BURLEIGH DODDS SERIES IN AGRICULTURAL SCIENCE

Microbial bioprotectants for plant disease management

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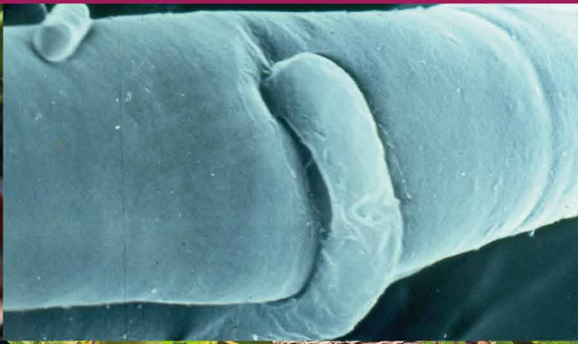

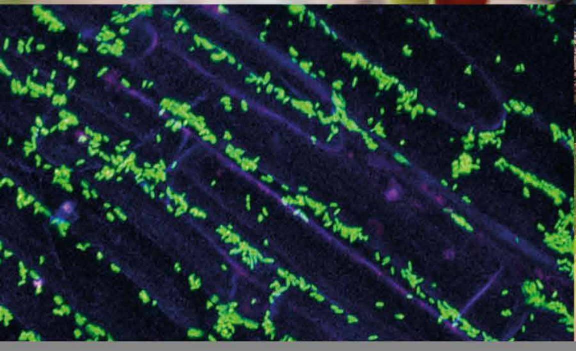

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Introduction

With growing concerns about the environmental impact of synthetic fungicides, increasing levels of fungicide resistance and increasing regulatory restrictions on fungicide use, the crop protection sector and farmers face mounting pressure to replace synthetic fungicides with environmentally friendly biological alternatives for disease control. This volume examines the recent advances in the development of ecologically balanced biological methods using bioprotectants based on beneficial microorganisms to control plant diseases.

Part 1 of the book focuses on product development of microbials, discussing aspects such as modes of action of microbial bioprotectants and developments in screening approaches. Chapters in Part 1 also examine methods to visualise and determine microbials, durability of efficacy of microbial bioprotectants, the commercialisation of microbial bioprotectants, advances in production of commercial microbials and the regulation of microbials in the European Union. Part 1 concludes with a chapter on microbial bioprotectants and the marketplace. Part 2 examines the use of biological control agents to control plant disease, focusing on bacterial, fungal and viral agents. Part 3 provides examples of the use of bioprotectants, specifically highlighting the use of bioprotectants to protect food from aflatoxin contamination and the use of *Verticillium albo-atrum* WCS850 to control Dutch elm disease. Part 4 of the book concludes with a discussion of the future of bioprotectants, focusing on their role in integrated crop protection approaches.

Part 1 Product development of microbials

Part 1 opens with a chapter that focuses on the advances in understanding modes of action of microbial bioprotectants. Plant-associated microorganisms are involved in important functions related to growth, performance and health of their hosts. Understanding their modes of action is pivotal for the development and application of microbial bioprotectants and biostimulants. Chapter 1 summarises current knowledge about beneficial plant-microbe interactions, discusses recent insights into the functioning of the plant microbiome and beneficial plant-microbe networks. It shows that the use of microorganisms and the exploitation of beneficial plant–microbe interactions offer promising and environmentally-friendly strategies to achieve sustainable agriculture on a global scale.

Chapter 2 examines advances in screening approaches for the development of microbial bioprotectants to control plant diseases. The chapter begins by discussing screening of microorganisms for biological control of plant diseases, specifically focusing on exclusive and inclusive approaches. It then goes on to discuss the nine-step approach to screening for biocontrol agents, which is then followed by an analysis of non-traditional biocontrol agents of plant diseases such as entomopathogenic fungi, bacteria, bacteriophages and mycoviruses. Sections on niche and regional markets for biocontrol agents are also included. The chapter also provides an overview of the formulation of biocontrol agents, the role of the microbiome in biocontrol and the use of microbiome engineering for disease control.

