010). Indeed, antibiotic production can benefit a particular microorganism (the introduced BCA) by inhibiting rhizosphere competitors thus facilitating its own colonization. However, this positive effect contradicts the idea that preserving greater diversity in plant microbiota contributes to

increase its health. From a practical perspective, several issues should therefore be considered. On the one hand, occurrence of antibiotic resistance in the target pathogen, obviously leading to loss of biocontrol effectiveness, is a risk that can take place under natural conditions (Mazzola et al. 1995). On the other hand, a range of changing (a)biotic factors in a dynamic scenario such as the rhizosphere can largely influence the biosynthesis of antimicrobial metabolites (Lugtenberg and Bloemberg 2004). Finally, it is worth mentioning that the potential utilization under field conditions of genetically modified microorganisms with enhanced antibiosis ability have raised public concern (i.e. undesirable effects on non-target organisms and ecosystems; Mercado-Blanco and Bakker 2007). However, according to diverse studies, the introduction of engineered strains can lead to changes in the microbiota composition, although these seem to be minor and transient compared to common agricultural practices such as crop rotation (Viebahn et al. 2005; Timms-Wilson et al. 2005). Antibiotic production, root colonization and biocontrol ability are strongly interconnected. Besides, they can be affected by diverse environmental, physiological and genetic factors. Thus, in the context of soilborne plant pathogens management, it is difficult to establish a hierarchical relevance ranking for these processes.

Bacteriocins are another class of antibiotics produced by some plant-associated beneficial bacteria. They are usually proteins able to inhibit related strains of the same species because of their high degree of specificity. These compounds may also play an important role in colonization, stressing once again the fact that successful colonization and biocontrol effectiveness are processes necessarily interconnected. For instance, a *Pseudomonas fluorescens* SF39a mutant impaired in bacteriocins production showed to be less competitive than the wild-type strain in wheat rhizosphere colonization (Godino et al. 2016). Yet, the involvement that these metabolites may have in the biocontrol of plant pathogens and pests has not been sufficiently investigated.

3.3.2 Predation and Parasitism: A Specific Mechanism of Fungal BCA

In the case of parasitism, the pathogen is directly killed by the BCA. A single fungal pathogen can be attacked by multiple mycoparasites. In contrast to parasitism, microbial predation is a more general, non-specific mechanism and, overall, it provides less predictable level of disease control. Some BCA exhibit predatory behaviour under nutrient-limiting conditions. For instance, *Trichoderma* spp. are well-known fungal antagonists and BCA that produce a range of enzymes targeting cell walls of pathogenic fungi (Heydari and Pessarakli 2010). Chitinases produced by *Trichoderma* species are involved in antagonistic activity and biocontrol of pathogenic fungi such as *Botrytis cinerea* by degrading the cell wall and inhibiting spore germination (Markowich and Kononova 2003). These lytic enzymes can hydrolyze a broad range of polymeric compounds, including cellulose,

hemicelluloses, proteins, interfering with the pathogen growth and/or its activities. Sometimes, these enzymes act synergistically with antibiotics thereby enhancing the antagonistic effect on phytopathogenic fungi. As mentioned above, root exudates and mucilage-derived nutrients attract both harmful and beneficial microorganisms. Thus, to favour BCA rhizocompetence and root colonization, the ability to produce and secrete this type of secondary lytic enzymes and other compounds will subsequently improve its biocontrol activity (Compant et al. 2010; Rahman et al. 2018).

3.3.3 *Competition for Nutrients and Niches*

To achieve successful colonization of the rhizosphere (or any other belowground microhabitat) and effective biocontrol, a BCA must be competitive in both nutrient acquisition and spot occupancy (e.g. usual infection sites by pathogens). Indeed, competition for nutrients and niches (CNN) must be viewed as a primary biocontrol mode of action (Pliego et al. 2011, see Chap. 1), although its contribution to biocontrol might have been considered of less importance than other mechanisms here summarized. Being the first (or more rapid) to colonize a niche represents an advantage over other components of the soil-borne microbiota. Likewise, being more metabolically versatile than deleterious microorganisms in microhabitats such as the rhizosphere, root infection sites, vascular tissues, etc., confers a huge competitive advantage to the BCA. In summary, arriving more rapidly, displacing your competitors, and feeding more efficiently than your opponents are relevant strategies to counteract the efficacy of pathogen attacks. CNN can obviously rely on other mechanisms mentioned in this chapter. For instance, antibiosis contributes to displace and/or eliminate competing microorganisms (niche competition). Likewise, the more efficient acquisition of limiting (micro)nutrients such as iron (see below) provides a competitive advantage for better colonization of specific niches (nutrient competition). For instance, in addition to produce volatiles with antifungal effect, the fungus *Coprinopsis urticicola* is able to colonize the root cortex of wheat seedlings more rapidly than the pathogen. Thus, its success as BCA depends on its ability to internally colonize wheat roots by an active CNN mechanism (Gholami et al. 2019).

