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Biopesticides for sustainable agriculture

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Introduction

With increasing concern about the environmental impact of synthetic pesticide use, including their impact on beneficial insects, the problem of insect resistance and the lack of new products, there has been an increasing interest in developing alternative biopesticides to control insect and other pests. This collection reviews the wealth of research on identifying, developing, assessing and improving the growing range of biopesticides. Part 1 reviews research on developing new biopesticides in such areas as screening new compounds, ways of assessing effectiveness in the field and improving regulatory approval processes. Part 2 summarises advances in different types of entomopathogenic biopesticide including entomopathogenic fungi and nematodes and the use of Bt genes in insect-resistant crops. The final part, Part 3, assesses the use of semiochemicals such as pheromones and allelochemicals, peptide-based and other natural substance-based biopesticides.

Part 1 General

The first chapter of the book begins with a discussion of improving methods for developing new microbial biopesticides. Chapter 1 highlights the role and economics of biopesticides, analyses the strategic framework for biopesticides development and provides an overview of the biopesticide development process. The chapter then focuses on microbial exploration and discovery and draws attention to the biological characterisation and development of biopesticides. A section on fermentation and formulation is also provided, focusing on various fermentation methods and a selection of formulations useful to biopesticide production. The chapter concludes by providing an overview of the future of biopesticides as an emerging industry as well as giving recommendations for future research.

Chapter 2 reviews the implementation of biopesticides as part of an integrated pest management (IPM) programme. It begins by highlighting the way biopesticides and other biocontrol agents can be integrated into IPM programmes. The chapter then discusses regulatory assessment of when it is safe and appropriate to use biopesticides and the use of biopesticides in different crop production systems. The chapter also provides a case study of the French government's programme to move its agricultural sector from reliance on synthetic pesticides to the use of more sustainable biocontrol techniques.

Moving on to the final chapter of Part 1, Chapter 3, the subject is improving regulatory approval processes for biopesticides and other new biological technologies in agriculture. The chapter begins by discussing how a regulatory framework for biopesticides can be established. It moves on to review the regulation of pesticides in the European Union (EU) and how the biopesticide sector and new regulatory requirements have been developed. The chapter also highlights the challenges faced in improving the regulatory framework for biopesticides and discusses current EU regulation and global national initiatives in biopesticides. The chapter concludes by reviewing how good regulatory practice can be developed.

Part 2 Microbial biopesticides, entomopathogenic nematodes and mites

Part 2 begins with Chapter 4, a discussion of the advances in the use of entomopathogenic fungi as biopesticides in suppressing crop pests. Hypocrealean ascomycetes have a set of properties such as their unique mode of action by direct penetration through the cuticle, potential for mass production, and their newly discovered ecological roles as endophytes and/or plant growth promoters which put them at the forefront of the global development of alternative control strategies. The chapter first addresses general aspects related to the natural occurrence and biodiversity, life cycle and delivery methods of the entomopathogenic fungi. It uses case studies to review the use of entomopathogenic fungi as biopesticides for the control of a wide range of insect and mite pests including locust and grasshoppers, soil dwelling insect pests, piercing and sucking insect and mite pests, stored-grain pests, forestry pests, invasive pests, and medical and veterinary pests. Finally, the advantages and limitations of entomopathogenic fungi as biopesticides together with a market overview are provided, with a concluding section indicating possible future directions in research on these fungi.

The next chapter, Chapter 5, addresses the advances in the use of entomopathogenic bacteria/microbial control agents (MCAs) as biopesticides in suppressing crop insect pests. The chapter first provides an overview of commercialised entomopathogenic bacteria and then discusses commercialised spore-forming bacterial entomopathogens. It also draws attention to non-spore forming entomopathogenic bacteria with potential for commercialisation and provides a section on microbial control agents (MCAs). A section on the advantages and drawbacks on the use of bacterial biopesticides is included before the chapter concludes by discussing research needs and emerging initiatives and providing future research trends in the subject.

