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Biological control in the microbiome era: Challenges and opportunities

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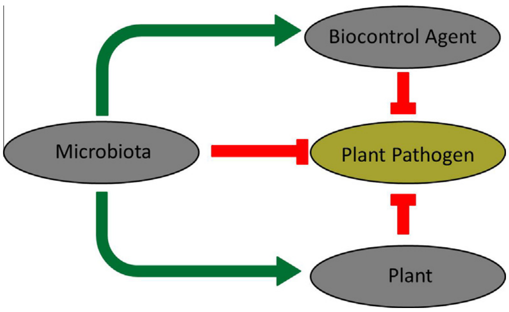
HIGHLIGHTS

- The plant microbiota has been neglected in biocontrol research.
- New sequencing technologies allow the study of microbiota and its role in biocontrol.
- Innovative biocontrol methods against plant pathogens will emerge.

GRAPHICAL ABSTRACT



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ABSTRACT

Biocontrol research has long been focused on the study of single strains of biocontrol agents (BCAs) and on their interaction with pathogens and host plants. Further focus on plant-associated microbial communities was suggested several years ago, but significant advances only occurred recently. The advent of high-throughput sequencing (or next-generation sequencing – NGS) technologies is now driving a paradigm change that allows researchers to integrate microbial community studies into the traditional biocontrol approach. This integration could answer old scientific questions, and will raise new biocontrol hypotheses. Microbial communities could impact disease control through their interaction with host plants, pathogens, and BCAs. A better understanding of these interactions will provide unexpected opportunities to develop innovative biocontrol methods against plant pathogens. For example, formulation or timing of BCA application can be improved, "helper" microbial strains can be selected, or molecules driving the microbiota to a pathogen-resistant composition ("prebiotic" approach) can be developed. The five main challenges of microbiome implementation in biocontrol research are also described, i.e. (i) the management of technical errors and biases, (ii) the growing importance of bioinformatics, (iii) the adaptation of experimental schemes, (iv) the appropriate interplay between NGS and other technologies, and (v) the need to complete current genome databases.

1. Introduction

From a historical point of view, studies on plant–microbe interactions have so far mainly focused on binary or ternary interactions like plant–pathogen, plant–mycorrhizal fungi or plant–pathogen–biocontrol agent. These studies significantly improved our understanding of these associations, but with a major drawback: plant roots and leaves harbor very diverse and abundant bacterial and fungal communities, so these interactions do not occur on a sterile surface as in most laboratory experiments. These communities are called microbiota when referring to the ecological community of microorganisms within a defined environment, or microbiome when referring to the collective genomes of all microorganisms from a given environmental niche. These microbial communities have been estimated at 10^6–10^7 cells/cm^2 in

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the phyllosphere (Lindow and Brandl, 2003) and 10^6–10^9 cells/g in the rhizosphere (Whitman et al., 1998).

Plants can therefore be considered as super organisms harboring very diverse microbial communities that provide specific functions and traits to plants. For more details, (de Bruijn, 2013) and (Vorholt, 2012) reviewed the role and function of the rhizosphere and phyllosphere microbial communities, respectively. These functions can be summarized as five key roles: (i) improving nutrient acquisition and growth, (ii) sustaining plant growth under biotic and/or abiotic stress, (iii) inducing resistance against pathogens, (iv) interacting with plant or human pathogens, and (v) interacting with other trophic levels like insects. The plant microbiota can be considered as a key factor for plant health and productivity. In parallel, plants drive microbiota selection through leaf or root anatomy and morphology or production of exudates.

Plant microbial communities hold a sizeable place in biological control as they are the source of the majority of biocontrol agents (BCAs). After washing and plating, cultivable microbes are isolated, and individual strains are screened for their biocontrol properties against plant pathogens. BCAs generated great enthusiasm as a sustainable control method of plant pathogens. Nevertheless, their practical application is often hampered by their lower or non-reproducible efficacy compared to chemical treatment. More comprehensive studies of BCA survival and efficacy on plants are still needed.

Research in biocontrol has mainly been focused on single BCA strains. Several authors suggested to focus more broadly on plant-associated microbial communities (Benítez and McSpadden Gardener, 2009; Kim et al., 2011; Smalla et al., 2001). However, this approach received little attention for the development of biocontrol methods. It was an unattainable objective due to the absence of techniques to survey microbial communities and their evolution in a holistic manner at affordable costs. Recent developments in high-throughput sequencing (or Next Generation Sequencing – NGS) technologies and in bioinformatic analyses have drastically changed the course of events.