3.3.3.1 **The Specific Case of Competition for Iron: Production of Siderophores**

Despite its abundance, iron is mostly unavailable for microorganisms living in soil. Thus, they have developed a sophisticated strategy for its assimilation based on the biosynthesis of low molecular weight, iron-chelating molecules named siderophores (Höfte and Bakker 2007). These molecules have a high affinity for ferric iron (Fe^{3+}) and its production, and that of protein receptors that recognize

Fe³⁺-siderophore complexes, is up regulated during iron-limiting conditions. Competition for Fe³⁺ is probably the best-reported mechanism of nutrient competition employed by BCA. It works by depriving the pathogen from iron thus favouring better colonization of the niche by BCA. Siderophore production can be strongly influenced by, among others, environmental factors, the root exudate composition, or the own plant phytosiderophores (Shaikh and Sayyed 2015). As previously mentioned for other biocontrol mechanisms, we would like to stress the close link between colonization and biocontrol. Indeed, being able to produce siderophores confers a selective and competitive advantage to the BCA over soil-inhabiting microorganisms unable to synthesize these molecules, which further favours BCA rhizocompetence and root colonization efficacy. Therefore, to enhance rhizosphere competence of a given PGPM unable to produce siderophores, the introduction of heterologous iron-regulated siderophore promoters and/or siderophore receptor genes could be a successful strategy. Genome analysis has revealed that some bacterial BCA, such as *Bacillus amyloliquefaciens* FZB42 (Chen et al. 2007) or *Pseudomonas fluorescens* Pf-5 (Paulsen et al. 2005), harbour large gene clusters responsible for siderophore biosynthesis (as well as for antibiotics and detoxification compounds). Since these compounds are also related to improved colonization ability of the host plant (displacing microbial opponents by antibiosis and/or CNN), the link between colonization and biocontrol is once again highlighted. In this sense, “-omics” approaches are providing an invaluable assistance in biocontrol research (Massart et al. 2015).

3.3.4 Induction of Systemic Resistance

Plants can reach an enhanced defensive state denominated induced systemic resistance (ISR) when correctly stimulated (Pieterse et al. 2014). Some PGPM have been identified as ISR elicitors because of their ability to induce resistance in plants against pathogenic fungi, bacteria and viruses (Patel et al. 2016), triggering a response/signal that spreads systemically throughout the plant. This enhances the defensive capacity of distant tissues to subsequent infection by pathogens. ISR, systemic acquired resistance (SAR) and plant priming by stimuli show phenotypic resemblances. They operate through different plant hormone signalling pathways (i.e. the jasmonate/ethylene pathway is key for ISR, and salicylic acid for SAR), although it must be stressed that salicylic acid, jasmonate and ethylene pathways cross communicate, pointing to the fact that these defence responses show diffused limits (Conrath et al. 2015; Mauch-Mani et al. 2017; Gupta and Vakhul 2018). Some BCA secrete antifungal metabolites (AFM) that can also trigger ISR. Efficient colonization is required to ensure AFM are systemically distributed through the plant. While we have stressed the primary importance of colonization for successful biocontrol, in the case of BCA operating by ISR a reduction in root colonization does not necessarily imply loss of biocontrol effectiveness since certain AFM (e.g. antibiotics) trigger this defence response by themselves (Compant et al. 2010; Rahman

et al. 2018 and references therein). To unquestionably demonstrate that ISR is the responsible mechanism of biocontrol, the BCA and the pathogen must be spatially separated. Specific experimental designs (i.e. split-root study systems) allows to exclude the possibility that direct contact between the BCA and the pathogen takes place during the interaction, thereby confirming that disease suppression is truly mediated or not by the plant (e.g. Gómez-Lama Cabanás et al. 2017).