Chapter 6 focuses on the advances in the use of Bt genes in insect-resistant crops. Insecticidal toxins derived from the soil bacterium Bacillus thuringiensis (Bt) have been widely used as microbial pesticides to selectively control juveniles of Lepidoptera, Coleoptera, Diptera and soil-dwelling nematodes. Synthetic genes derived from different strains of Bt have been used to produce genetically-modified (GM) plants resistant to insects. The chapter begins with an introduction to *Bacillus thuringiensis* (Bt) insecticidal toxins and moves on to discuss how Bt genes can incorporated into integrated pest management (IPM). It also reviews the importance of effective insecticide resistance programs before providing a section on potential areas for future research and sources for further information.

The subject of Chapter 7 is on plant growth-promoting bacteria (PGPBs) and how they can be used as biocontrol agents against invertebrate pests. Plant growth promoting bacteria (PGPBs) represent an important resource for agricultural crops, providing several benefits to the plant such as optimizing the use of environmental resources, improving plant health and resistance to biotic and abiotic factors, and directly acting against plant pathogens and invertebrate pests. The chapter begins by reviewing plant growth promotion and how plant growth-promoting bacteria (PGPBs) can be used against plant pathogens. It also addresses their potential against invertebrate pests and applications in agriculture and forestry. The chapter provides a case study on the soil-dwelling bacterium *Pseudomonas protegens* as a PGPB with dual action of biocontrol against both plant pathogens and invertebrate pests. It concludes by providing potential areas for future research.

Chapter 8 reviews advances in the use of entomopathogenic viruses as biopesticides in suppressing crop insect pests. It begins by discussing insect pests and their impact on the environment and how insect pest control has developed over time. It also highlights how viruses have been used as potential insect control agents. The chapter first analyses RNA viruses, specifically focusing on tetraviruses and reoviruses. It then looks at DNA viruses, focusing on Parvoviridae, Poxviridae, Nudiviridae and Baculoviridae. As baculoviridae is the most studied group of insect-specific viruses, the chapter also provides information on attributes of baculoviruses, an analysis of baculovirus genomics and molecular biology, a discussion on biopesticide development and reviews baculovirus production and formulation. It also provides a paragraph on the development of host-resistance to baculoviruses. The chapter concludes by providing an overview of how insect-specific viruses have potential for development as biological control agents of insect pests in integrated pest management (IPM) systems.

The next chapter, Chapter 9, discusses advances in the use of entomopathogenic nematodes (EPNs) as biopesticides in suppressing crop insect pests. Entomopathogenic nematodes have been commercialized as biopesticides since the 1980s. Since the 1990s, research on the application, biology, and ecology of these biocontrol agents has seen exponential growth, and since the mid-2000s basic research on EPNs has further expanded due to the use of the nematodes and their symbiotic bacteria as tractable model systems for many basic biological and ecological questions. The emphasis of the chapter is on the use of EPNs as biopesticides in a wide range of agricultural and other commodities. However, the chapter also provides overviews on EPN biology and ecology and mass production and application technology and interactions with other management tools as they are necessary for a better understanding of the strengths and limitations of EPNs as biopesticides. The chapter also provides a section on potential areas for future research.

Chapter 10, the final chapter of Part 2, reviews advances in the use of entomopathogenic oomycetes as biopesticides in suppressing crop insect pests. It begins by discussing how oomycetes have evolved from invertebrate pathogens to plant pathogens, what they are commonly known as today. The chapter also provides a case study on *Lagenidium giganteum* as a biocontrol agent against insects. The chapter moves on to review how the mode of infection can be established by entomopathogenic oomycetes, and how oomycetes can be used as sources of novel biopesticidal metabolites. It concludes by providing potential sources for further information and directs researchers to key conferences in the field.

Part 3 Natural substance-based biopesticides

The final part of the book begins by addressing the advances in the use of semiochemicals in integrated pest management, specifically focussing on pheromones. Semiochemicals enable intra- and interspecific chemical communication in insects. Among them, pheromones are species-specific and have been used in the management tactics for control of many insect pests for the last 50 years. During this time, important advancements have been made in understanding pheromones and their uses, particularly pheromone receptor neurons and pheromone inhibitors. Chapter 11 presents the latest developments of pheromones and their applications as key elements to specifically control insect pests within IPM programs. It also includes a section which provides researchers with potential sources for further information on the subject.