The subject of Chapter 3 is visualising plant colonisation by beneficial bacteria, which is considered a key step to improve the understanding of plant-microbe interactions. Plants contain diverse microorganisms that interact with their hosts and with each other. Beneficial bacteria can be utilised on crops to protect plants against biotic and abiotic stresses and to stimulate plant growth. However, the behaviour of specific microorganisms on and within plants is still underexplored. The chapter begins by highlighting the methods that can be used to visualise and determine microbial colonisation of plants. The chapter then goes on to examine colonisation of beneficial bacteria from the soil to the root surface and to root internal tissues. A section on the colonisation of the aerial parts of plants is also provided, which is then followed by a discussion on improving applications by understanding colonisation processes. These techniques are crucial in the selection process of biocontrol agents and the development of a product formulation and method of application.

Chapter 4 addresses the durability of efficacy of microbial bioprotectants against plant diseases. The chapter highlights current knowledge concerning erosion of efficacy of microbial bioprotectants against plant pathogens and its possible consequences for field applications. It starts by reviewing the durability of plant protection against bio-aggressors, then goes on to discuss the main modes of action of microbial bioprotectants against plant pathogens in relation to potential mechanisms of resistance developed by plant pathogens. The chapter also reviews the diversity of susceptibility within plant pathogen populations to microbial bioprotectants and the related risk of emergence of resistance to microbial bioprotectants in plant pathogens as well.

The next chapter reviews the recent commercialisation of microbial bioprotectant products containing bacteria, fungi, yeast and bacteriophages for the control of plant diseases. Chapter 5 also summarises recent development activities of new bioprotectant products based on microorganisms or their metabolites, including induced resistance products, single domain antibody proteins produced by microorganisms, and protozoans (amoebae). Production, mainly by submerged fermentation, and formulation processes of microbial bioprotectants are discussed. Key factors influencing the fermentation, formulation and the scale up for industrial production of such microorganism as bioprotectant products are also addressed, including stability and viability of the active substances produced by liquid fermentation processes.

Chapter 6 examines the key issues in the regulation of microbial bioprotectants in the European Union, focusing specifically on the challenges and solutions to achieve more sustainable crop protection. Microbial bioprotectants, like chemical pesticides, are required to pass a risk assessment and risk management procedure prior to use in plant protection, which in many countries is an obstacle for market access, in particular, the European Union. Administrative issues and data requirements, adapted from those used for chemicals, cause issues for both applicants and evaluators. These issues are reviewed and improvements are proposed. Biology should be the basis of the evaluation and data requirements for microorganisms, with an emphasis in this chapter on microbial compounds and testing methods. Political actions involving the use of pesticides are reviewed and recommendations are made on how to improve the system for microbial bioprotectants, including new uses of microorganisms. New legislation is suggested for all microorganisms used in agriculture and related uses based on the assumption that well-known microorganisms are of low risk to human health and the environment.

The final chapter of Part 1 reviews microbial bioprotectants and the marketplace. Chapter 7 begins by discussing the latest figures in terms of microbial bioprotectants global market value. It also highlights the different types and ways these bioprotectants can be used. The chapter also discusses the trends and drivers in the microbial market, focusing specifically on why microbials dominate and the factors that drive bioprotectant adoption. A section on the myths about the bioprotectant market is also included, which is then followed by a discussion of the limitations for using microbial bioprotectants. Future opportunities and threats for microbial bioprotectants are also highlighted.