Many NGS technologies have been developed so far, and new technologies are still being developed. These technologies, their performances and limitations have been reviewed in detail elsewhere (Knief, 2014), so they will not be specifically addressed here. As far as microbial community studies are concerned, NGS methodologies can be divided into three approaches. Amplicon sequencing-based technologies are currently the most popular ones for plant microbiome studies. A specific genomic region is targeted, amplified and sequenced in order to describe the microbial community within a sample. Usually, the selected genes are the 16S rDNA gene for bacteria and the 18S rRNA gene or Internal Transcribed Spacer (ITS) region for fungi. Metagenome sequencing is a second approach. It is based on shotgun sequencing. DNA is extracted from the sample, sheared into small fragments, and directly sequenced. The sequences are further assembled into contigs and annotated. The third approach, called metatranscriptomics, is based on shotgun sequencing to study the gene transcripts of the whole microbial community. RNA is sheared, retrotranscribed, and then sequenced. Importantly, rRNA must be removed to focus the sequencing on other RNAs (messenger RNAs or non-coding RNAs). The sequences are further assembled into contigs and annotated. Recently, several studies using NGS, and mainly the amplicon approach, contributed to a more complete characterization of the composition of plant microbial communities. They boosted the interest for this neglected field and for the impact of these communities on plant growth, plant health and food safety. There are already several reviews on the impact of NGS on rhizosphere and phyllosphere microbial communities (Berlec, 2012; Bulgarelli et al., 2013; Knief, 2014; Rastogi et al., 2013).

The specific objective of this paper is to analyze the existing and future contribution of NGS technologies to plant microbiome studies in the context of biocontrol research, as illustrated in Fig. 1. In this figure, we underline the main emerging scientific questions related to the study of plant microbial communities and their effect on pathogens, plants and/or BCAs within the framework of biological control. Microbial communities may directly influence pathogen development through antibiosis, parasitism or competition. The microbiota may also have an indirect role by stimulating plant defenses or BCA survival and activity. A better understanding of the microbiome will also allow researchers to identify strains (considered as "helper microbial strains") or molecules (some could be considered as plant prebiotics) involved in improving these direct or indirect effects against plant pathogens. A better understanding of plant microbial community assembly, roles and ecosystem services could bring about unexpected opportunities to develop innovative control methods of plant pathogens.

2. Available tools to study the plant microbiome

In Fig. 2, the current tools used in microbiome studies are classified following two axes. The vertical axis corresponds to the focal degree of the approach from a single target to a holistic analysis. The horizontal axis classifies approaches depending on the information they provide: from community description and characterization to functional analysis of community member interactions.

This figure illustrates the opportunities offered by NGS technologies. For the first time, it is possible to study microbial communities at a very fine resolution while using holistic approaches. These advances were made possible by huge research projects on the human microbiome, but also by the Earth Microbiome Project (www.earthmicrobiome.org), a massive multidisciplinary effort to analyze microbial communities across the globe. The project aims to sequence and analyze 200,000 samples from these communities to produce a global Gene Atlas describing gene functions, proteins, and environmental metabolic models for each biome. The Earth Microbiome Project also releases protocols and standards for sample preparation and data analysis.

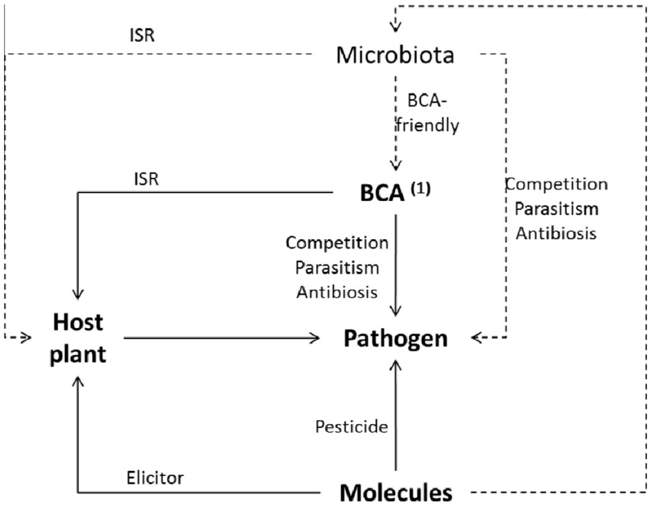

Fig. 1. Current and future roles of the microbiota in the control of plant pathogens. Full arrows represent current research areas and control measures. Dotted arrows correspond to future research areas and control measures that can arise from a better understanding of the role of the microbiota using NGS methodologies. ISR: induced systemic resistance. (1): BCAs are frequently isolated from plant microbiota.