The next chapter, Chapter 12, discusses the possible use of allelochemicals in integrated pest management (IPM). Allelochemicals are produced and released into the environment by one species and have an effect on another species. They include attractants, repellents and toxic defence compounds which could provide valuable tools for use in IPM. The chapter begins by reviewing allelochemicals for pest management and also discusses botanical pesticides. Sections on repellents, attractants and defence activators are also provided. The chapter concludes by discussing the importance of overcoming barriers to improve the use of allelochemicals in pest management (IPM).

Chapter 13 reviews the use of peptides as novel biopesticides. Venom peptides are small toxic proteins originated from animal venoms. They have attracted great interest as a route to develop the next generation of novel, effective and safe biopesticides. The chapter begins by reviewing the characteristics and discovery of a range of venom peptides. It shows how inhibitory cysteine knot (ICK) type peptides, characterized by strong selectivity/specificity, stability and capacity for production at high volume, were used to develop the first commercialised peptide-based insecticide: SPEAR®. It also discusses other commercialised peptides and techniques to incorporate venom into plants to protect against insect and fungal attack.

The final chapter of the book, Chapter 14, reviews the development of plant-derived compounds as biopesticides. It begins by providing an overview of the legal framework and authorization of plant-derived plant protection products, then goes onto discuss how these products are developed. The chapter focuses on how plant-derived substances with activity against plant pathogens can be identified, as well as the efficacy of botanicals and novelty of invention and IP protection. It also highlights the importance using raw material of a high quality for plant-derived plant protection products. The chapter also provides a section on the extraction process that is used to produce extracts suitable for plant protection products. The chapter concludes by highlighting the growing interest in environmentally-friendly produced agricultural products, including plant-derived plant protection products, and also provides potential sources of further information on the subject.

Part 1

General

Chapter 1

Improving methods for developing new microbial biopesticides

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1 Introduction

Biopesticides are attracting global attention as pest management tools that have a role in sustainable pest management. They are broadly defined as the deliberate use of living organisms, such as bacteria, fungi, viruses and nematodes, and their natural products (bioactive compounds produced as secondary metabolites) that directly or indirectly harm weeds, reduce the impact of plant diseases, or control the insect pests. Although the scientific community has been involved in biopesticide technology development for decades, it is only in recent years that it has been recognized as a viable emerging technology that is acceptable for mainstream use, rather than niche market products (Vincent et al., 2007; Bailey et al., 2009; Glare et al., 2012, 2016; Morán-Diez and Glare, 2016). They have generally been accepted as environmentally friendly technology because they are naturally occurring and thus considered attractive alternatives to synthetic pesticides. This is not to say that all naturally derived microbes are safer than chemicals because some of the mycotoxin-producing fungi can be considered very toxic to humans and animals.

2 The role of biopesticides

Several factors have precipitated the necessity for developing biopesticides as pest control strategies to synthetic pesticides. These include the deregistration or

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phasing out of older chemistries for agricultural and horticultural crop protection by the regulators and phasing out of numerous synthetic pesticides in lawns and gardens (Bailey et al., 2009; Boyetchko, 2017). Government legislation was enacted to ban synthetic pesticides for cosmetic use in urban municipalities in Canada through the Pest Management Regulatory Agency (PMRA). For example, Halifax, Nova Scotia, became the first Canadian city to ban chemicals within city limits in 2000 (Bailey et al., 2009). More than 100 urban municipalities in Canada followed suit, while the provinces of Ontario, Quebec, and Manitoba have ratified similar bans for cosmetic use of pesticides within urban municipalities. These regulations have thus generated a demand for biopesticides. However there are not sufficient biopesticide products to replace those that have been forbidden for use by the public and/or city employees. Therefore there is an even more urgent need to conduct future research for the discovery, development, and registration of new biopesticide products. The legislation accordingly supported policy changes within the Canadian regulatory body to register lower-risk pest control products. Biopesticides were thus classified under the Reduced-Risk Products Initiative (http://www.hc-sc.gc.ca/cps-spc/pest/index-eng.php; Bailey et al., 2009). This program helped expedite the registration of currently registered biopesticides in the United States which led to the harmonization of regulations with the US EPA and Canadian PMRA. During this time, there was a significant increase in PMRA registrations because registrants were allowed to undergo a pre-submission consultation process and the length of time it took to register biopesticides was reduced. In addition to the changes in federal legislation, the federal government created the Pest Management Centre (PMC) in 2003, an entity that helped in the delivery of new biological control products by jointly funding research to aid in their registration. PMC also provided support to encourage the adoption of these new pest management tools.