Part 2 Biological control agents

The first chapter of Part 2 focuses on the use of *Bacillus* spp. as bacterial control agents to control plant diseases. Biocontrol agents (BCAs) based on plant growth promoting rhizobacteria have recently been developed as alternatives to chemical pesticides. Among those beneficial bacteria, *Bacillus* spp. are one of the most promising BCAs. A wide range of bioactive secondary metabolites (BSMs) are involved in biocontrol via antibiosis to plant pathogens and/or via elicitation of systemic resistance in their host plants. Chapter 8 illustrates the diversity of pathosystems in which BCA based on *Bacillus* spp. have proved effective. It describes the mechanisms underpinning this biocontrol activity via production of a wide range of enzymes, proteins and small-size BSMs. As these BSMs are clearly involved in pathogen control, the chapter emphasises the importance of understanding the ecological factors influencing their production. The final section of the chapter highlights the potential interactions between *Bacillus* spp. and other soil microorganisms in developing consortia of biocontrol agents combining species with synergistic activities for plant health improvement.

Chapter 9 examines the use of *Pseudomonas* spp. as bacterial biocontrol agents to control plant diseases. The chapter starts by highlighting recent advances in *Pseudomonas* taxonomy and a summary of its most important biocontrol traits. It then provides an overview of the most important *Pseudomonas* groups and subgroups harbouring biocontrol strains. Based on their activity, *Pseudomonas* biocontrol strains come in three types. The first type, represented by *P. chlororaphis*, *P. protegens*, *P. corrugata* and *P. aeruginosa* (sub)group strains, produces an arsenal of secondary metabolites with broad antimicrobial activity. The second type is found in the *P. putida*, *P. fluorescens*, *P. koreensis*, *P. mandelii*, and *P. gessardii* (sub)group. The spectrum of biocontrol properties of these strains is less diverse and involves siderophores and cyclic lipopeptides. The third type colonizes above-ground plant parts. Strains from this type mainly belong to the *P. syringae* group and are used to control postharvest pathogens. Examples of well-characterized and representative biocontrol strains show the links between phylogeny, ecology and biocontrol traits. The chapter concludes by reviewing commercially-available biocontrol strains.

Moving on from Chapters 8 and 9, Chapter 10 focuses on bacterial bioprotectants besides *Bacillus* and *Pseudomonas* species. The chapter begins by discussing the taxonomy of non-*Bacillus* and *Pseudomonas* (NBP) bioprotectant strains, including enterobacteria, actinomycetes, *Sphingomonas, Methylobacterium, Agrobacterium-Rhizobium* and *Lactobacillus*. The chapter reviews their mechanisms of action against plant pathogens. Sources of isolates and methods of isolation are discussed in building strain collections. The chapter then reviews procedures for screening antagonistic bacteria candidates as bioprotectants using biochemical and molecular markers, including the example of lactic acid bacteria. The chapter also covers strain improvement to increase fitness and efficacy in the field through physiological and genetic manipulation. Since they are essential for commercial development, biosafety issues are discussed, followed by an overview of patented substances and commercialized products. The chapter concludes with a summary and future trends in research section on non-*Bacillus* and *Pseudomonas* species.

The next chapter addresses the use of *Trichoderma* spp. to control plant diseases. *Trichoderma* is one of the most studied genera of ascomycetous fungi due to the beneficial effects it has on plants. *Trichoderma* spp. are involved in the production of cell wall-degrading enzymes and metabolites with antimicrobial activity. It also produces volatile compounds that act together as direct biocontrol agents to protect plants against phytopathogenic fungi, oomycetes, nematodes and bacteria. Chapter 11 first reviews the various biocontrol mechanisms that can be used, specifically focusing on mycoparasitism, antibiosis and competition between the soil microbiota and plants for resources such as nutrients. The chapter also examines systemic defence and growth promotion in plants and how these can both have beneficial effects on the plant. A section on the importance of registration and commercialisation of *Trichoderma* strains is also provided as well as an overview of successful uses.