3.3.5 Reducing Germination of Inoculum

Reducing the germination of primary pathogen's inoculum is an interesting mode of action by BCA. For instance, it has been reported that root application of *Arthrobacter* sp. FP15, *Blastobotrys* sp. FP12 and *Paenibacillus alvei* K-165 reduce *Verticillium dahliae* microsclerotia germination in the rhizosphere of eggplants. Additionally, some BCA are able to parasitize and block microsclerotia formation of this soil-borne pathogen (Antonopoulos et al. 2008; Papisotiriou et al. 2013). Mycofumigation with volatile organic compounds produced by certain BCAs (e.g. non-pathogenic *Fusarium oxysporum*) efficiently suppressed (by means of reducing inoculum density) Verticillium wilt of cotton (Zhang et al. 2015). When non-pathogenic fungi are used as BCA, and its inoculum concentration is increased, their hyphae can attach to the root earlier than those of the pathogen can. Spore germination of non-pathogenic fungal BCA can be induced by plant root exudates thus contributing to outcompete pathogen biomass also present in the root system (Narayanasamy 2013).

3.4 Plant Growth Promotion: Consequence of Effective Biocontrol or Primary Cause for Its Success?

A host plant showing good development and nutritional status, and growing in the absence of stressing conditions must be, in principle, better prepared to overcome pathogen attacks. It is well known that a number of PGPM able to colonize the plant root system (and the surrounding environments) positively contribute to the host fitness, growth and development by mechanisms like phosphate solubilisation, nitrogen fixation, production of siderophores, synthesis of 1-amino-cyclopropane-1-carboxylate deaminase and production or degradation of phytohormones. Besides, some soilborne PGPM can help the plant host to cope with abiotic stresses such as the presence of heavy metals, recalcitrant organic pollutants and elevated saline conditions (Lugtenberg 2015). These beneficial components of the belowground plant-associated microbiota can behave, in addition, as effective BCA. Indeed, while BCA contribute to plant growth by counteracting the negative effects caused by deleterious agents, plant growth promotion traits they can deploy upon host colonization may also help to confront subsequent pathogen attacks due to a good and

equilibrated nutritional status of the plant. Consequently, while plant growth promotion is not a biocontrol mechanism by itself, optimal plant fitness as a consequence of the presence of PGPM/BCA prior to the introduction of the pathogen contributes to efficiently deal with its negative effects.

3.5 Concluding Remarks

Root colonization by an introduced BCA, and its subsequent endurance in this organ, are key factors for the efficient protection against soilborne pathogens. Efficient colonization, long persistence and enhanced rhizocompetence by BCA are complex processes influenced by many (a)biotic parameters (see Chap. 7). With so many factors affecting the activity of beneficial microbes, it is not surprising that an individual BCA may differ in its effectiveness and success. These outcomes largely depend on, not exclusively though, the diverse pedological environments a BCA faces when released into the soil. Hence, effective biological control of soilborne diseases by microbial inoculants can show inconsistency, especially when applied under field conditions. This unpredictability may be due to inefficient root colonization by the BCA, a result of our limited understanding of the (a)biotic factors affecting this process. Therefore, there is an urgent need for a comprehensive knowledge of these factors in order to improve colonization and, as a necessary consequence, biocontrol effectiveness at the belowground level, thereby helping to achieve greater success with the application of microbial-based formulations. Furthermore, there are additional major gaps in our knowledge in this research field. Indeed, many aspects of the underlying mechanisms involved in biocontrol are still unknown. The same can be said about the complex regulatory networks ruling the expression of biocontrol modes of action. The available multi-omic tools, combined with other methodological approaches such as microscopy, is now providing useful information to fill these gaps. These powerful approaches will undoubtedly help to overcome the problems and inconsistencies frequently observed. For instance, on the one hand, genomics and transcriptomics are contributing in the identification of genes and routes related to plant-microbe interactions (e.g. adhesion, colonization, chemotaxis, etc.), competition for nutrients and/or space, antibiotics production, etc. Besides, comparative genome-wide analyses serve to predict and discover novel potential traits associated with biocontrol and plant growth promotion. On the other hand, the integration of (meta)genomics, (meta)transcriptomics, (meta)proteomics and metabolomics will allow to understand the complexity of the belowground microbiota composition, structure and functionality, as well as the intricate interactions its members establish with the plant and the environment. In this regard, most of the available studies, as well as currently available biopesticides, are based on formulations of a single microorganism or combinations of few of them. A better understanding of the belowground microbial communities will encourage more holistic approaches in which consortia of BCA, synthetic communities or even customized microbiomes will be used in the future to improve biocontrol strategies within integrated and sustainable disease management frameworks.

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