The general public has a greater awareness for environmentally friendly and safe products in agriculture and forestry, and for use in home gardens.(Ott et al., 1991; Magnusson and Cranfield, 2005; Bailey et al., 2009; Glare et al., 2012) . Therefore there is a greater expectation for organic and pesticide-free products. Agricultural practices such as the continued use of chemicals that pollute soil, water, and food supplies are putting more pressure on industry to provide suitable products for pest management and this practice has forced the industry to evaluate current pest management practices and how chemical pesticide loads have affected our environmental performance. Changes in consumer acceptance of biopesticide technology are thus driving the need for such products and are leading the initiatives into the development of 'green' technologies for food production in grocery stores and farmer's markets.

Another major issue of concern related to traditional chemical pest control products is the development of pesticide-resistance to chemicals making them ineffective to the pests they were intended to control (Boyetchko and Rosskopf, 2006; Lazarovits et al., 2007; Bailey, 2010; Bailey et al., 2009; Glare et al., 2016). Repeated use of synthetic pesticides has created this problem, but researchers and the industry involved in their manufacture were skeptical to believe that pesticide-resistance could occur until the scientific community reported that such problems were occurring. For example, there are numerous examples of (i) insecticide-resistance to pyrethroids, organophosphates, and carbamates with insects; (ii) herbicide-resistance to acetyl-CoA carboxylase (ACCase), acetolactate synthase (ALS), and 5-enolpyruvylshkimate-3-phosphate (EPSPS or glyphosate) with weeds; and (iii) fungicide-resistance to QoI and azole fungicides with plant pathogens (Hawkins et al., 2019).

3 The economics of biopesticides

The discovery of new synthetic chemicals with novel modes of action is becoming more expensive and difficult. It is estimated that their discovery is decreasing by 1–2% per annum, while the biopesticide industry is expected to grow by over $1 billion by 2010, and projections indicate the biopesticide market to exceed $4 billion by 2023 (Oerke, 2006; Thakore, 2006; Bailey et al., 2009; Glare et al., 2012). The cost to develop a new synthetic pesticide is more than $250 million and takes at least 10 years for a product launch (Oerke, 2006; Kelly and Allen, 2011; Glare et al., 2012). Conversely, it takes fewer microbial strains to discover a new biopesticide and can cost approximately $3–5 million to commercialize in the United States and take 3 years to get to market. In the EU, it typically takes 5–7 years to get a biopesticide to market and there are fewer biopesticide-active substances registered in the EU versus the United States, mainly due to greater complexity of EU-based biopesticide regulations (Balog et al., 2017).

In the past, the multinational companies did not invest in many biopesticide products. The commercialization of the majority of biopesticides was engaged by small to medium-sized companies (Bailey, 2010; Bailey et al., 2009; Boyetchko, 2017). Investment in infrastructure for the discovery of synthetic chemicals is expensive and facilities and equipment can be specialized for their discovery. Large firms invested in discovery and development of synthetically derived compounds whereas a change in infrastructure investment is required for microbial-based technology, which requires access to fermentation capabilities. Moreover, formulation of chemical pesticides may or may not be compatible with biopesticide technology. Therefore, small- to medium-sized enterprises (SMEs) were often responsible for biopesticide development. It was anticipated that multinational companies would tackle the screening of microorganisms and their secondary metabolites once they acquired small to medium-sized biopesticide companies (Glare et al., 2016). For example, Becker Underwood was acquired by BASF, and Bayer CropScience purchased

Agraquest and Prophyta, in addition, Syngenta purchased Valent BioSciences and Pasteuria BioScience. This investment by multinationals demonstrated their recognition of the growing value of the biopesticide market. Nonetheless, multinationals have the resources to devote to R&D however, their focus has been on major crops such as corn, soybean, rice, potato, and cotton being the main emphasis. Other markets that remain overlooked are pulse crops, small and large fruit crops and high value field and greenhouse vegetable crops and other horticultural crops. This therefore creates an opportunity for biopesticides to fill this niche.