The subject of Chapter 12 is the use of *Clonostachys rosea* to control plant diseases. The fungus *C. rosea* was recognized as an aggressive parasite on other fungi already in the late 1950s. Research into its potential use in biological control of plant diseases soon followed, resulting in several commercial products. The chapter begins by reviewing the taxonomy and sources of *C. rosea*, then goes on to discuss the range of mechanisms of action involved in combating *C. rosea*. A section on the lessons that can be learnt from genomics and transcriptomics and *C. rosea* is also provided, which is then followed by a review of *C. rosea* product development and commercialisation. The chapter also analyses the delivery and action of *C. rosea* as a biocontrol agent, before concluding with an overview of why the use of *C. rosea* for plant disease control is a promising prospect.

Chapter 13 looks at the use of bacteriophages to control plant diseases. Bacteriophages are viruses that kill target bacteria without affecting another microorganism and environment. Bacteriophage efficiency on the phyllosphere is mainly affected by ultraviolet (UV) light. The chapter first discusses the advantages of using bacteriophages for biocontrol over other strategies, then goes on to examine the early research on bacteriophages. The chapter also reviews the sources of bacteriophages and the interactions they can have with host cells. A section on the concerns in using bacteriophages as biocontrol agents is also provided which is followed by an analysis of the facts that can affect the efficacy of bacteriophages for plant disease control. Approaches for optimum efficacy of bacteriophage for plant disease control are discussed and the bacteriophage therapy challenges that need to be addressed are also included. A list of applications of bacteriophages is also provided.

The next chapter focuses on the use of mild viruses for the control of plant pathogenic viruses. Plant virus management strategies have largely been limited to the application of hygiene protocols, the control of viral vectors such as insects and nematodes and the use of resistant varieties. However, these approaches are often insufficient to prevent infections. The rapid control of newly emerging viral diseases remains challenging. Chapter 14 focuses on cross-protection using mild viruses as active substances in biocontrol. The chapter begins by describing the theoretical modes of action of cross-protection. It then goes on to discuss crucial elements in the development of a cross-protection strategy, taking into account new insights based on commercial application of cross-protection. The chapter also provides case studies in which cross-protection has been applied in commercial crops, in particular vaccination strategies to control Pepino mosaic virus (PepMV) in greenhouse tomato. Finally, developments that may impact future research into the control of emerging viral pathogens are discussed.

The final chapter of Part 2 looks at the possibility of mycoviruses as a means of biocontrol. Chapter 15 discusses the characteristics of mycoviruses and the conditions that need to be met to make a successful virocontrol agent. Virocontrol involves biocontrol of pathogens via hypovirulence-causing mycoviruses. The chapter describes one of the success stories so far, that of virocontrol of *Cryphonectria parasitica*, the chestnut blight pathogen. The chapter ends by reviewing future trends and where to find more information on mycoviruses and virocontrol.

Part 3 Examples of use of microbial bioprotectants

Part 3 opens with a chapter that focuses on the development and scale-up of bioprotectants to keep staple foods safe from aflatoxin contamination in Africa. Aflatoxins pose a significant public health risk, decrease productivity and profitability and hamper trade. To minimise aflatoxin contamination a biocontrol technology based on atoxigenic strains of *Aspergillus flavus* that do not produce aflatoxin is used widely in the United States. The technology, with the generic name Aflasafe, has been improved and adapted for use in Africa. Aflasafe products have been developed or are currently being developed in 20 African countries. Aflatoxin biocontrol is being scaled up for use in several African countries through a mix of public, private, and public-private interventions. Farmers in several countries have commercially treated nearly 400 000 ha of maize and groundnut achieving >90% reduction in aflatoxin contamination. Chapter 16 summarises the biology of aflatoxin-producing fungi and various factors affecting their occurrence, including climate change. Various management practices for aflatoxin mitigation are then discussed. These include biological control, which is increasingly being adopted by farmers in several countries. The chapter also discusses biocontrol product development and commercialization in various African countries. Subsequently, the chapter highlights some barriers to adoption and other challenges.