4 Strategic framework for biopesticide development

One of the major challenges to the development and commercialization of biopesticide products is how to manufacture them at an affordable cost and in a consistent manner. Fermentation systems, formulation technology, and spray application or delivery of biopesticides has often been ignored in the R&D phase by researchers. It should be borne in mind that the initial screening of microbial organisms for biopesticides represents the first step in the discovery phase. The perception of finding a suitable organism that effectively controls the target pest by senior managers and the general public unfamiliar with the complexities of biopesticide development often assumes that once the organism has been discovered, that is the end of the process. However, the microbe must be mass-produced through fermentation systems, subsequently formulated into a stable form and applied using spray application systems to facilitate their delivery to the soil, seed, and/or foliage (Boyetchko and Peng, 2004; Hynes and Boyetchko, 2006). Developing a suitable fermentation system requires an understanding of the microbial physiology of the organism and a suitable formulation often involves the knowledge of the microbe’s mode of action in order to facilitate delivery of the biopesticide. Contrary to the popular belief that once a microbial organism has been discovered with biopesticidal properties in the laboratory, the final biopesticide product is imminent, a biopesticide product is one where all the processes related to its manufacture are considered, including platform technologies related to fermentation, formulation, and spray application (Fig. 1; Boyetchko and Peng, 2004). The taxonomy, biological characterization, mode of action, and efficacy are among the factors that are core to the selection of the biopesticide organism. The platform technologies are interrelated and closely linked and their complexities have been underestimated (Boyetchko and Peng, 2004; Boyetchko et al., 2007; Bailey, 2014). They are integral factors to consider with the biopesticide product development. The goal is to find ‘nature’s best’ with respect to the microbe selected for biological control. However, fermentation or formulation may not necessarily improve a mediocre microbe, but a superior microbial agent may

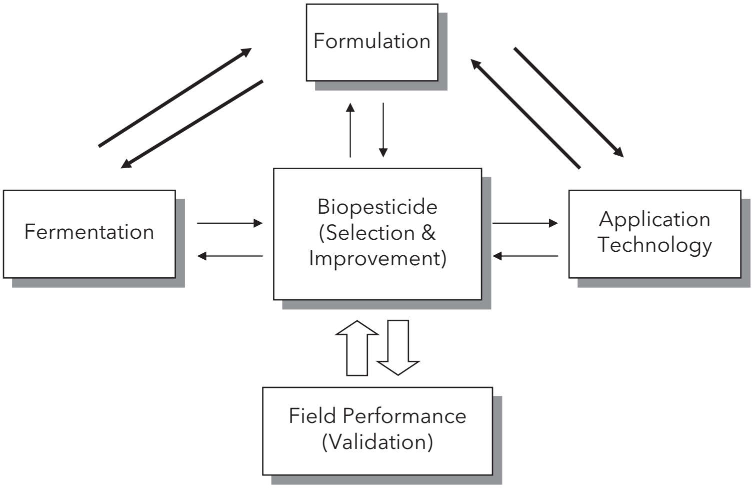

Figure 1 Strategic framework for evaluation and development of biopesticides. Source: adapted from Boyetchko and Peng (2004).

have to be sacrificed if it cannot be readily grown in suitable fermentation media or it cannot be readily formulated. Compromises are sometimes made in the selection process.

The earlier prototype models of biopesticides were manufactured using simple formulations but the intricacies between the platform technologies are much more complicated (Auld and Morin, 1995; Fravel, 1999; Auld et al., 2003). Lack of appropriate formulation may have led to ineffectual or inconsistent field performance and fermentation of the microbial organism may be too expensive or scale-up unacceptable that the product commercialization was abandoned which led to an 'orphaned' biopesticide product (Butt et al., 2001; Hallett, 2005; Hynes and Boyetchko, 2006).

4.1 The process of biopesticide development

Biopesticide discovery and development follows a process of incremental steps that are unique for each target pest-biopesticide system. Different research or industry groups follow different stepwise protocols to facilitate in the selection of promising biopesticide candidates (Boyetchko and Svircev, 2009; Bailey et al., 2009; Bailey, 2010; Köhl et al., 2011; Ravensberg, 2011; Glare et al., 2012). The 'Biopesticide Innovation Chain' (Fig. 2) was developed in response to a need for several researchers in the disciplines of weed science, entomology, and plant pathology to follow a similar blueprint to develop microbial biopesticides. The innovation chain depicts nine critical stages for developing a biopesticide product using a series of 'Go vs. No-Go' criteria in order to make decisions on

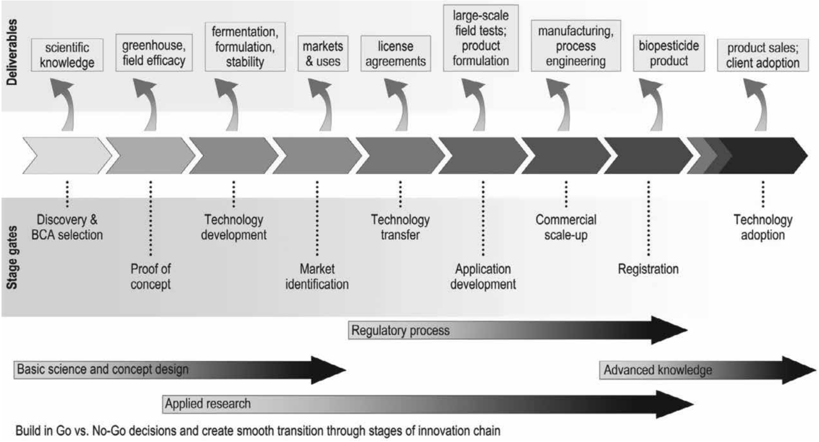

Figure 2 Solution for delivery of biopesticides: AAFC biopesticide science innovation chain. Source: adapted from Boyetchko and Svircev (2009).

the feasibility of the organisms and the target pest. This concept is applicable to any type of biopesticide (i.e. bacteria, fungi, viruses, natural products) and is appropriate for any type of crop pest (i.e. weed, invertebrate/insect pest, plant pathogen). The stages of the biopesticide innovation chain include (i) discovery and selection; (ii) proof of concept; (iii) technology development; (iv) market identification; (v) technology transfer; (vi) application development; (vii) commercial scale-up; (viii) registration; and (ix) technology adoption. The innovation chain describes the process for developing a biopesticide without considering the scientific terminology specific to each crop protection discipline; everyone will be describing the process using similar terminology. This road-map forces the researcher to proceed to the subsequent steps while not being fixated on one or two stages, thus preventing him/her from advancing toward the end goal: developing a biopesticide.

The early stages of discovery involve characterization of the biopesticide agent, assessing growth characteristics, basic understanding of the biology, genomics, and initial in vitro and in vivo screening bioassays that measure efficacy on the target pest (Bailey et al., 2009; Bailey, 2010). Proof of concept entails evaluation of biopesticide performance on live plants under controlled-environmental conditions where light, temperature, and relative humidity/moisture conditions can be monitored. It is then essential to bring the evaluation to the field, where possible, in order to demonstrate the adequacy of the biopesticide performance. Technology development involves the bioprocesses of fermentation/mass-production, formulation, and application technology for delivery of an efficacious, stable biopesticide with qualities that contribute to an 'adequate' shelf life for a microbe that is easy to grow and deliver to the target pest. As previously indicated, these platform technologies that are critical to biopesticide development are often linked. Recent advances have been publicly available, but, unfortunately, industry has often kept fermentation and formulation as proprietary or trade secret information. Several fermentation and formulation may tested before a final process is selected (Bailey, 2014).

The importance of market considerations cannot be underestimated since they will dictate the success or failure for commercialization. Although this area is not necessarily a research consideration to scientists, it is important because it defines the field of use and the market the biopesticide is being developed for. It will pre-determine the experimentation and scientific data required to register the product. For example, will the biopesticide be developed for use in the horticultural or greenhouse market, in large acreage field crops, or for use in forestry or for the home gardener? Another key feature of the innovation chain is that it encourages additional applications and crop pests into other potential use patterns, thus broadening to other target pests and production systems (Boyetchko and Rosskopf, 2006; Hynes and Boyetchko, 2006). A great deal of understanding of the microbial physiology and biochemistry is also required, but the business side of the research is an essential factor.