Chapter 17 highlights the potential of using *Verticillium albo-atrum* WCS850 to control Dutch elm disease, specifically focusing on its prominence in Europe. The chapter first describes the development of the *Verticillium albo-atrum* WCS850 strain, which first started in the 1980s. The chapter then goes on to review the production, method of application, registration and use of the *Verticillium albo-atrum* WCS850 strain as a biocontrol agent. A section on testing long-term efficacy of the biocontrol strain is also provided, before concluding with an analysis of how Dutch elm disease treatment has changed since it was first identified.

Part 4 Future outlook on microbial bioprotectants

Chapter 18 addresses the role of bioprotectants for disease control in integrated crop protection approaches. Bioprotectants have the potential to replace chemical pesticides in agricultural cropping systems and crop protection approaches. Development of new bioprotectants in combination with more restricted use of chemical crop protection will result in their much stronger market position in the future. Bioprotectants fulfil particular roles in current and future crop protection approaches, primarily reducing pesticide residues in harvested products in conventional systems, as well as being the first and preferred control option in integrated pest management programs and organic farming, and complementing resident microbiomes in future resilient cropping systems. The process of developing bioprotectants until market access can take from up to 10 to 15 years. The chapter gives a brief overview of the role of bioprotectants in current and future crop protection approaches to stimulate discussion within the biocontrol industries, and amongst scientists and funding agencies on the need for new generations of bioprotectants for an agriculture industry undergoing transition.

The final chapter of the book highlights the future outlook on microbial bioprotectants in agriculture. Microbial bioprotectants have the potential to play a major role in the future of crop protection. Agriculture needs to become more sustainable and still provide food security within planetary borders. New technologies and scientific discoveries can unravel the interactions between the plant, the microbiome and the soil and provide new opportunities for crop protection and more resilient cropping systems. Regulatory issues delay and hamper exploitation and research of genetic resources. Chapter 19 describes the factors that promote the use of microbial bioprotectants as well as those that hamper their further adoption. A sustainable and resilient agriculture depends on the microbial interactions between plants in promoting plant growth and combatting biotic and abiotic threats. The transition to a resilient agriculture requires big changes in policy, regulation and farming practices. The chapter assesses the future outlook for the methods for controlling plant diseases described in this book as well as the factors determining their uptake and success.

Chapter 1

Advances in understanding modes of action of microbial bioprotectants

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1 Introduction

Human activities substantially affect the environment. According to the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES), for example, species extinctions are currently up to 1000 times higher than normal (ipbes.net). Intensive agriculture has contributed significantly to these long-lasting anthropogenic impacts (Crutzen, 2002). The conversion of atmospheric nitrogen to ammonia by the Haber–Bosch process, for efficient production of fertilizers, has fundamentally altered the global nitrogen cycle. Overuse of chemicals leads to the development of resistant plant and human pathogens, which are difficult or impossible to suppress. Current agricultural practices focus on increased productivity to ensure high crop yields with maximized profitability. Growing levels of fertilizer and pesticide inputs globally have led to the contamination of the environment as well as of food, resulting in negative impacts on so-called *One health* or planetary health (Flandroy et al.,

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2018). We have already exceeded planetary boundaries for nitrogen supply, land use, as well as biodiversity loss (Rockström et al., 2009).

Microbial bioprotectants and biostimulants could provide a solution to these problems (Köhl et al., 2019). Plants and soil naturally harbor specific microorganisms that fulfill functions, such as nutrient, mineral, and vitamin supply, and protect against biotic and abiotic stress (Bakker et al., 2020). Such beneficial microbes can be categorized according to their modes of action into bioprotectants and biostimulants (Lugtenberg and Kamilova, 2009; Dunham Trimmer, 2017). In this chapter, we follow the definition of bioprotectants provided by the International Biocontrol Manufacturers Association (IBMA) (https://ibma-global.org/what-is-biocontrol), in order to avoid the ambiguity that might be caused by different national and continental definitions.