The later stages in the innovation chain (i.e. technology development, technology transfer) test the robustness of the earlier decisions by actively working with various stakeholders involved in the development of biopesticides. These include the industry partner, other collaborators, and regulators necessary to develop the data required for the registration package. Moreover, involvement of additional stakeholders generates ‘buy-in’ of the new technology which will help with the adoption of the biopesticide into the field of use. For example, the innovation chain can be used as a tool for the training of farmers and crop protection consultants in the safe and effective use of biopesticides. Moving into commercial scale-up can still affect the final stages of product development and is more often led by industry, often requiring significant investment back into technology development to registration, and technology adoption, which is usually directed by the industry partner and extension agrologists. The champion of the technology is usually the lead scientist or inventor who should work collaboratively with industry until the technology adoption has occurred. The corporate memory or ‘know-how’ and intuition of the lead scientist, along with his/her scientific expertise, can ease in the transition phases toward commercialization. Do not assume that the technology will be successful once it has been handed off to industry, without input from the scientist who originally discovered the biopesticide. The lead scientist can provide guidance into the overall idea and direction of the scientific discovery, along with a number of unsuccessful trials that had been attempted but failed during the process of developing the biopesticide.

5 Microbial exploration and discovery

If we are to identify the 'nature's best', a systematic approach is essential during the exploration and discovery phase to thoroughly evaluate the microbial biodiversity. This biodiversity provides excellent opportunities for finding microbial strains with suitable traits for biocontrol potential (Boyetchko and Peng, 2004).

Numerous discoveries of biopesticides have taken place in university and government institutions resulting in the licensing of the technology to industry. Many of these institutions may be in possession of undervalued but extensive microbial culture collections which can be exploited for the discovery of future biopesticide candidates. To add value to these collections, it is essential to conduct additional mass-screening of these same microbes against additional target pests, whether they are for insecticidal, fungicidal, or herbicidal activity using established in vitro and in vivo screening protocols. Over the years, a researcher can accumulate numerous future biopesticide candidates which can be stored under −80°C conditions until required. Future collections can thus access potential microbes based on the taxonomy of related pests they are known to be inhibitory to. Different strains to the same genus species may perform differently from one another and should not be readily discarded. Glare et al. (2012) described how Trichobank™, a culture collection of over 2000 isolates of 21 *Trichoderma* spp. has been effectively utilized to select for promising biopesticide candidates with various growth characteristics for control of several soil-borne diseases. Similarly, Bailey et al. (2009) explained their philosophy behind screening for microorganisms as bioherbicides. Once the target weed is selected, several hundreds to thousands of fungi and bacteria that have been collected through surveys were isolated from a broad range of environmental conditions and evaluated to select those microbes that possess a list of desirable characteristics. Therefore, a systematic approach for selection of appropriate biopesticide candidates is conducted. For example, the fungal pathogen, *Phoma macrostoma*, was initially discovered to be a foliar pathogen, but its bioheribicidal potential to control Canada thistle and chickweed was only discovered when it was found to be effective when applied to the soil (Bailey, 2014). The potential for *P. macrostoma* as a bioheribicide was only discovered when the researchers understood what was the best method to apply the fungal pathogen and what was its mode of action. No longer are potential biopesticide candidates developed by a fortuitous discovery, as was the case with *Colletotrichum gloeosporioides* f.sp. *malvae* to control round-leaved mallow (Boyetchko et al., 2007; Bailey, 2010). Experienced researchers are developing their own road-map and selection process for screening biopesticide organisms.

Endophytic bacteria and fungi found in vascular plants are an underexplored bioresource as promising biological control agents (Vega, 2008; Glare et al., 2012; Eljounaidi et al., 2016; Rybakova et al., 2016; De Silva et al., 2019). Endophytes grow inside the plant for part or all of its life cycle but do not cause visible symptoms. Almost all vascular plants examined to date have been found to harbor bacterial and/or fungal endophytes. It is believed that many endophytic microbes may have originated in the rhizosphere and phylloplane and they gained entry through natural openings or plant wounds. Due to the diversity of species discovered to be endophytic, it is postulated that they may prove to be very suitable biopesticides and plant-growth promoters. Endophytes can be introduced as biocontrol agents through seeds and propagated plant material such as those introduced via tissue culture (Eljounaidi et al., 2016). Once endophytes enter the plant, they will not be subjected to environmental stresses that many other nonendophytic biocontrol agents can succumb to. This may mitigate the need to develop formulations to protect them from unfavorable environmental conditions. Some endophytes may demonstrate their biocontrol potential by secreting antifungal and antibacterial metabolites, thereby inhibiting the competition of pathogens, or they may exhibit mycoparasitic activity (i.e. parasitism of one microorganism by another microorganism) (de Silva et al., 2019). Those endophytic microbes that are entomopathogenic are surmised to act as a feeding deterrent or by antibiosis. Commonly isolated bacteria that can become endophytic belong to the genera *Bacillus, Pseudomonas, Agrobacterium, Paenibacillus* and *Enterobacter*. Common endophytic fungal microbes belong to *Fusarium oxysporum, Trichoderma* spp., *Colletotrichum gloeosporioides*, and *Phomopsis* spp.

Many biopesticide products were developed because they targeted a single major pest (Glare et al., 2012; Bailey, 2014; Boyetchko, 2017). Narrow host-range possessed by these biopesticides was initially considered an asset, if not essential. A narrow host-range contributes to environmental safety and limits the risk of a biopesticide affecting nontarget crops and other beneficial microbiota and microfauna. This trait has also restricted its success to a niche market product. However, it was later considered a hurdle to commercialization because industry was hesitant to invest in technology with such a small market potential. It was recognized that it is important to select a product that has activity against multiple pests in order for industry to get a return on their investment. Moreover, it is not practical or economical to develop a crop protection product for use by farmers on a single target pest when agriculture and forestry ecosystems are comprised of multiple crop pests. For example, bioinsecticide products containing *Bacillus thuringiensis* have had remarkable success in the marketplace because they kill several invertebrate pest species (Côté, 2007). Similarly, the fungus *Chondrostereum purpureum* was developed as a broad-spectrum bioherbicide to control a number of woody tree species and shrubs for vegetative management in forests and riparian lands and utility-rights-of-way (Hintz, 2007). Control of a broad spectrum of plant diseases is also possible with the commercial product Serenade®, hence adding to its commercial value (Marrone, 1999, 2002).

5.1 Biological characterization and development

The discovery of new and promising microbial candidates as active ingredients for biopesticides has far out-paced the knowledge and related technology required to bring them to commercialization. Although there are numerous biopesticides registered for use globally (Vincent et al., 2007), including over 100 biopesticide-active ingredients in the United States and more than four different microbial-based active ingredients registered in Canada since 1972 (Fravel, 1999; Hynes and Boyetchko, 2006; Bailey et al., 2009; Kabaluk and Gazdik, 2011), the public still wonders why more biopesticides are not commercially available. It has been pointed out that scientific efforts in biological control have not contributed to the lack of commercial success of biopesticides, but that researchers have failed to focus on resolving critical technical elements such as formulation and methods for stabilization of living organisms (Zorner et al., 1993). It is true that a great deal of research effort has focused on the search for microbial agents with pest control potential, but technological challenges that include economical methods for mass production and scale-up, viable formulation and application strategies, and shelf life have been encountered and have contributed to the lack of commercial success (Auld and Morin, 1995; Boyetchko and Peng, 2004). However, the long-term commitment toward biological control has been predominantly supported by public research institutions such as government and universities (Boyetchko, 2017), while investment by industry to commercialize this technology has been sorely lacking. Access to public funding has been one constraint to this area; biopesticide research requires long-term commitment to funding, while many funding agencies provide financial support for projects that are 3–4 years in duration. In addition, the teams of researchers dedicated in understanding the fundamentals of pest biology and ecology and the agronomists necessary to implement pest management practices, have not been active participants in the application and implementation of biopesticide technology. It should be recognized that the success of biopesticides will depend on the involvement of multidisciplinary research teams consisting of crop protection specialists in either entomology, weed science, or plant pathology, microbiologists, natural products chemists, agronomists, molecular biologists, and economists with a vested interest in biopesticide adoption (Fig. 3). Biopesticides should not be considered a stand-alone pest management tool. A holistic approach using various tools for controlling pests can often lead to an additive or

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