Bioprotectants based on beneficial microbes can potentially replace chemical pesticides. Gram-positive bacteria with spore-forming ability are currently the main group being commercialized; however, they represent only a small proportion of the potential microbial community. The commercial use of bioprotectants depends on the reliability of microbial formulations, their modes of action, as well as their safety. A low shelf life and inconsistent effects in the field characterize many of the products that have been commercialized so far. There have been recent developments in methods for the analysis of microbial communities based on next-generation sequencing (Jansson and Baker, 2016; Berg et al., 2020). They have resulted in an improved knowledge base in terms of understanding the modes of action of biocontrol agents (Spadaro and Droby, 2016). This knowledge is important during all stages of the development of microbial products, from their discovery to risk and efficacy assessment.

Microbiome research has revolutionized our understanding of the way plants function and opened new possibilities to develop more sustainable agriculture (Berg et al., 2020). This new field of research has contributed to our understanding of the modes of action of binary plant–microbe interactions as well as the interplay within the whole plant microbiome. Promising results from microbiome research have provided a boost for the whole ‘microbiome market’ as well as private investment in companies and startups (global-engage.com). Advances in the engineering of environmental microbiomes are predicted to replace potentially toxic chemicals in agriculture in the future, stimulate a more sustainable use of environmental resources, and improve food processing. Currently, agricultural products based on the microbiota are one of the fastest-growing sectors in agronomy, with a compound annual growth rate (CAGR) of 15–18% and a predicted value of over $10 billion US dollars by 2025 for the whole biocontrol sector (Dunham Trimmer, 2017).

This chapter summarizes current knowledge about beneficial plant–microbe interactions and discusses recent insights into the functioning of the plant microbiome and beneficial plant–microbe networks. It shows that the use of microorganisms and the exploitation of beneficial plant–microbe interactions offer promising and environmental-friendly strategies to achieve sustainable agriculture on a global scale.

2 Modes of action of plant-associated microorganisms with their host

Over the past 125 years, research and practical applications have both repeatedly demonstrated that microorganisms can have an intimate interaction with their host plants and are able to promote plant growth as well as suppress plant pathogens (Hiltner, 1902; Whipps, 2001; Berg, 2009). Plant-beneficial effects can result either from direct interactions of microorganisms with their plant hosts or indirectly by the suppression of plant pathogens. Under *in vitro* conditions, the principles of these interactions, their regulation, and stimulation are well understood at the molecular level. However, in nature, they often occur in combination and direct mechanisms are difficult to differentiate from indirect disease suppression (Haas and Défago, 2005). The ability of a microorganism to colonize plant habitats is essential for all successful plant–microbe interactions (Lugtenberg et al., 2002). Distinct steps in the initiation of such interplay include recognition, adherence, invasion (only endophytes and pathogens), colonization, and growth to establish interactions (Hardoim et al., 2015). Figure 1 provides an overview of tripartite interactions between beneficial microorganisms and plants, including the native microbiome as well as pathogens. It also illustrates the modes of action that are involved. Box 1 includes examples of studies on modes of action of plant-beneficial microorganisms with their plant hosts.

Box 1 Selected reviews of the modes of action of plant-associated microorganisms with their host and pathogens

Reviews describing modes of action in general were published by Whipps (1988, 2001), Weller et al. (2002), Berg (2009), and Lugtenberg and Kamilova (2009). Moreover, reviews focusing on modes of action of specific genera are also available, for example, for *Pseudomonas* (Haas and Défago, 2005), *Burkholderia* (Compant et al., 2008), *Bacillus* (Santoyo et al., 2012), and *Paenibacillus* (Rybakova et al., 2016); all of them are representatives of common plant-beneficial genera. Köhl et al. (2019) discussed the modes of action especially with regard to the potential risk for human and environmental health.

There are three general types of direct beneficial interaction of microorganisms with their plant hosts:

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